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ORIGINAL PAPERS

INVESTIGATING THE BIOCHEMICAL POTENTIAL OF QUANTITATIVELY DOMINANT *ULVA* SPECIES FROM THE ROMANIAN BLACK SEA COAST

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Abstract. *Green macroalgae of the genus Ulva are commonly found along the Romanian Black Sea coast, with Ulva rigida and Ulva intestinalis emerging as the quantitatively dominant species in the past years. This study investigates the biochemical composition and antioxidant activity of U. intestinalis and U. rigida collected from the Romanian Black Sea coast during summer 2024. Both species exhibited high moisture content (~80%), with U. intestinalis showing higher protein (10.8% DW) and carbohydrates (9.09% DW) levels compared to U. rigida (7.61% DW and 3.46% DW, respectively). Total lipid content was low in both species. Ulvan extraction revealed significantly higher yields in U. rigida (up to 14.73% DW) than in U. intestinalis (1.75% DW). Catalase activity, used to assess antioxidant potential, showed no significant differences between species. These findings highlight the valorization potential of Ulva spp. as a sustainable marine resource.*

Keywords: Romanian Black Sea coast, green macroalgae, *Ulva* spp., biochemical composition.

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1. Introduction

Seaweeds are an important source of proteins, which, together with peptides, show interesting bioactivities, with multiple applications such as food and pharmaceutical compounds. Furthermore, they also contain considerable quantities of pigments, which, besides exhibiting biological properties with potential benefits for human health, such as antioxidant, anticancer, anti-inflammatory, or biomedical applications, among others, are a promising alternative in the pharmaceutical, agriculture, and textile sectors and as an artificial food coloring [1].

Species of green macroalgae from the genus *Ulva* (Phylum Chlorophyta, Class Ulvophyceae, Order Ulvales, Family Ulvaceae) are among the most abundant seaweed species, being omnipresent in coastal communities around the world.

From an economic point of view, *Ulva* spp. biomass has long been recognized as sustainable and valuable. It contains valuable metabolites, including bioactive compounds, which can be used in the food, pharmaceutical, nutraceutical, or biorefinery industries [2]. Green algae species such as *Ulva intestinalis, U. fasciata, U. lactuca,* and *U. rigida* are well known for their exceptional nutritional profiles, owing to their high concentrations of carbohydrates, proteins, lipids, minerals, and vitamins, essential nutrients for human health. Studies have shown that the biochemical composition of marine algae varies significantly depending on species, habitat, maturity stage, environmental factors, and the time of collection [3], genetic differences and populations as well as environmental conditions, such as temperature, salinity, irradiance and nutrient composition of the water [2].

Ulva species contain unique polysaccharides that give them pharmaceutical value and physiological benefits, particularly in the prevention of conditions such as inflammatory disorders, viral and bacterial infections. These compounds also act as immunomodulatory and hypolipidemic agents, with effects that are primarily preventive rather than curative. Among these, ulvans, structurally distinct heteropolysaccharides found in the cell walls of *Ulva*, exhibit a wide range of bioactive properties, including immunomodulatory, antioxidant, anticancer, anti-inflammatory, antiviral, antidiabetic, anticoagulant, and cytotoxic activities [4].

The most common *Ulva* species found year-round along the Romanian Black Sea coast include *U. rigida, U. intestinalis, U. compressa, U. flexuosa, U. linza,* and *U. prolifera.* Among these, *U. rigida and U. intestinalis* were quantitatively dominant in the past years [5].

The aim of this study is to highlight the biochemical profile of the most quantitatively abundant *Ulva* species along the Romanian Black Sea coast. The selected species were chosen based on their high availability in the area, taking into account their abundance, seasonal occurrence, and their distribution across the bathymetric gradient. These characteristics make them promising candidates for future biotechnological applications. In this context, the present work focuses on the detailed analysis and characterization of two local *Ulva* species, namely *U. rigida* and *U. intestinalis*.

2. Material and Methods

2.1. Biological material

Primary biochemical analysis (dry weight - DW, ash, lipids, carbohydrates, proteins) and enzymatic antioxidant activity were performed on *Ulva* spp. samples collected during summer season of 2024 from Pescărie area (44.218293, 28.650746). The selected area is characterized by high macroalgal diversity, with

abundant populations of green algae from the genus *Ulva*, alongside species of red and brown algae. Furthermore, the location facilitated efficient transfer to the laboratories, thereby preventing deterioration of the algal material prior to biochemical analysis. The samples were harvested from depths between 0 and 3 meters, cleaned with seawater to remove sand, debris, epiphytes, and other impurities. In the laboratory, samples were sorted, rinsed with distilled water to eliminate surface salts, taxonomically identified and stored at -20°C until analysis. Ulvan extraction was performed on freeze-dried *Ulva* material. Two extracts were obtained from samples collected during the summer seasons of 2023 and 2024. The sampling periods were similar in both years, spanning July to September.

2.2. Dry weight and ash content determination

For each species (*U. rigida* and *U. intestinalis*), one g of fresh algal material was weighed in triplicate and washed with ammonium formate to remove salts and impurities. To determine dry weight, the samples were oven-dried at 100°C in successive intervals: 1 hour, 2 × 2 hours. After each drying step, the samples were weighed until a constant dry mass was reached. Subsequently, the dried samples were heated in an electric oven at 450°C for 4 hours to determine the ash content. After cooling in a desiccator, the ash content and organic matter were quantified by weighing the remaining material [6].

2.3. Total lipids

Lipid extraction was performed using a modified Bligh and Dyer method (1959), as adapted by Kates and Volcani (1966) and Mercz (1994) [7, 8, 9]. For each species, one g of fresh algal material was weighed in triplicate and washed with ammonium formate to remove salt. Excess water was removed by blotting, and samples were frozen at −80°C for 30 minutes to disrupt the rigid cell structure and improve handling. The frozen material was then ground using a mortar and pestle. A solvent mixture of methanol: chloroform: deionized water (2:1:0.8) was added (5.7 mL), followed by centrifugation at 4000 rpm for 15 minutes. The supernatant was collected, and a second extraction was performed on the residual biomass using the same solvent volume and conditions. The two supernatants were combined, vortexed with 3.3 mL deionized water, and then mixed with 3 mL chloroform (≥99% purity). The mixture was stored at 4°C for 24 hours to allow phase separation. After stratification, the upper aqueous phase (containing non-lipid components) was discarded. Seven drops of toluene were added to each tube to remove residual water. The lower chloroform phase (containing lipids) was transferred to pre-weighed tubes and evaporated using a nitrogen concentrator at 38°C for 35 minutes. The tubes were then weighed to determine the lipid content (Figure 1).

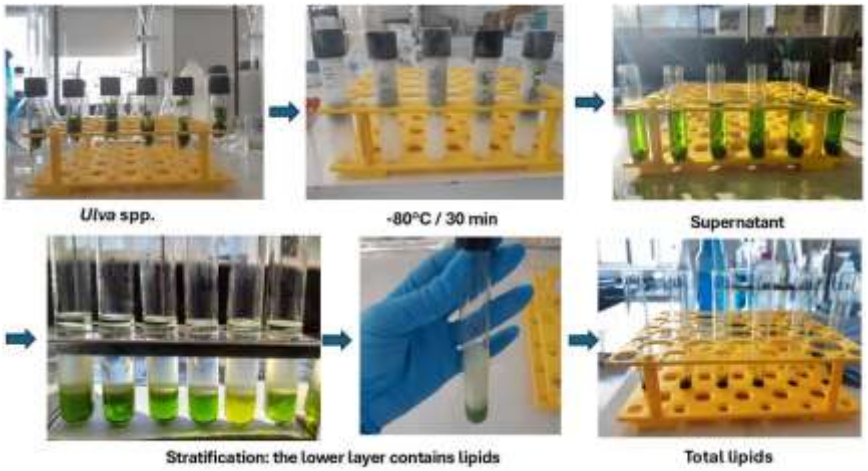

Fig. 1. Total lipids extraction from *U. intestinalis* and *U. rigida* – main steps

2.4. Carbohydrates

One gram of fresh algal material from each species was weighed in triplicate, washed with ammonium formate, blotted to remove excess water, and ground. Samples were frozen at -80°C for 30 minutes. Then, 5.5 mL of 1 M H_2SO_4 was added in two steps: 0.5 mL before and 5 mL after homogenization. Samples were heated at 100°C for 60 minutes, centrifuged at 4000 rpm for 10 minutes, and 2 mL of supernatant was mixed with 1 mL of 50 g/L phenol solution. After quick mixing, 5 mL of concentrated H_2SO_4 was added, homogenized, and cooled at room temperature for 30 minutes [6]. Absorbance was measured at 485 nm using 1:100 diluted samples, and concentration was determined using a glucose calibration curve (0 - 200 µg/mL) (Figure 2).

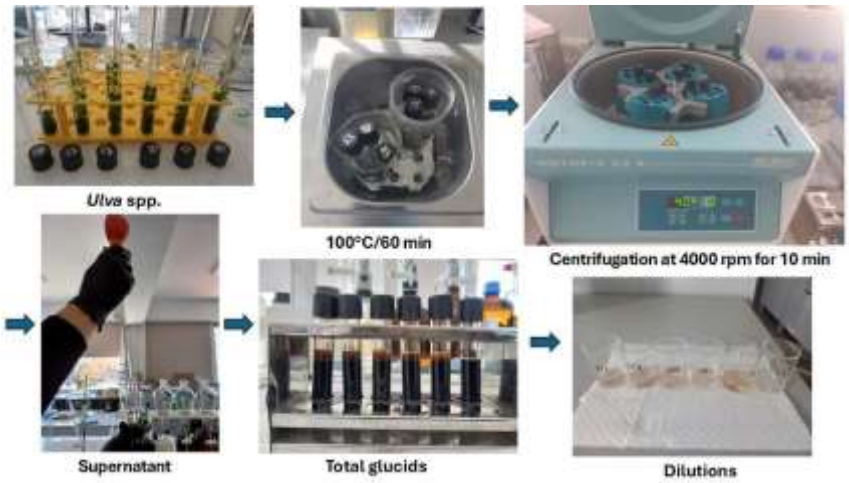

Fig. 2. Total glucids extraction from *U. intestinalis* and *U. rigida* – main steps

2.5. Soluble proteins

One gram of fresh algal material from each species was weighed in triplicate and washed with ammonium formate. A standard curve was prepared using serial dilutions of a bovine serum albumin solution (0-100 µg/mL). Protein extraction was performed using 0.5 M NaOH in a 1:10 biomass-to-reagent ratio (1 g algae:10 mL NaOH). Samples were sonicated for 4 hours, then centrifuged at 4000 rpm for 15 minutes [10]. Soluble proteins in the supernatant were quantified using the Lowry method [11]. Absorbance was measured at 660 nm using 1:100 diluted samples (Figure 3).

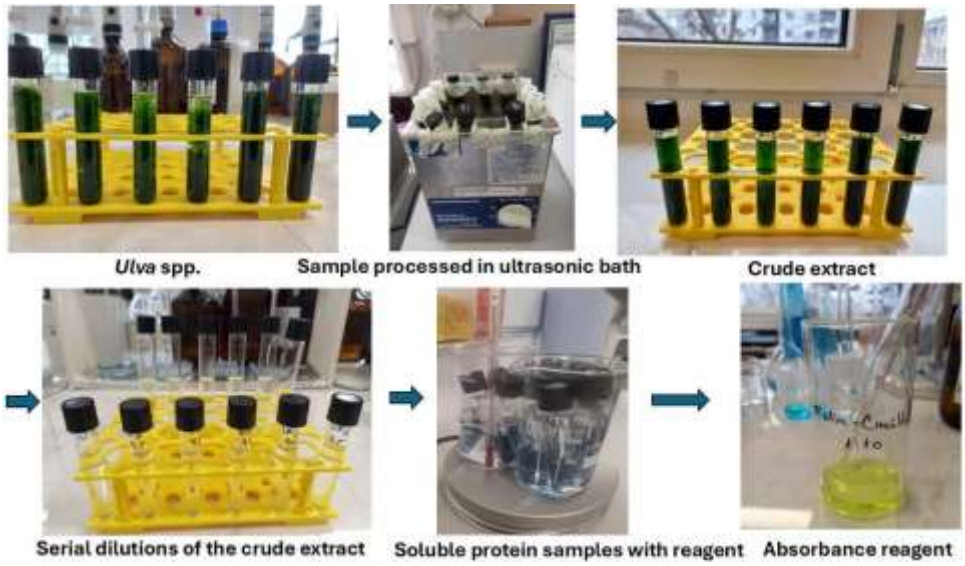

Fig. 3. Soluble proteins extraction from *U. intestinalis* and *U. rigida* – main steps

The algal material, carefully pre-processed, was subjected to primary biochemical analysis (Figure 4).

Fig. 4. Sample preparation for biochemical extraction: *U. intestinalis* (first 3 tubes) and *U. rigida* (last 3 tubes) in each image

2.6. Ulvan extraction

Eight grams of freeze-dried biomass from each species were weighed. The algal biomass was suspended in 200 mL of 0.01 M HCl (pH=2) and heated at 95°C for 4 hours. After cooling to room temperature, the mixture was centrifuged at 4000 rpm for 10 minutes. The supernatant was collected and dialyzed using a Spectrum membrane for 48 hours, then freeze-dried. The lyophilized fraction was redissolved in 15 mL of deionized water and precipitated with 60 mL of cold ethanol. The mixture was centrifuged at 2500 rpm for 20 minutes. The precipitate, containing various concentrations of ulvan, was collected, weighed, and freeze-dried (Figure 5) [12].

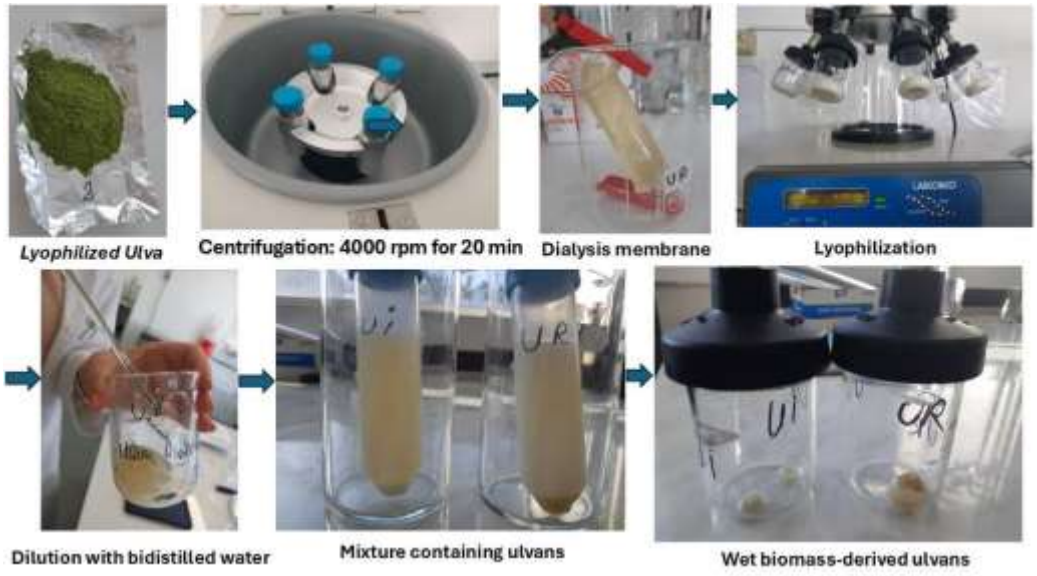

Fig. 5. Ulvan extraction from *U. intestinalis* and *U. rigida* – main steps

2.7. Antioxidant activity

Antioxidant activity in *U. intestinalis* and *U. rigida* was evaluated through catalase analysis, an enzyme that decomposes hydrogen peroxide into water and oxygen. Catalase activity was measured using Sinha's method (1972) [13], based on the reduction of potassium dichromate by H_2O_2 to chromic acetate, detectable at 570 nm. One gram of fresh algal biomass from each species, in triplicate, was washed, ground, and used for analysis. A standard curve was prepared using 0.01 M phosphate buffer (pH 7) and 0.08 M H_2O_2 (0-80 µmol/mL). The enzyme extract reacted with H_2O_2 for 60 seconds, then the reaction was stopped with dichromate reagent. Samples were boiled for 10 minutes, cooled, and absorbance was read at 570 nm. Catalase activity was expressed as µmol H_2O_2 decomposed/min/mg protein (Figure 6).

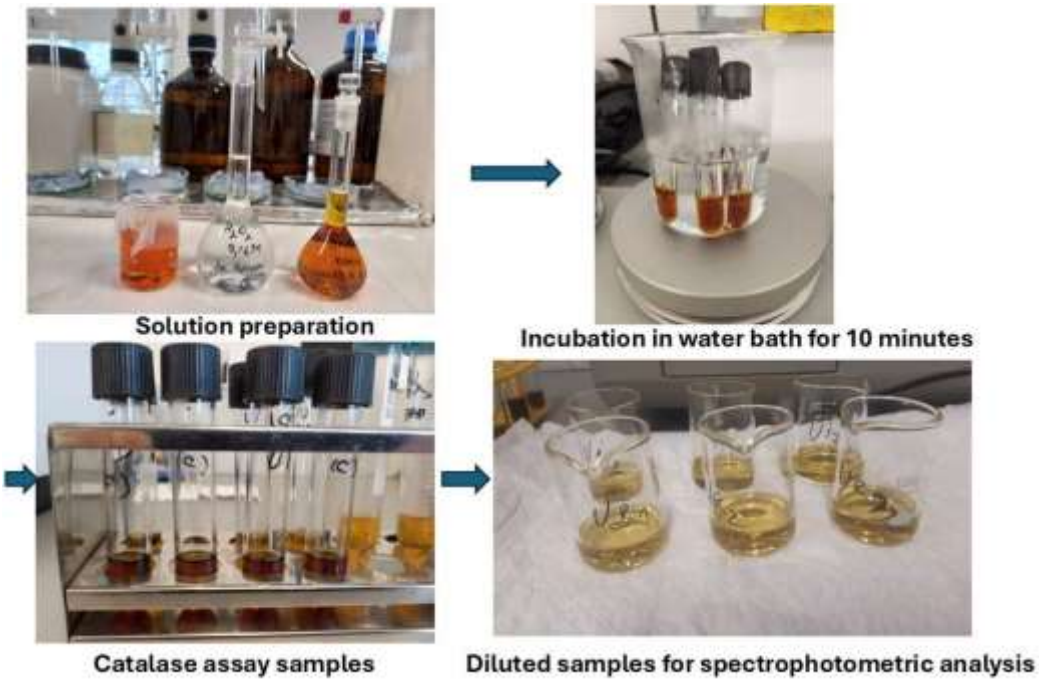

Fig. 6. Antioxidant Activity in *U. intestinalis* and *U. rigida* – main steps

2.8. Statistical analysis

Data analysis was performed using XLSTAT 2021.3.1 and JASP [14, 15]. To assess data consistency and potential differences between replicates, descriptive statistical analyses (mean, standard deviation) were applied, and appropriate statistical tests were used for comparisons. Results are expressed as mean ± standard deviation of three replicates and reported relative to dry algal biomass. Statistical significance was set at $p < 0.05$. The normality and homogeneity of variance for each data group were assessed using the Shapiro-Wilk normality test and Levene's test. To compare differences between datasets (across species and biochemical components), analysis of variance (ANOVA) and the parametric two-sample t-test were applied.

sample t-test were applied.

3. Results and Discussions

The nutritional value of *Ulva* species is determined by their biochemical profile. Their potential for use across various sectors, such as pharmaceuticals, industry, and cosmetics is closely related to the biochemical composition. Table 1 presents the biochemical profile of the two species, *U. intestinalis and U. rigida*.

Table 1. Seasonal biochemical characterization of *U. intestinalis* and *U. rigida* (summer 2024)

Components	***Ulva intestinalis***	***Ulva rigida***
Moisture	79.47±3.47	76.95±2.97
Organic matter	4.93±2.37	1.21±0.71
Dry weight (DW)	20.53±3.47	23.05±2.97
Ash	15.60±1.25	21.84±2.71
Main biochemical components (% DW)		
Total lipids	2,.66±0.80	2.26±0.57
Carbohydrates	9.09±1.25	3.46±1.90
Soluble proteins	10.80±1.95	7.61±1.46

* Values are expressed as mean ± SD; n = 3.

The water content of the two species is similarly high - approximately 80% in *U. intestinalis* and around 77% in *U. rigida*. The remaining portion represents dry weight, of which 5% is organic matter and over 15% ash in *U. intestinalis*, while *U. rigida* contains only 1.21% organic matter and about 22% ash. Generally, the water content of marine algae can be influenced by various factors, including tissue structure and morphology, thallus size, sampling location, collection period, and even laboratory sample preparation [16].

Ulva is considered an important source of minerals, with a relatively high ash content of 14% to 52% DW depending on the species and the growth conditions. The values recorded for *Ulva* spp. collected in summer 2024 from the Romanian coast fall within these ranges (Table 1) and suggest their potential as a valuable resource. The high ash content could be explained by the accumulation of essential minerals, which can be due to the presence of anionic carboxyl, sulphate, and phosphate in their cell wall [1].

Globally, green algae of the genus *Ulva* have high valorization potential due to their rich content of proteins (6 - 29% DW), carbohydrates (9 - 62% DW), lipids (0.5 - 8% DW), and ash (17 - 60% DW) [17].

In our study, among all the biochemical constituents, protein content was the highest in both species: 10.8% in *U. intestinalis* and 7.61% in *U. rigida* (Table 1). Regarding carbohydrates content, significant differences were observed between the two *Ulva* species from the Romanian coastal area, as *U. intestinalis* showed a higher carbohydrates level (9.09% DW) compared to *U. rigida* (3.46% DW). In general, macroalgae are reported to have lipid content of less than 5% DW [18]. Thus, for the two species from the Romanian coastline, the total lipid content fell within this range, being slightly higher in *U. intestinalis* (2.66% DW) compared to *U. rigida* (2.26% DW) (Table 1).

The use of quality protein from macroalgae in aquaculture, animal feed and human nutrition is expanding [10]. Protein accumulation in cells depends on environmental factors and relates to nutrient uptake for metabolism, photosynthesis and growth. According to Nissen and colab., higher temperature and irradiance resulted in a decrease in protein content, whereas addition of dissolved inorganic nitrogen increased the protein and fatty acid content while decreasing the ash content [17]. The highest protein content, 29% (DW), was recorded in *U. lactuca* collected from North Yorkshire in the United Kingdom and the lowest protein content was reported in the same *Ulva* species collected in Tunisia during the summer, which suggests a role of temperature in regulating protein content, similar to land plants [2]. The results of this study are in good accordance with the literature, which suggests that proteins represent less than 15% DW in seaweed.

Ulva spp. biomass is relatively low in energy due to a low lipid content (1 to 12% DW) [2, 19]. The differences in the reported quantities of extracted lipid could have been due to many factors, such as geographical and seasonal factors, or climate change as well as the development stage of the macroalgae. According to Moustafa and Batran [18], as the temperature increased, the lipid content in macroalgae decreased and remained almost stable until the end of the growing season. Since the samples were collected in the summer of 2024, when sea surface temperatures reached unusually high levels (27 - 28°C), this may explain the low lipid content observed in *U. intestinalis* and *U. rigida* in the present study.

The assumption of normality and homogeneity of data was met for lipid, carbohydrates, and protein content in the case of the two analyzed species. The parametric two-sample t-test showed no statistically significant differences between *U. intestinalis* and *U. rigida* in terms of lipid content ($p=0.518$) and protein content ($p=0.086$). However, there were significant differences in carbohydrates percentage ($p=0.013$) (Figure 7), with *U. intestinalis* exhibiting a higher carbohydrates content compared to *U. rigida* (Table 1). The absence of statistical differences may also be a consequence of the fact that the two species are generally found in association, occupying the same ecological niche and thus responding similarly to the availability of nutrients.

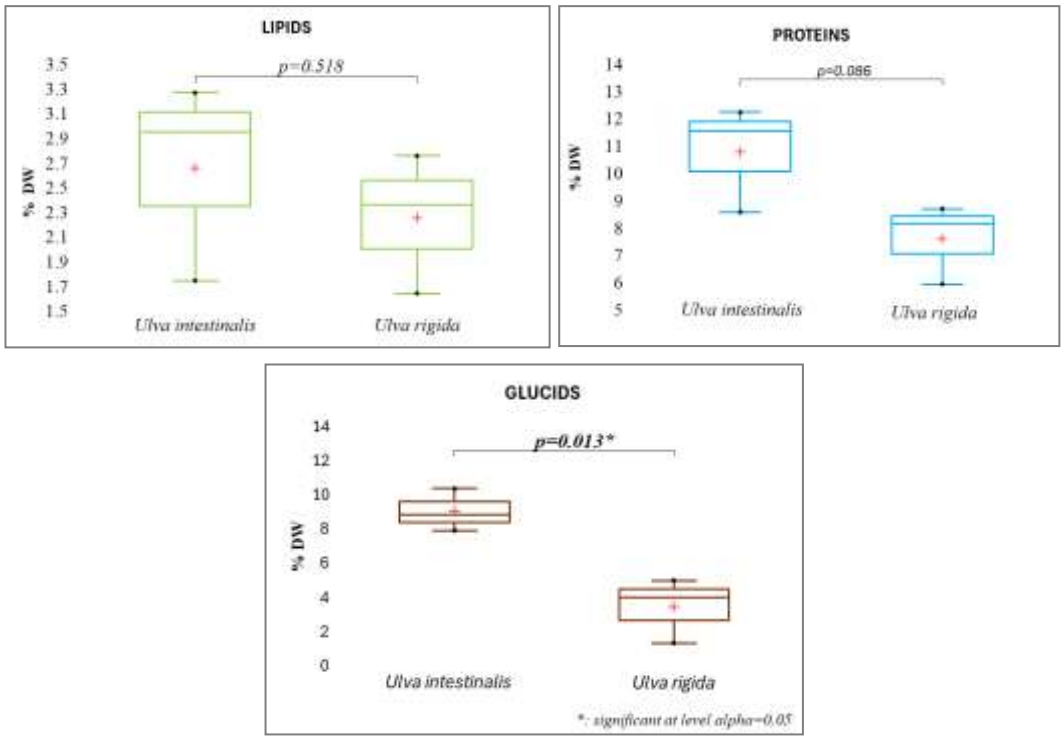

Fig. 7. Statistical analysis of the main biochemical constituents in *U. intestinalis* and *U. rigida*

A series of one-way ANOVAs were conducted to assess the effect of species on four biochemical parameters: humidity, organic matter, dry weight, and ash content. No significant differences were found for humidity or dry weight ($F_{(1, 4)}$ =0.909; p=0.394). Organic matter showed a marginally non-significant trend ($F_{(1, 4)}$ =6.780; p=0.060), suggesting a potential species effect that warrants further investigation. In contrast, ash content differed significantly between species ($F_{(1, 4)}$ =13.05; p=0.023), highlighting a species-specific influence on this parameter.

Ulva spp. are rich in dietary fibre, which supports gastrointestinal health and may reduce the risk of chronic diseases. The fundamental active constituent of *Ulva* is the soluble fibre ulvan, a gelling sulfated polysaccharide with biological activities including immunomodulating, antiviral, antioxidant, antihyperlipidemic and anticancer. Ulvan also has the capacity to modulate cellular signaling processes in both plant and animal systems, leading to beneficial effects on productivity and health. Consequently, ulvan is of significant interest as a constituent in human health, agricultural, and biomaterial products [12]. Ulvan compounds exhibit a wide range of applications, including use as food additives, in packaging, cosmetics, pharmaceuticals, and agriculture. Their versatility lies in their ability to form hydrocolloid particles, gels, 3D hydrogels, and nanofibers, enabling diverse functional applications [20].

The sulfate polysaccharides derived from marine algae, including ulvans, are unique compounds since they combine the chemical diversity and biocompatibility of polysaccharides with unrivaled bioactivities such as antimicrobial, anti-fouling, antioxidant, anticancer, and anti-coagulant that are not found in any other chemical compounds. Ulvan has been shown to have antimicrobial activity. For example, the antimicrobial activity of ulvan obtained from *U. reticulata* demonstrated potent antimicrobial activity, with an inhibition zone diameter of 20 mm against *Enterobacter cloacae* and 18 mm against *E. coli* [21]. However, to utilize ulvan as a supplement, nutraceutical, and therapeutic agent, it is imperative to elucidate the potential cytotoxicity and delineate therapeutic dosages for effective application. The assessment of toxicity typically demands the conduct of in vitro inquiries involving cellular systems as a preclinical test step before in vivo analyses utilizing controlled experimental cohorts of living organisms [22]. The maximum ulvan extraction efficiency was not related to the maximum ulvan content in the seaweed, but with the active growth period of the seaweeds. Ulvan chemical and macromolecular structures are genetically controlled and modulated by growth conditions. From the viewpoint of industrial application, collection of *Ulva* for the production of gelling ulvan would be best during the active growth periods of the algae and particularly in spring [23].

Significant interspecific differences were observed in ulvan content from the lyophilized biomass of *U. intestinalis* and *U. rigida*, both in 2023 (*U. intestinalis* 1.75% DW; *U. rigida* 14.73% DW) and in 2024 (*U. intestinalis* 0.21% DW; *U. rigida* 9.25% DW) (Figure 8). Notably, *U. rigida* yielded a considerably higher ulvan fraction, approximately 12% DW, compared to *U. intestinalis*, which showed only about 1% DW (Figure 8).

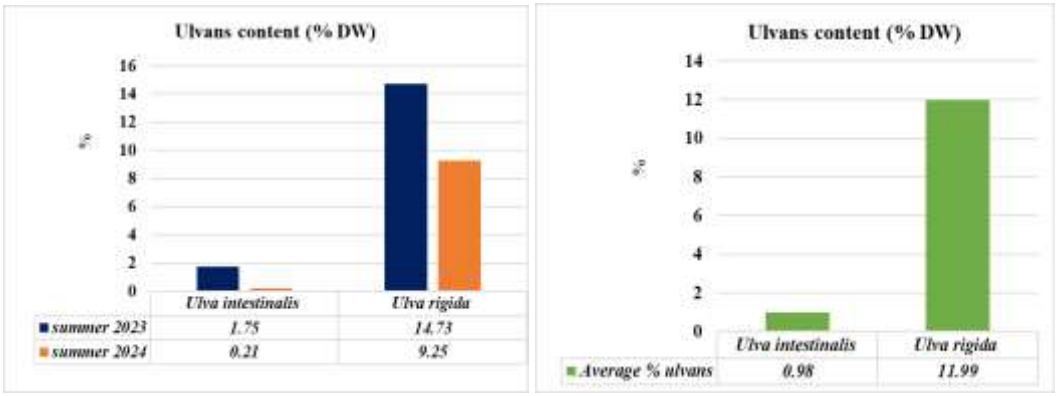

Fig. 8. Ulvans (% of dry weight) – (left) seasonal variation; (right) species variation

A one-way ANOVA showed no significant effect of species on catalase activity ($F_{(1, 4)}$ =0.193; p=0.683). *U. intestinalis* exhibited a catalase activity of 2.76 ± 0.34 µmol H_2O_2/mg protein, while *U. rigida* showed a slightly lower value of 2.65 ± 0.26 µmol H_2O_2/mg protein.

Conclusions

(1) *Ulva intestinalis* and *Ulva rigida* collected from the Romanian Black Sea coast in summer 2024 exhibit distinct biochemical profiles, with *U. intestinalis* showing higher protein and carbohydrates content, while *U. rigida* yielded significantly more ulvan.

(2) Both species demonstrated low lipid conten, less than 3% DW.

(3) Both species represent promising candidates for further investigation as potential sources of ulvans.

(4) The biochemical composition of these *Ulva* species supports their potential for valorization in food, pharmaceutical, and agricultural applications.

Acknowledgment

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