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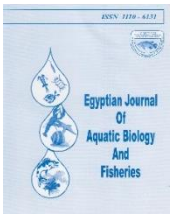

Antioxidant Activity, Nutritional Composition, and Proximate Analysis of the Moroccan Alga *Ulva Lactuca* from the Mehdia Coast

Mariame Taibi*, Marouane Aouji, Sara Razzak, Hiba Benchehida, Lalla Aicha Lrhorfi, Rachid Bengueddour, Oussama Hassani

Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Science, Ibn Tofail University, B.P. 133, Kenitra, Morocco

***Corresponding Author:** mariame.taibi1@uit.ac.ma

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ABSTRACT

The investigation of seaweeds to identify their bioactive metabolites and potential broader applications has gained significant relevance. This study aimed to analyze the proximate composition, mineral content, fatty acid profiles, water and oil-holding capacities, and antioxidant activity of various solvent extracts of *Ulva lactuca*. The results indicated that *Ulva lactuca* is characterized by its fiber content (12.68 ± 0.5%), carbohydrates (61.12 ± 1.42%), proteins (22.15 ± 1.7%), and lipids (1.3 ± 0.1%). It has a higher proportion of unsaturated fatty acids, an excellent ω-3/ω-6 ratio, and a high nutritional value. Additionally, it serves as a significant source of essential minerals, particularly potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), and phosphorus (P). The methanolic extract exhibited higher levels of phenolics (45.69 ± 3.24mg gallic acid equivalents/g DM), flavonoids (15.49 ± 0.064 mg quercetin equivalents/g DM), and tannins (22.52 ± 8.23mg catechin equivalents/g DM) compared to other extracts. Using total antioxidant capacity, DPPH (2,2-Diphenyl-1-picrylhydrazyl), and reducing power assays, the results demonstrated that all three examined extracts possessed antioxidant properties. Notably, the methanolic extract showed the highest antioxidant activity, with a half-inhibitory concentration of 33.6 ± 1.31µg/ mL in the DPPH radical scavenging assay. The study also revealed that the water and oil-holding capacities of *Ulva lactuca* varied with temperature and were comparable to those of many commercial fiber-rich products. Sourced from the coastal waters of Kenitra, *Ulva lactuca* presents a viable option for applications in nutrition, pharmaceuticals, and the agri-food industry due to its biochemical composition, functional characteristics, and antioxidant properties.

INTRODUCTION

Based on their chlorophyll and auxiliary pigments, macroalgae, often known as seaweeds, are divided into three major groups: green, brown, and red algae. These organisms are offered for sale as raw or processed goods and constitute an industry worth over 6 billion dollars annually **(FAO, 2018)**. They are widely recognized as a valuable source of essential nutrients and bioactive compounds beneficial to human health. Abundant in proteins, peptides, amino acids, minerals, and polysaccharides, these marine

organisms provide a diverse array of essential nutrients **(Ruan *et al.*, 2023)**. Algal extracts are commonly integrated into food preparation and have a long history of direct use in the food habits of the population in East Asia and the Pacific Islands. The growing popularity of healthy foods has increased Western consumers' interest in the benefits of algae, which have traditionally been consumed by coastal and artisanal communities. There is a particular emphasis on the presence of macronutrients, micronutrients, and bioactive compounds that have the potential to treat human diseases **(Vaghela *et al.*, 2023)**. These components, which help to create functional foods and nutraceuticals, consist of peptides, polysaccharides, fatty acids, pigments, and polyphenols. Antiviral, anti-inflammatory, antitumor, antidiabetic, antibacterial, anticancer, and anticoagulant characteristics are among the functions of these bioactive substances **(Bayomy, 2022)**. Among the polyphenolic compounds thought to be non-toxic and risk-free antioxidants are flavonoids. High consumption of natural phenolics in food has been linked in numerous studies to better endothelium function, lowered blood pressure, longer life expectancy, and a decreased potential for certain chronic illnesses like diabetes, obesity, and cancer **(Yan & Asmah, 2010)**.

To find natural bioactive molecules to create new medications and nutritious foods, several marine bio-sources have gained attention in recent years. Brown, red, and green algae have been shown to have substances with antioxidant, antiviral, antifungal, antibacterial, anticancer, and anti-inflammatory properties; moreover, seaweeds contain four primary types of antioxidants: pigments, polysaccharides, vitamins, and polyphenolic chemicals, such as tannins, flavonoids, and phenolic acid **(Cox *et al.*, 2010)**.

Algae's antioxidant content is influenced by light, salinity, seasonality, nutrition availability, and growth depth, among other external environmental variables. Furthermore, they rely on intrinsic variables such as the type of tissue, age, length, and algal species **(Lopez-Santamarina *et al.*, 2020)**.

The edible seaweed genus *Ulva* is an essential supper in many countries in Southeast Asia. As far as we are aware, *Ulva lactuca* has not been studied in the Kenitra region. The purpose of this study was to analyze *Ulva lactuca*'s chemical composition, nutritional profile, functional properties and the antioxidant properties for possible applications in the food, cosmetic, dietary supplement, and healthcare industries.

MATERIALS AND METHODS

1. Sample collection and preparation

Fresh seaweed was gathered by hand from the Mehdia coast (latitude: 9 °440 N and longitude: 79 °000 E), cleaned thoroughly in seawater to remove external matter such as sand, epiphytes, and other algae contaminants; then placed in polyethylene plastic bags and transported to the laboratory promptly to minimize deterioration. In the laboratory, the samples were washed again three times with tap water and one more time with distilled

water. Samples were dried at 40 ± 2°C for 78 hours, and then kept in dark bottles at 4°C, after being mechanically blended into a fine powder for future analysis and applications.

2. Phytochemical screening

The extraction was performed using a cold maceration method (5%, w/v) in three solvents of varying polarities. The mixture was subjected to magnetic stirring at 6000rpm for one hour, and then left at room temperature for 24 hours with occasional manual stirring to extract as many polar compounds, such as polyphenols, as possible. After filtration through Whatman No. 1 paper, the obtained filtrates were stored in dark glass bottles at 4°C for further analysis **(Dhasarathan & Theriappan, 2011)**.

Phytochemical screening was conducted using various chemical tests to identify the presence of specific classes of compounds **(Edeoga *et al.*, 2005)**:

- **Alkaloids:** Detected using Mayer's, Wagner's, and Dragendorf's reagents.
- **Carbohydrates:** Identified using Fehling's test.
- **Cardiac Glycosides:** Tested with Legal's reagent.
- **Flavonoids:** Detected using an alkaline reagent.
- **Tannins:** Determined with the Ferric Chloride test.
- **Phytosterols and Triterpenoids:** Identified using the Salkowski test.
- **Proteins:** Detected with the Ninhydrin test.
- **Anthraquinones:** Assessed using the modified Borntrager test.
- **Saponins:** Tested with the foam test.

3. Proximate analysis

3.1. Moisture content

According to **AOAC (2000)**, the algae powder's moisture content was assessed by drying it at 103 ± 2°C until a consistent weight was reached.

3.2. Ash content

Ash content was calculated following the guidelines of **AOAC (2000)** by incineration of the algae powder at 550 ± 25°C until the contents of the crucibles turned grayish white.

3.3. Crude protein content

Thus, the formula P% = 6.25 x N% represents the link between the total nitrogen concentration and the conversion coefficient of 6.25, which determines the protein content. The Kjeldhal approach was used to calculate the crude protein content (N x 6.25) and total nitrogen **(AOAC, 2000)**.

3.4. Total lipid content

The Folch approach was used to figure out the total lipid content **(Folch *et al.*, 1957)**: 10g of the previously dried algae sample was added in the presence of 60ml of Folch reagent, a solvent combination of methanol and chloroform (2/1; v/v), and the amount of fat was measured.

3.5. Carbohydrate content

The carbohydrate content in the sample was obtained by dividing the difference between the dry matter content and the total amount of fat, ash, and protein content already obtained **(Adrian *et al.*, 1995)**. PC% = 100 - (P% + F% + A%), where P%: Protein content , F% : Fat content , A% : Ash content

3.6. Dietary fiber dosage

The method used is the one proposed by WEENDE, quoted by **Adrian *et al.* (1995)**, for determining fiber content. It consists of treating the sample successively with boiling solutions of sulfuric acid and potassium hydroxide before incineration at 550°C in the muffle furnace.

3.7. Energetic value

The *Ulva lactuca*'s calories were calculated from its fat, carbohydrate, and protein content using the conversion factors of Atwater: For protein and carbohydrates, 4 kcal g^{-1}; for fat, 9 kcal g^{-1} **(FAO, 2003)**.

4. Determination of total phenolic content

The Folin-Ciocalteu method was used to determine the extracts' polyphenol concentrations according to **Ribéreau-Gayon and Peynaud (1968)** and modified by **Li *et al.* (2007)**. The absorbance was then read with a spectrophotometer (UV-VIS spectrophotometer) at 765nm. For the blanks, the extract was replaced with methanol, and a calibration curve was established using a gallic acid dilution series (0.01 to 0.2mg/ mL). The same protocol was applied to measure the samples. Results were expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Determination of total flavonoid content

The extracts' flavonoid content was calculated using the aluminum trichloride technique cited by **Meda *et al.* (2005)**. Absorbance was read at 430nm. The results were expressed in equivalent milligrams of quercetin per gram of dry matter (mg QE g^{-1} DM).

5. Determination of total condensed tannins

Condensed tannins were determined by the vanillin acid method **(Hagerman, 2002)**. The absorbance was read at 510nm. A calibration curve was based on a catechin dilution series (0.01 to 0.3mg mL^{-1}). The same protocol was used to measure the samples. Results were expressed in milligram equivalents of catechin per gram of dry matter (mg CE g^{-1} DM).

6. Determination of pigment content

Total carotenoids, chlorophyll *a*, and chlorophyll *b* were measured according to **Arnon (1949)**, with a few minor adjustments: 1g of algal powder was stirred for 1 hour in 10ml of acetone (100%), and the pigments were extracted. The homogenate was stored at - 4°C for 18 hours. After centrifuging the extract for ten minutes at 5000rpm, the pigment-containing supernatant was recovered and the pellet was discarded. The following formulas were used to compute the pigment concentrations based on the absorbance measured at 663, 645, and 480nm: (For the carotenoids concentration formula, the correction factor is 4).

$$Chlorophyll\ a = \frac{[12.7\ (A663) - 2.69\ (A645)] * vol.of\ extraction}{Weight\ of\ the\ sample}$$

$$Chlorophyll\ b = \frac{[22.9\ (A645) - 4.68\ (A663)] * vol.of\ extraction}{Weight\ of\ the\ sample}$$

$$Total\ chlorophyll = \frac{[20.2\ (A645) - 8.02\ (A663)] * vol.of\ extraction}{Weight\ of\ the\ sample}$$

$$Carotenoids = \frac{4 * (A480) * vol.of\ extraction}{Weight\ of\ the\ sample}$$

7. Estimation of lipids

GC-MS spectrophotometry (BRUKER 456-GC EVOQ) was utilized to ascertain the fatty acid composition of lipids after transmethylation. Fatty-acid methyl esters were produced via a modified version of **Milinsk *et al.* (2016)**'s transmethylation procedure. The lipid extract sample was put in screw-capped tubes and mixed with n-hexane until the lipid content was completely dissolved. Following the addition of KOH to methanol ((0.1 M) 2 $mol.L^{-1}$), the liquid underwent extensive agitation. After the layers were separated, the top layer was put into bottles. The GC-MS was operated under the following conditions: a temperature ramp of 10°C/min to 200°C for 2 minutes, followed by a hold for 2 minutes, and then a ramp at 5°C/min to 250°C for 9 minutes; the injection temperature was set at 250 °C with a split ratio of 20:1 and an injection volume of 1µL; helium was used as the gas carrier, with a solvent delay of 3 minutes; the column dimensions were 30 m × 250µm; the scan range was from 50 to 500 Da; and the transfer and source temperatures were set at 250 and 200°C, respectively.

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8. Estimation of minerals content

Following a 550°C calcination and ash solubilization in 70% nitric acid, the elements Ca, Mn, K, Mg, Na, Cu, Fe, and Zn were determined using the ICP-OES (Perkin Elmer Optima 8000) given the setting: 1500 watts of radio frequency power, 8L min^{-1} of plasma gas flow, 0.2L min^{-1} of auxiliary gas flow, axial view size, 45 minutes of playback and copying, and 15 minutes of copying **(Allen *et al.*, 1997)**.

9. Colorimetric determination of phosphorus (P)

This technique makes use of the vonadomolybdic reagent. When phosphorus is present, the latter creates a yellow-colored phosphovonadomolybdic complex **(Lurent, 1991)**. At 430nm, the optical densities were read. For the calibration curve, a stock solution of potassium dihydrogen phosphate (KH_2PO_4) was used to create a standard phosphorus range.

10. Techno-functional properties

The WHC and OHC were measured to test the physicochemical characteristics of *Ulva lactuca* powder.

10.1. Water holding capacity (WHC)

The technique outlined by **Beuchat *et al.* (1975)** was employed to determine the water absorption capacity: into graduated conical centrifuge tubes (25mL), 1g of sample was weighed, then distilled water (10mL) was added. The mixture was centrifuged at 200×g for 30 minutes after being allowed to stand at 30 ± 2°C for an hour. Upon the decantation of the supernatant, the sample was weighed immediately. The proportion of water absorption represented the weight change according to the initial weight of the sample.

10.2. Oil holding capacity (OHC)

Sosulski's approach was used to determine oil absorption capability as expressed by **Abbey and Ibeh (1988)**. 1g of powder was measured and the weight was recorded in a centrifuge tube. 10mL of corn oil (density= 0.925g cm^{-3}), was appropriately added to the tube and stirred. For fifteen minutes, the suspension was centrifuged at 350 × g. After dismissing the supernatant, the tube and its contents were weighed again. The weight change given as a percentage of oil-bound refers to the oil absorption capacity of the powder.

11. Antioxidant assays

11.1. DPPH radical scavenging capacity

The method suggested by **Aouji *et al.* (2023)** was adjusted to assess the seaweed extracts' capacity to scavenge free radicals: 2.5mL of a methanol solution containing 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) was combined with a 0.5mL every extract (at doses of 0.015 to 1 mg mL^{-1}); the combination was then incubated for 30 minutes at room temperature in the dark. At 517nm, absorbance was then measured. The DPPH solution was used as a control, with ascorbic acid serving as a standard to determine the extent to which the samples and the reference standard scavenged radicals. The fraction of DPPH scavenging was calculated using the equation below:

$$\%\text{DPPH scavenging} = [(1 - \text{ABS}_{\text{sample}})/\text{ABS}_{\text{Control}}] \times 100$$

The absorbance of the test sample (the sample combined with the DPPH solution) is denoted as ABS sample, while ABS control refers to the absorbance of the control (DPPH solution without the sample). The inhibitory concentration at which 50% of the DPPH radicals were scavenged is measured as the IC50 value (µg/mL).

11.2. Total antioxidant capacity (TAC)

For this experiment, 2500µL of reagent (4 mM ammonium molybdenum, 28 mM sodium phosphate, and 0.6 M sulfuric acid) was combined with 50µL of each *Ulva* extract. After 90 minutes of incubation at 95°C, the reaction mixture was allowed to cool at room temperature, and the absorbance at 695nm was determined in comparison to a blank. The extract's overall antioxidant potential was measured in milligrams of ascorbic acid equivalent per gram of dry matter (mg EAAS.g^{-1} DM). L-ascorbic acid served as the standard **(El-Moussaoui *et al.*, 2019)**.

11.3. Estimation of total reducing capacity (TRC)

The extracts' reducing power was ascertained using the Oyaizu method **(Oyaizu, 1986)**. Potassium ferricyanide (1%, w/v) was incorporated into 2.5mL of phosphate buffer (0.2 M, pH 6.6) after the extracted sample (1mL) had been combined with it. For twenty minutes, the mixture was incubated at 50°C. Then, 2.5mL of 10% w/v trichloroacetic acid was incorporated, and the mixture was centrifuged at 3000rpm for 10 minutes. 2.5mL of the supernatant was combined with 0.5mL of ferric chloride (0.1%, w/v) and 2.5mL of distilled water. After 10 minutes of incubation, the absorbance was determined at 700nm. A higher reducing power was demonstrated by the reaction mixture's increased absorbance.

12. Statistical analysis

The average and standard deviation (SD) of the analysis' findings were displayed. Every experiment that was conducted yielded triplicate results. One-way analysis of variance (ANOVA) was carried out to analyze the obtained data, and then Duncan's multiple distribution test was performed.

RESULTS

1. Phytochemical screening

Different kinds of bioactive chemicals detected in *Ulva lactuca* extracts were identified based on physicochemical methods used in phytochemical screening. Table (1) highlights the particular physicochemical tests that were used to identify the significant secondary metabolites.

Table 1. Qualitative organic tests of *Ulva lactuca*

COMPOUND	TEST	METHANOL	CHLOROFORM	HEXANE
CARBOHYDRATES	FEHLING	++	++	+
TRITERPENES	SALKOWSKI	+++	+	-
PHYTOSTEROLS		+++	+	-
PHENOLS	IRON CHLORIDE	+++	++	-
CATECHIC TANNINS	STIASNY	+++	+++	-
GALLIC TANNINS		++	++	-
PROTEINS	BIURET	++	++	-
GLYCOSIDES	BORNTRAGER MODIFIED	+++	+	+
SAPONINS	FROTH	+	+	+
ANTHRAQUINONES	BORNTRAGER	+++	+	+
ANTHOCYANINS	NaOH	++	-	+
FLAVONOIDS	ALKALINE REAGENT	++	+	+
ALKALOIDS	DRAGENDORFF	+	-	-
	WAGNER	+++	++	-
	MAYER	++	+	-

(+): Positive test **(-): Negative test**

The methanol extract included the recognized 13 types of chemicals. The chloroform extract was found to include the following types of components in the highest number: anthraquinones, flavonoids, carbohydrates, glycosides, triterpenes, phytosterols, phenolic compounds, and saponins and catechin tannins. The presence of flavonoids, anthraquinones, anthocyanins, carbohydrates, and glycosides was detected by hexane extract.

2. Proximate analysis

By emphasizing moisture and ash, the algal biochemical composition was ascertained, revealing the amount of protein, fat, carbohydrate, dietary fiber, and energetic value. *Ulva lactuca* had a reasonably typical moisture content of 23 ± 1.3%, and the calculated crude ash percentage for 5g was 15.43 ± 0.191%.

Proteins, lipids, and carbs are among the three major biochemical components of seaweed. *Ulva lactuca* had a protein level of 22.15 ± 1.7% algal DM in the current investigation (Fig. 1). Since they serve as the primary source of energy for the metabolic pathways of algae, carbohydrates are regarded as the most significant biochemical component of algae. In our investigation, proteins were generally less common than carbohydrates having an amount of 61.12 ± 1.42%.

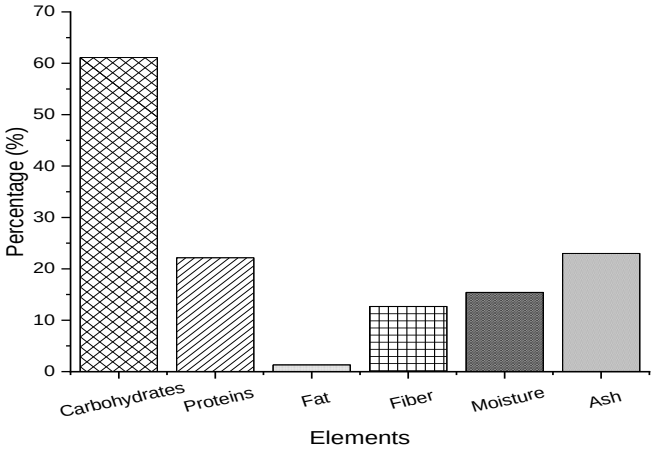

Fig. 1. Proximate analysis of *Ulva lactuca*

On average, total lipids were found to be low (1.3 ± 0.1%) of the dry matter of the algae (Fig. 1).

Table 2. Fatty acids profile of *Ulva lactuca*

Fatty acids	**% of total fatty acids**
Myristic acid C14:0	8.342 ± 0.35
Pentadecanoic acid C15:0	11.564 ± 0.02
Palmitic acid C16:0	34.876 ± 0.31
Stearic acid C18:0	14.267 ± 0.12
Docosanic acid C23:0	1.312 ± 0.01
Saturated fatty acids (SFA)	**70.361**
Tetradecanoic acid C14:1 (ω5)	1.235 ± 0.01
9 Hexadecenoic acid C16:1 (ω7)	3.233 ± 0.02
Heptadecenoic Acid C17:1 (ω7)	0.344 ± 0.45
Oleic acid C18:1 (ω9)	9.230 ± 0.28
Erucic Acid C20:1 (ω9)	0.045 ± 0.08
Monounsaturated fatty acids (MUFA)	**14.087**
Linoleic acid C18:2 (ω6)	1.175 ± 0.09
α Linolenic acid C18:3 (ω3)	0.487 ±0.12
Arachidonic acid C20:4 (ω6)	1.633 ± 0.18
Eicosapentaenoic acid (EPA) C20:5 (ω3)	1.243 ± 0.09
Docosadienoic Acid C22:2 (ω6)	0.753 ± 0.05
Docosahexaenoic Acid (DHA) C22:6 (ω3)	0.238 ± 0.02
Polyunsaturated fatty acids PUFA ω6	**3.561**
Polyunsaturated fatty acids PUFA ω3	**1.968**
Ratio ω6/ ω3	**1.8**

Values are means of three replicates ± standard deviations SD.

Based on the content of fatty acids, 16 compounds were found in different amounts (Table 2). The predominant ratio of saturated fatty acids (SAFA) was seen in *Ulva lactuca*. The predominant fatty acid (C 16:0) was found to be 34.87 ± 0.31% of the total fatty acid composition. Whereas myristic acid (C 14:0) measured 8.34 ± 0.35%, pentadecanoic acid (C 15:0) recorded 11.56 ± 0.02%. Docosanic acid (C 23:0) was 1.31 ± 0.01% and stearic acid (C 18:0) was significant at 14.26 ± 0.12%. In this investigation, polyunsaturated essential fatty acids (PUFA) were also found. *Ulva lactuca* has a linoleic acid ω6 (C 18:2) content of 1.17 ± 0.09%. Decosahexadecanoeic acid (DHA) ω 3 (C22:6) (0.23 ± 0.02) and eicosapentaenoic acid (EPA) ω 3 (C 20:5) (1.24 ± 0.09%) were the representative fatty acids of the ω3 group. There were six identified monounsaturated fatty acids (MUFA).

3. Mineral composition

Upon examining the mineral content, it was revealed that *Ulva lactuca* is composed of a diverse array of minerals, as shown in Fig. (2).

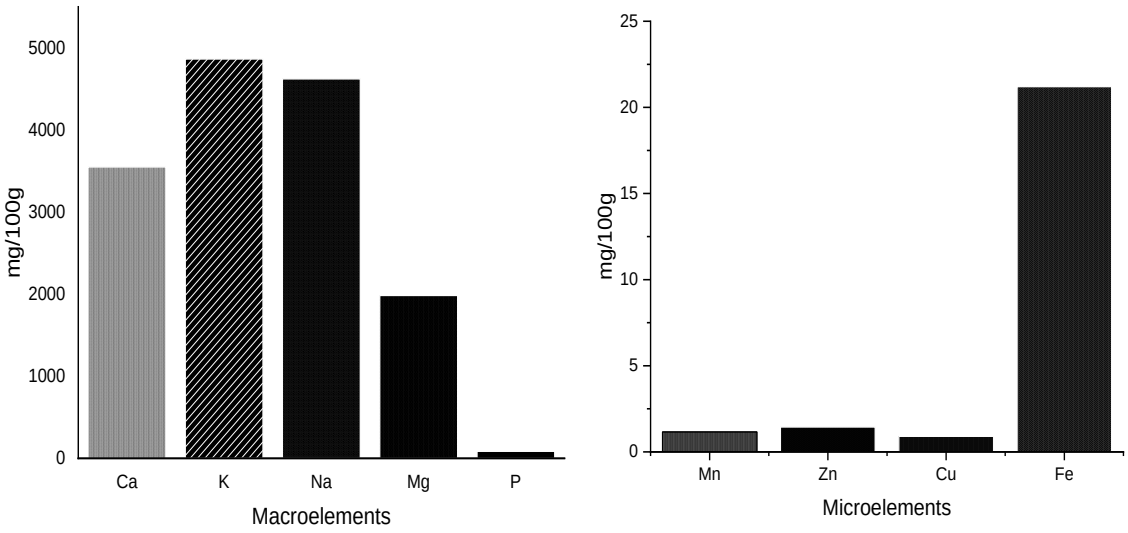

Fig. 2. The mineral composition of *Ulva lactuca*

The following is a list of the minerals' contents: K > Na > Ca > Mg >Fe > Zn >Mn

4. Total content of phenols (TPC), flavonoids (TFC) and tannins (TTC)

One of the most significant groups of natural antioxidants is thought to consist of phenolic chemicals. The results, which were obtained using particular methods, are displayed in Table (3).

Table 3. Effect of different solvents on total polyphenols, flavonoids, and tannins content

Biochemical content	**Methanol extract**	**Chloroform extract**	**Hexane extract**
Total phenol content (mg GE g^{-1} DM)	45.69 ± 3.24 [a]	16.61 ± 0.064 [b]	4.383 ± 0.064[c]
Flavonoids content (mg QE g^{-1} DM)	15.49 ± 0.064 [a']	1.29 ± 1.029 [b']	1.19 ± 0.582 [b']
Tannins content (mg CE g^{-1} DM)	22.52 ± 8.23 [a'']	11.46 ± 4.118 [b'']	0.36 ± 4.267 [c'']

Lowercase letters a, b, and c were used to mark the significance of the difference ($p < 0.05$). The same letters between solvents mean insignificant differences. Different letters between solvents mean significant differences.

The analysis of the extracts for phenolic compounds showed that the methanol extract from *Ulva lactuca* is rich in these compounds (45.69 ± 3.24 mg GAE g^{-1} DM). Moreover, the quantitative determination of total flavonoids reveals that the methanol extract was the richest in flavonoids, with contents that can reach 15.49 ± 0.064 mg QE g^{-1} DM. In the same context, the analysis of algal extracts in total tannins reveals that the methanol extract has the highest tannin contents, with levels that can reach 22.52 ± 8.23 mg CE g^{-1} DM .

The total amount of flavonoids, phenols, and tannins of *Ulva lactuca* were substantially different at $P \leq 0.05$. Methanol extract had the highest value (45.69 ± 3.24), followed by hexane extract (4.38 ± 0.064) and chloroform extract (16.61 ± 0.064mg GAE g^{-1} DM). The three extracts' flavonoid contents were discovered to adhere to the same pattern, with values of 15.49 ± 0.064, 1.19 ± 0.58 and 1.29 ± 1.02mg QE g^{-1} DM, and tannins content of 22.52 ± 8.23, 0.36 ± 4.26 and 11.46 ± 4.11mg CE g^{-1} DM, respectively.

5. Pigments composition

The pigment composition of *Ulva lactuca* is shown in Fig. (3):

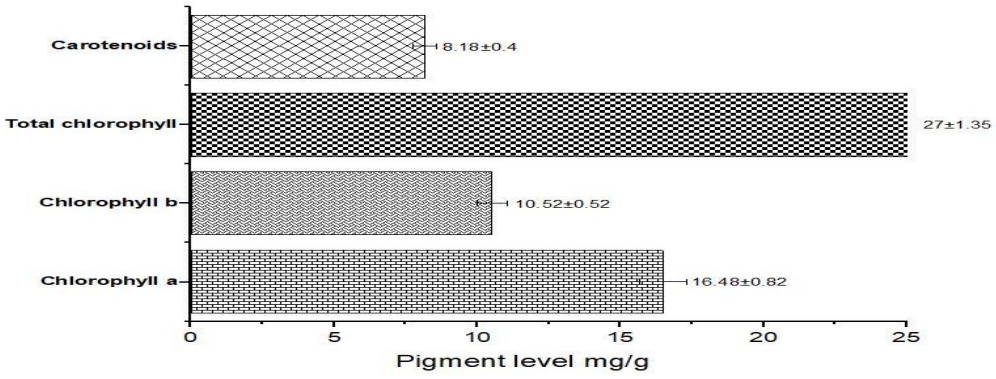

Fig. 3. Pigment composition in *Ulva Lactuca*

Results show that *Ulva lactuca* contained high levels of chlorophylls. This green algae produces photosynthetic pigments; consequently, the growth medium's nutrient composition has a big impact on that process.

6. Techno-functional properties

Water holding capacity (WHC) measurements was used to identify hydration characteristics. The variations in *Ulva lactuca* powder's water-holding capacity (WHC) were between 25 and 37°C, as shown in Fig. (4).

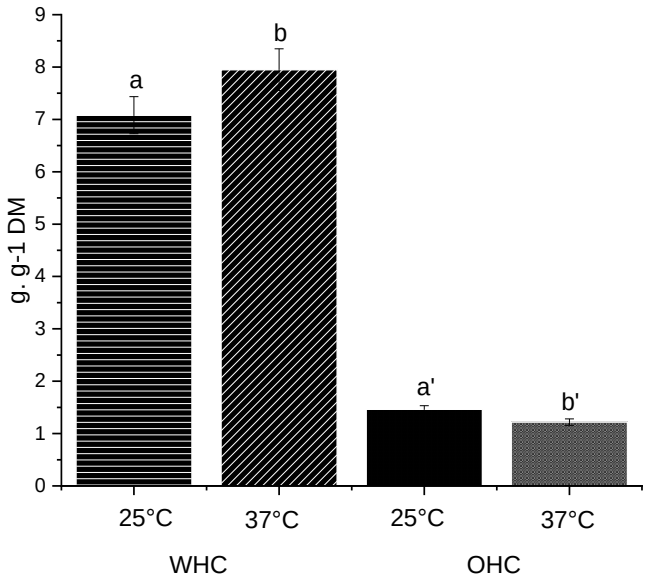

Fig. 4. Water holding capacity (WHC) and oil holding capacity (OHC) of *Ulva lactuca* The letters show the significant difference (*P<0.05*) in this case

Temperature-dependent changes in the water-holding capacity of *Ulva lactuca* powder resulted in values of about 7.08 ± 0.12 and 7.95 ± 0.3g of water.g^{-1} DM at 25 and 37 °C, respectively.

Fig. (3) depicts an increase in the *Ulva lactuca* algal powder's oil-holding capacity (OHC) at two different temperatures (25 and 37°C). We discovered that when the temperature increased, the green seaweed powder's ability to contain oil reduced slightly (1.46 and 1.22 g oil.g^{-1} DM, respectively, at 25 and 37 °C).

7. Antioxidant properties

7.1. Total antioxidant capacity

Both in the standards and the extracts, the activity depended on concentration. The three solvents that were utilized showed differences in their antioxidant capability. The findings from the assay measuring total antioxidant capacity for methanol, chloroform, and hexane extract revealed that the total antioxidant activity was 7.68 ± 0.1mg AAS g^{-1} extract, 3.98 ± 0.2mg AAS g^{-1} extract, and 1.05 ± 0.4mg AAS g^{-1} extract, in that order.

7.2. DPPH assay

Antioxidants' ability to scavenge radicals is the basis for this kind of analysis assessment **(Zaatout *et al.*, 2019)**. *Ulva lactuca* extracts in methanol, chloroform, and hexane at varying concentrations were measured for the percentage of DPPH inhibition. The results for methanol extract (81.6 ± 0.45%) were comparable to those for ascorbic acid standard (98 ± 0.04%), and they were followed by chloroform extract (60.56 ± 1.3%) and hexane (52.76 ± 0.6%).

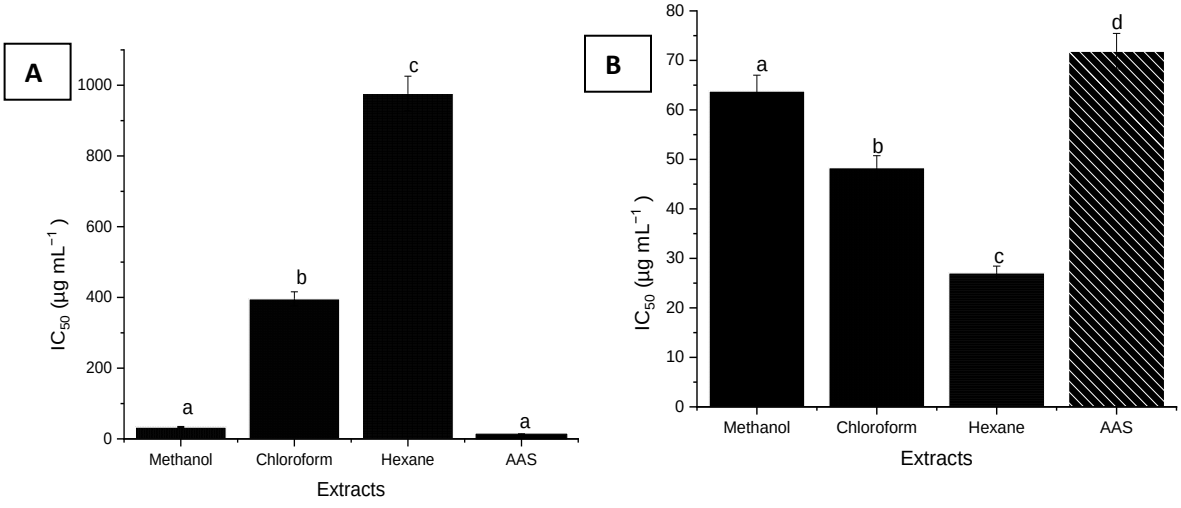

Fig. 5. IC_{50} of (**A**) DPPH and (**B**) FRAP assays of *Ulva lactuca* extracts and ascorbic acid. The letters show the significant difference (P<0.05) in this case

To measure the DPPH radicals' ability to scavenge, the IC_{50} value was found. The extract has the highest capacity to absorb DPPH radicals when its IC_{50} value is at it its lowest. The methanol extract's IC_{50} value of 33.6 ± 1.31µg mL^{-1} was found to be notably strong and comparable to that of ascorbic acid, the positive control, which had an IC_{50} value of 13.49 ± 0.353µg mL^{-1} (Fig. 5).

7.3. Reducing power assay

At 700nm, *Ulva lactuca* methanol extract's reducing potential showed noticeably greater absorption than that of the other solvents. With an IC_{50} value of 63.75 ± 0.05µg mL^{-1}, the methanol extract in this study showed good ferrous-reducing activity; however, the reducing power increases as the *Ulva lactuca* extract concentration increases (Fig. 5). To compare the reduction activity, ascorbic acid was utilized as a reference antioxidant (71.86 ± 1.48µg mL^{-1}).

8. Correlation

At the 0.01 level, there was a significant correlation found between the phenolic, flavonoid, and tannin concentrations in the methanol extract and the DPPH antioxidant activity (Fig. 6). The TAC assay demonstrated an identical pattern of antioxidant action with less dependence on phenolic and flavonoid contents at the 0.05 level. At the 0.01 level, there was a strong positive correlation between the flavonoid levels and the antioxidant activity of FRAP.

For the chloroform extract, the phenolic and flavonoid contents exhibited the same pattern as methanol extract to the DPPH, TAC, and FRAP assays, except for tannins contents that do not show any correlation to the DPPH and TAC assays, moreover, tannins do not show any correlation to the polyphenols and flavonoids contents. On the other hand, the flavonoid content was significantly correlated with polyphenols contents at 0.01 level, for the methanol and chloroform extracts.

Methola	Polyphenol	Flavonoids	Tannins	TAC	DPPH	FRAP
Polyphenol	1					
Flavonoids	0.878**	1				
Tannins	0.788**	0.797**	1			
TAC	0.786*	0.795*	0.797*	1		
DPPH	0.992**	0.896**	0.994**	0.796*	1	
FRAP	0.799*	0.986**	0.987*	0.789*	0.891*	1

CHLORORM	Polyphenols	Flavonoids	Tannins	TAC	DPPH	FRAP
Polyphenols	1					
Flavonoids	0.864**	1				
Tannins	0.464	0.464	1			
TAC	0.745*	0.776*	0.464	1		
DPPH	0.956**	0.876**	0.464	0.793*	1	
FRAP	0.763*	0.963**	0.686*	0.763*	0.963**	1

HEXANE	Polyphenols	Flavonoids	Tannins	TAC	DPPH	FRAP
Polyphenols	1					
FlavonoidS	0.168	1				
Tannin	0.253	0.996**	1			
TAC	0.786*	-0.168	-0.253	1		
DPPH	0.613*	0.158	0.243	-0.967**	1	
FRAP	0.854**	0.168	0.253	-0.954**	0.889**	1

Fig. 6. Correlation matrix of total phenolic content, total flavonoid content, tannin content, and the antioxidant activity measured by the three assays

*Correlation is significant at the 0.05 level (2 -tailed); **Correlation is significant at the 0.01 level (2 -tailed).

Oppositely, for hexane extract, flavonoids and tannins content were insignificantly correlated with the DPPH and FRAP activities, while TAC was negatively correlated to DPPH and FRAP assays, and polyphenols were highly significantly correlated with DPPH assay.

DISCUSSION

The natural products made from marine sources, green algae have great nutritional potential since they contain bioactive elements such as carbs, lipids, proteins, and dietary fibers. After analyzing the data, we discovered a strong correlation with the literature, which led us to speculate about the possible nutritional value and health benefits of these algae for people.

Ulva lactuca has a high carbohydrate content of 61.12 ± 1.42%. These findings are consistent with the amounts of carbohydrates in the green seaweed found in other marine environments, as stated by **Hentati *et al.* (2020)** in their studies on green seaweed from the Mediterranean Sea off the coast of Tunisia; these outcomes are comparable to those of **Ortiz *et al.* (2006)** and **Yaich *et al.* (2011)**.

Ulva lactuca has a high protein concentration determined by its nitrogen content. These outcomes surpass those of **Rasyid (2017)** (13.6%), who conducted research on *Ulva* sp. from the Pameungpeuk seas in Indonesia.

Moreover, *Ulva lactuca* has a low percentage of lipids; this number is similar to the 1.18 percent obtained by **Oucif (2019)** for the same species. Since the total lipid content of seaweeds was shown to be less than 4% in several studies, they were typically not considered a valuable source of lipids. However, in contrast, the amount of polyunsaturated fatty acids is similar to that seen in plants. The current findings are corroborated with the outcomes of previous studies **(Ortiz *et al.*, 2006; Francavilla *et al.*, 2013; Saroja, 2016)**.

With a percentage of 34.87%, palmitic acid (C 16:0) dominated the saturated fatty acid (SFA) composition of the *Ulva lactuca* sample under investigation. Other authors have reported that in the case of *Ulva lactuca*, palmitic acid predominates **(Ortiz *et al.*, 2006; Yaich *et al.*, 2011)**. Stearic acid (C 18:0) (14.26%) and myristic acid (C 14:0) (8.34%) were found in very small amounts in *Ulva lactuca*. The most prevalent monounsaturated fatty acid (MUFA) at 9.23% was oleic acid (C 18:1). The amount of linoleic acid (1.72 ± 0.91%) in *Ulva lactuca* that **Ortiz *et al.* (2006)** reported was less than what we found for the identical species. SFA and unsaturated fatty acids (USFA) were found in the same algae, according to **Metwaly *et al.* (2023)**; however, their levels are comparable and slightly lower than those found in the current investigation.

In addition, polyunsaturated fatty acids were 5.52%, which consisted of important fatty acids in human nutrition, especially docosahexaenoic acid (DHA; C 22:6) and eicosatetraenoic acid (EPA; C 20:5). The known health advantages of ω 3 PUFA, specifically EPA and DHA, have been extensively studied **(Lauritzen *et al.*, 2001)**. The ω-3 and ω 6 PUFA were higher in *Ulva lactuca*, and the ω-3/ ω-6 ratio was consistent with the ratio (> 0.1) advised by **WHO (2005)**.

The ω3/ω6 ratio has been presented as a practical tool to evaluate the relative nutritional advantages of dietary oils. Increased ω 3/ω 6 ratios have been associated with better nutritional value in numerous studies **(Sirbu & Negreanu-Pirjol, 2019)**. WHO recommendations **(WHO, 2005)** stated that a healthy human diet should have a daily ω 3/ω 6 ratios of less than 1:5. While *Ulva lactuca*'s ω 3/ω 6 ratio falls within the recommended range (0.32), it is nonetheless high. The maximum value reported for the ω 6/ω 3 ratio was 4.0 **(Negreanu-Pirjol *et al.*, 2020)**; however, the ratio in *Ulva lactuca* was lower, at 1.8. According to this perspective, PUFAs have a critical role in the management of cardiovascular disease, inflammatory conditions, autoimmune diseases, and type 2 diabetes **(Bae *et al.*, 2023)**. These findings indicate that polyunsaturated fatty acids (PUFAs) are necessary for maintaining human health and ought to be found in food.

Ulva lactuca has an average proportion of total dietary fiber; these findings are consistent with other studies on the amount of dietary fiber in total, resembling those made by **Yaich *et al.* (2015)**, who addressed seaweed from the coast of Tunisia in the Mediterranean Sea.

Seaweeds are well known for being high in minerals. In general, mineral components are necessary for both general physical and mental health since they are elements of blood, muscle, bone, teeth, soft tissues, hemoglobin, and nerve cells **(Smith *et al.*, 2010)**. Reduced blood pressure and a lower risk of stroke are associated with potassium, calcium, and magnesium via boosting potassium absorption and decreasing sodium absorption in the gastrointestinal system **(Smith *et al.*, 2010)**. The dominant element determined in *Ulva lactuca* was Ca (4830 mg $100g^{-1}$). According to **Debbarma *et al.* (2016)**, *Ulva lactuca* had macronutrient contents that are lower than those found in our study, and the Na / K ratio of the *Ulva lactuca* had a 0.91 value, falling within the range of earlier studies **(Ruperez, 2002; El-Said & El-Sikaily, 2013)**. High Na food consumption can result in hypertension; thus, Na, K, and Cl are essential for maintaining the proper balance of bodily fluids **(Insel *et al.*, 2007)**. According to these findings, eating seaweed can help balance diets' Na / K ratios. In comparison to foods eaten on land, seaweeds are known to collect calcium in significantly higher amounts, which is a crucial ingredient for human health **(El-Said & El-Sikaily, 2013)**. Magnesium is also essential for the construction of chlorophyll and other metabolic activities in algae. In *Ulva lactuca*, magnesium was abundant (1983.4mg $100g^{-1}$). The most common components for nutrient absorption binding sites in seaweeds were Ca and Mg. These results imply that incorporating seaweed into diets can lessen specific ranges within the human body and hence lower the risk of disorders like heart disease, preeclampsia, and hypertension. The iron levels in seaweeds are higher than in many commonly consumed sources (such as meats and spinach) because of their metabolic ability to absorb elements directly from saltwater **(Smith *et al.*, 2010)**.

It was discovered that the mineral contents vary according to the kind of algae species, waving contact, seasonal fluctuations, physiological and environmental parameters,

mineralization routine, and processing method **(Mabeau & Fleurence, 1993)**. Ferrous is an essential component of hemoglobin and affects numerous other plant and animal metabolic processes **(Smith *et al.*, 2010)**. The study's findings demonstrated that the examined species had significant Fe concentrations (21.2mg $100g^{-1}$). These results are lower than the dietary recommended intake (DRI) levels for iron reported by **Gebhart and Thomas (2002)**, indicating that seaweeds may be used as a source of iron nutrition without running the risk of overdosing and toxicity. Comparable results were noted for copper, which had a 0.9mg $100g^{-1}$ concentration; this value was found to be within the ranges reported in earlier studies for the consumption of seaweed-based foods that contain copper **(Mabeau & Fleurence, 1993; Ruperez, 2002)**. However, manganese was linked to bone formation and the metabolism of carbohydrates, lipids, and amino acids, whereas zinc was linked to protein stability, enzyme function, and the control of gene expression **(Smith *et al.*, 2010)**.

The WHC properties of algae are generally related to the content and properties of their polysaccharides and proteins associated with polysaccharide cell walls **(Se-Know & Li, 2011)**. Previous work has described how temperature fluctuations can drastically alter the physicochemical properties of algae due to increased fiber solubility and the presence of proteins **(Yaich *et al.*, 2011)**. Algal WHC can be attributed to different protein content and an increased number and type of water-binding sites on protein molecules **(Yaich *et al.*, 2013)**. Additionally, **Wong and Cheung (2000)** found *Ulva lactuca* to have higher WHC values at 37°C (9.71g/ g DM), while being comparable to those previously reported for some agricultural by-products (fiber concentrates) (6.30–13.2 g/g DM) **(Grigelmo-Miguel & Martin-Belloso, 1999)**. Furthermore, the WHC of *Ulva lactuca* algae samples was comparable to some commercial high-fiber dietary supplements (6.60–9.00g/ g DM) **(GonAi & Martin-CarroAn, 1998)**. **Wong and Cheung (2000)** reported the oil holding capacity of *Ulva lactuca* to be 0.65g/ g DM lower than the value found in this study, moreover, this value was higher than that reported for orange pomaces dried **(Afrin *et al.*, 2022)**. A significant difference ($P<0.05$) in OHC was observed between the different temperatures investigated (25 and 37°C). Fundamentally, the OHC mechanism is primarily based on the physical retention of oil by capillary forces. In addition, protein hydrophobicity plays an important role in fat absorption.

Therefore, in algae samples, the variation in OHC may be partly due to the different ratio of polar side chains of amino acids on the surface of their protein molecules. Furthermore, **Fleury and Lahaye (1991)** reported that algal OHC was also related to particle size, total charge density, and hydrophilicity of individual particles. Furthermore, the OHC of green seaweed powder may also depend on the total protein content and total fiber content **(Wong & Cheung, 2000)**.

Determination of polyphenols, flavonoids, and tannins showed significant differences between extracts. This suggests that extraction solvents have a significant impact on the concentrations of secondary metabolites in algal extracts. The solvents used to extract plant compounds vary in polarity, which influences their ability to dissolve different types of compounds. Polyphenols, flavonoids, and tannins have diverse chemical structures, and their solubility can vary according to the polarity of the solvent. Some polar solvents can extract more hydrophilic compounds such as flavonoids, while other apolar solvents can extract more hydrophobic compounds such as tannins.

Regarding the amount of pigment findings, three varieties of chlorophylls a, b, and carotenoids in *Ulva lactuca* were recognized and expressed as milligrams per gram. *Ulva lactuca* has the highest concentration of chlorophyll *a* (16.48 ± 0.82mg g^{-1} DM). The findings are consistent with information published by **Negreanu-Pirjol *et al.* (2020)**, who investigated green seaweeds from the Black Sea coast of Romania and the Mediterranean, respectively. As the main pigments in photosynthesis, chlorophylls are essential for the absorption of light energy. The pigments chlorophyll a and chlorophyll b, which are found in the species *Ulva lactuca*, are essential to photosynthesis and help convert solar radiation into chemical energy **(Mhatre *et al.*, 2018)**. Chlorophylls play a role in photosynthesis and also have antioxidant qualities **(Lanfer-Marquez *et al.*, 2005).**

A total of 8.18 ± 0.4 mg g^{-1} DM of carotenoid content was discovered. The data from this study are in line with those of **Christaki *et al.* (2013)**, who elucidated that the total carotenoid content for Aegean Sea algae was 12.73 ± 1.32mg g^{-1}. Algal carotenoids also possess antioxidant properties that protect against oxidative stress-related human illnesses and the growth of cancer cells **(Collins *et al.*, 2016)**. The findings are consistent with those on total carotenes and chlorophyll a for *Ulva lactuca*, a green algae found in Mediterranean Sea waters **(Ozgun & Turan, 2015)**. It is commonly known that carotenoids have antioxidant qualities. Antioxidants are essential for preventing the damaging effects of free radicals, which in turn helps reduce oxidative stress and protect the integrity of cells and tissues **(Putra *et al.*, 2023)**.

Compared to some synthetic antioxidants, natural antioxidants are readily available and have fewer or no negative effects. One quick, easy, and affordable way to assess the antioxidant potential of algae as food is to employ the free radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH was frequently used to measure antioxidants in intricate biological systems and assess a compound's capacity to donate hydrogen or scavenge free radicals. The antioxidant activity of various seaweed components was evaluated and compared using the other two methods, which included reducing capacity (electron donors) and total antioxidant capacity.

The three *Ulva lactuca* extracts demonstrated antioxidant activity at 1000µl of each extract in each solvent; nonetheless, the methanol extract exhibited the highest level of

antioxidant activity with IC_{50} = 33.6 ± 1.31µg mL^{-1}. The increased concentration of primary and secondary metabolites in *Ulva lactuca* undoubtedly contributes to its enhanced antioxidant activity as established in **Meenakshi *et al.* (2012)**, postulating that *Ulva lactuca*'s antioxidant action is triggered by the presence of phenolic compounds, flavonoids, polysaccharides, chlorophylls, carotenoids, alkaloids, terpenes, and phytosterols.

To determine the IC_{50} value of the extract or standard (ascorbic acid), the percentage inhibition was initially computed. The percentage of inhibition indicates a substance's capacity to scavenge free radicals. In the current study, it is evident that upon increasing the ascorbic acid or the three *Ulva lactuca* extract concentrations, the capacity to scavenge free radicals increases. This is consistent with the pharmacodynamic principle, which states that a drug's response is directly correlated with its dose and concentration **(Gunawan *et al.*, 2007)**. *Ulva lactuca*'s methanolic extract's IC_{50} value was 33.61± 1.31µg mL^{-1}, whereas ascorbic acid served as the standard at 13.49 ± 0.353µg mL^{-1}. An antioxidant's activity is high if its IC_{50} value is less than 200µg mL^{-1}, low if it's between 200 and 1000µg mL^{-1}, and extremely low if it's greater than 1000µg mL^{-1} **(Molyneux, 2004)**. Considering these outcomes, the methanolic extract of *Ulva lactuca* has very strong antioxidant activity.

A reducing power of between 15 and 500mg mL^{-1} has been determined. L-ascorbic acid solutions within the identical range of concentration served as the control solutions. At 700nm in wavelength, absorbances were measured. All of the extracts employed in this investigation showed impressive antioxidant activity, as seen by the FRAP assay results, which showed no negative values. The findings regarding seaweeds' antioxidant activity are consistent with those of further researchers, including those published by **Farasat *et al.* (2014)** for seaweeds gathered from the shores of the northern Persian Gulf coasts. According to **Karawita *et al.* (2005)**, algae taken from South Korea's marine waters consistently reduce power.

The total phenol, flavonoid, and tannin concentration determines the antioxidant capacity. Three distinct methods were used to measure the antioxidant activity: TAC, DPPH, and reducing power. The phenol, flavonoid, and tannin levels, and consequently the antioxidant potential of seaweed extracts, were significantly influenced by the type of extractant used. Numerous significant correlations were noted among the biochemical and antioxidant parameters acquired in our investigation. The amounts of polyphenols, flavonoids, DPPH, and FRAP were shown to be strongly correlated, suggesting that these measures are useful for assessing the antioxidant properties of various extracts. There was a significant correlation ($P< 0.01$) between the phenolic, flavonoid, and tannin contents of the methanol extract and the DPPH antioxidant activity. However, a significant correlation was observed between the phenolic content of the hexane extract and the DPPH antioxidant activity, as well as between the phenolic and flavonoid contents of the chloroform extract.

On the other hand, there was no significant correlation found between the DPPH activity of hexane extract and the concentrations of flavonoids and tannins, and between the DPPH activity of chloroform extract and tannin content (Fig. 6). The TAC assay demonstrated the same pattern of antioxidant action, albeit with less reliance on the phenolic, flavonoid, and tannin components at the 0.05 level for the methanol extract. While, the FRAP antioxidant activity demonstrated a strong positive correlation with the flavonoid content at the 0.01 level for the methanol extract. FRAP was significantly correlated with the DPPH antioxidant assay at the 0.01 level, although the data showed favorably significant correlations between the FRAP-TAC and DPPH-TAC antioxidant assays at the 0.05 level. **Chakraborty *et al.* (2013)** found that various edible brown, green, and red seaweeds with high phenolic content exhibited a similar dependence on antioxidant activity. The total quantity of flavonoids and phenols in *Ulva lactuca* was consistent with its antioxidant activity. Many studies have indicated that among the most potent antioxidants in marine algae are phenolic compounds, and deduced that there is a strong correlation between high antioxidant activity and total phenolic content **(Zakaria *et al.*, 2011)**. In this context, **Bouba *et al.* (2010)** detected a favorable correlation between total phenolics and flavonoids in extracts of twenty Cameroonian spices.

CONCLUSION

According to its physicochemical characteristics and proximate composition, *Ulva lactuca* has a significant amount of ash, protein, carbohydrates, fatty acids, and minerals. The seaweed that was studied is said to have a lot of potential as food supplements and might be a source of components with a significant level of nutritional value for the food sector. *Ulva lactuca* products can have their commercial value increased by marketing them as value-added because it has been discovered to be a good source of critical nutrients. It does, however, have significant variations based on its makeup that make it more suitable in some situations. Because of its high chlorophyll and zinc content, it may therefore be a beneficial food for those on low-protein diets, those who need to lower their oxidative status, and those who need to increase their intake of these nutrients. On the other hand, *Ulva lactuca* displayed increased levels of carotenoids, PUFAs, and minerals including Mg and Fe. For every extract, various scavenging activity levels were targeted in tests such as DPPH, TAC, and FRAP assays. It was also noted that the *Ulva* extracts' potential antioxidant potential which has always depended on concentrationwas caused by their electron-donating and/or free radical-scavenging qualities. *Ulva lactuca*'s phenol and flavonoid levels have been noted as a possible source of natural antioxidants that are effective in preventing disorders linked to oxidative stress.

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