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Research Article

Phytochemical screening of *Ulva rigida* and evaluation of its antioxidant activity

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Abstract: *Ulva rigida* is a green macroalga widely distributed in marine ecosystems, rich in essential nutrients such as fibers, minerals, proteins, vitamins, polysaccharides and secondary metabolites such as polyphenols and flavonoids. This marine macroalga has promising biological properties. The aim of this study is to determine the main bioactive compounds of this macroalga and to evaluate its antioxidant power for use in the pharmaceutical, food and cosmetic sectors.

The extract was analyzed by Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC), which were used to identify the bioactive compounds. The compounds of *Ulva rigida* contribute significantly to the antioxidant properties of the seaweed. The antioxidant activity of the extract was evaluated *in vitro* using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) reduction assays with ascorbic acid as a positive control. Extract analysis revealed the presence of gallic acid, rutin and luteolin.

The DDPH and ABTS assays showed significant antioxidant activity with IC50s of 0.242 mg/mL and 0.016 mg/mL, respectively. *Ulva rigida* is a versatile and promising marine resource due to its composition. Its exploitation could meet the growing demand for sustainable natural products in various industrial sectors. However, further research into its mechanisms of action, including *in vivo* studies, is needed to maximize its exploitation.

Keywords: *Ulva rigida*, antioxidant, polyphenols, flavonoids, TLC, HPLC.

1. Introduction

Ulva spp., commonly known as sea lettuce in the food industry, are the main green macroalgae consumed worldwide. *Ulva* belong to the *Ulvophyceae* class and are renowned for their cosmopolitan presence, mainly due to their ability to withstand specific environmental conditions. Consequently, their wide distribution across many climatic and ecological conditions, as well as their opportunistic growth, makes them well-adapted and profitable. (Msuya et al., 2008 ; Marinho et al., 2013 ; Shpigel et al., 2017 ; Queirós et al., 2021).

Ulva rigida is a very common green marine alga that is distributed worldwide and used as a popular food ingredient in Asian countries as well as in North and South America. The high protein, lipid, mineral and vitamin 9content of marine *Ulva rigida* has encouraged its extensive use as a dietary supplement for humans and animals and as an organic fertilizer (Mezghani et al., 2016).

This widespread green macroalga is an important producer in marine ecosystems and thrives in coastal waters of temperate

and tropical regions worldwide. However, the importance of *Ulva rigida* goes far beyond its ecological role (Mutavski et al., 2024).

Algae production and processing can support the blue circular economy by contributing to its main drivers within the European Union (Lange et al., 2021; Hofmann et al., 2024).

Ulva rigida contains a variety of natural compounds that can be used in many fields, including pharmaceuticals, cosmetics, chemicals, energy and food. It is rich in fibers, minerals, polysaccharides, vitamins, trace elements, chlorophylls, proteins and polyphenols. Full utilization of the plant can be a cost effective approach by utilizing the various components of *Ulva rigida* (Mutavski et al., 2024).

The proximate composition, nutrients, and bioactive compounds present in seaweeds depend on several factors such as species, oxygen concentration, salinity of water, climatic season, intensity of UV radiation, and area of production (Kumar et al., 2021).

Over the past thirty years, the use of medicinal plants has become a major trend worldwide. Analysis of algae, plant extracts and natural products has shown that green algae is a potential source of income. Among its many biological properties, its antioxidant activity has a particular interest. It plays an essential role in neutralizing free radicals, thus helping to prevent various diseases linked to oxidative stress, including cardiovascular disease, premature aging and certain forms of cancer. What's more, this plant has significant antibacterial activity, which is an advantage over antibiotics, which are facing a crisis due to the rapid increase in bacterial resistance, as in the case of *Staphylococcus aureus* (Cadar et al., 2022).

In-depth, study of the composition and mechanisms of biological activities of this seaweed is therefore essential to explore its potential as a functional ingredient in food and health. Further research into the potential constituents of *Ulva rigida* would also be of interest to the food supplement industry. It can be used to enrich functional food formulations and supplement our diets with specific nutrients and bioactive ingredients. Knowledge of *Ulva rigida* can also pave the way for the development of new and sustainable applications, such as its potential as a source of biofuel production, offering a glimpse of an energy-efficient future landscape. The seaweed can be used as a renewable raw material for biofuel production as it contains lipids and carbohydrates (Mutavski et al., 2024) .

Ulva rigida is rich in nutrients such as vitamins and minerals, yet low in fat and calories compared to foods of animal origin, giving it a high nutritional value. In particular, *Ulva rigida* is a source of high quality protein containing 17 different amino acids, including all the essential ones, making it excellent for human consumption (Ouahabi et al., 2024).

Macroalgae have great potential as a sustainable raw material due to their high carbohydrate content (25-60% of dry weight) (Cesário et al., 2018; Tânia et al., 2023).

Ulva rigida could be a valuable alternative source of essential macronutrients.

Indeed, seaweeds have been proposed as an alternative ingredient for the formulation of nutritional supplements that could cover various dietary needs (Echave et al., 2021).

This article examines the biochemical composition of *Ulva rigida* and investigates its antioxidant activity, highlighting its potential role as a natural resource in the pharmaceutical industry.

2. Materials and Methods

2.1 . Plant material

The *Ulva rigida* (sea lettuce) plant was collected on 8 October 2024 from the coast of the Sahel region (Sayada) in Tunisia. The latitude and the longitude of Sayada site is 35.661334; 10.904428. The water is not very deep in this station.

The algae collected was attached to the rocks. The plant was transported in a plastic box to the laboratory of pharmacognosy in the faculty of pharmacy Monastir.

The plant was washed with distilled water and air dried on filter paper for 5 days. A reference sample was kept in the laboratory and I worked on the other part.

2.2 . Microscopic study

We added a small amount of fresh algae with a small drop of water and covered them with a microscope slide. Observations were made under a light microscope at magnification x10 and x40.

2.3 . Preparation of the extract

After drying for 5 days, the plant was extracted by Soxhlet: 15 g of the drug was placed in a cartridge in the Soxhlet body and 130 ml of methanol was added to the heated flask. The solvent circulates continuously: it evaporates, condenses and comes into contact with the drug, then returns to the flask via the siphon. This process is repeated several times over a period of 6 hours. After cooling the flask, the solvent was evaporated by Rotavap at a temperature of 60°C for 15 minutes.

2.4. TLC (Thin Layer Chromatography) extract study

This is a widely used analytical method for the separation and identification of chemical compounds. The factor involved in the partitioning of molecules between the stationary and mobile phases is polarity.

The extract is applied to a silica plate attached to aluminum, which is the stationary phase. The plate is then placed in a vessel saturated with a mobile phase of formic acid/water/ethyl acetate (2/3/95). The plate is dried at room temperature and the staining of the components is observed with the naked eye, under UV (254 and 365 nm) and after spraying the plate with aminoethanol-R-diphenylborate.

The frontal ratio is calculated for the spots found:

RF= distance travelled by the compound / distance travelled by the solvent front.

2.5. Chromatographic study of the extract by HPLC

High-performance liquid chromatographic method with gradient elution and diode-array detection was used to quantify a phenolic acid (gallic acid) and flavonoids (rutin, luteolin) in the extract. HPLC analyses were performed with Shimadzu LC-2030 3D Plus (Prominence-I) chromatograph. The mobile phase contains 1% aqueous acetic acid solution (solvent A) and acetonitrile (solvent B), the flow rate was adjusted to 0.7 mL/min, the column was thermostatically controlled at 28°C, and the injection volume was kept at 10 µL. Gradient elution was performed by varying the proportion of solvent B to solvent A: from 10% to 40% B for 28 min, from 40 to 60% B for 39 min, and from 60 to 90% B for 50 min. The mobile phase composition was set back to the initial condition (solvent B: solvent A:

10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. Chromatographic separation was performed on a Chrom-Clone TM C18 column (5 µm particle size, 250 × 4.6 mm), and the detection was conducted using a diode-array UV detector at 254 nm. (Ouahabi et al., 2024)

Standard solution of gallic acid, rutin, and luteolin was prepared in methanol (0.1 mg/mL). The sample solution was prepared by dissolving 1 mg of the extract in 1 mL of methanol. Both the standard and sample solutions were filtered through a Whatman 0.45 µm syringe filter. The responses were measured as peak areas versus concentration.

2.6. Study of antioxidant activity

2.6.1. ABTS method

The study of the ability of the extracts to trap the radical cation of 2, 2′-azinobis-3ethylbenzothiazoline-6-sulfonate ($ABTS^+$) was performed by the method of Yahyaoui et al. (2017). The $ABTS^+$ radical was generated by a chemical reaction of an aqueous solution of ABTS (7 mM) and potassium persulfate (2.45 mM) in the dark for 16 h and then diluted with Methanol to an absorbance at 734 nm of 0.700 ± 0.02. An aliquot of 25 µL of extract at different concentrations (1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125 mg/ml, 0.0625mg/ml) was mixed with 975µL of diluted ABTS. Then the mixture was vigorously incubated for 6 min in the dark at room temperature. The absorbance was measured at 734 nm against a blank (negative control) and compared to a reference standard (positive control), Vitamin C whose absorbance was measured under the same conditions as the samples studied. The $ABTS^+$ radical scavenging capacity was determined by the IC50 and expressed in (mg/L) which represents the values of the concentration necessary to scavenge 50 % of the $ABTS^+$ radicals. A low IC50 value corresponds to the high efficiency of the extract. The samples were analyzed in triplicate.

% Inhibition = (Abs initial - Abs (ABTS + extract) / Abs initial) *100

2.6.2. DDPH method

Radical scavenging activity was performed according to the protocol described by Yahyaoui et al. with some modifications, which is based on the reduction of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). 810 µL of methanolic DPPH solution was mixed with 190 µL of each concentration of the sample after 30 min of incubation in the dark at laboratory temperature. The absorbance was measured against a blank at 520 nm. The positive control was represented by a solution of a standard antioxidant which is the Vitamin C. The free radical scavenging activity was determined by the IC50 as previously described for ABTS. The tests were performed in triplicates.

% Inhibition = (Abs initial - Abs (DDPH + extract) / Abs initial) *100

The instrument used for the 2 methods is Jenway spectrophotometer UV/Visible 6305. Curves, IC50 and P values were determined using GraphPad Pris2m 10 software.

3. Results

3.1. Microscopic observation

Under the microscope, as observed in figure 1 *Ulva rigida* cells are rectangular or polygonal in shape and arranged in a regular pattern. Adjacent cells are connected by thick cell walls visible under the light microscope. The cells contain numerous cup-shaped chloroplasts, often arranged around the periphery of the cell.

These chloroplasts give an intense green color, characteristic of chlorophytic algae, and are responsible for photosynthesis. The nucleus may be visible in special stains or in fine preparations. Vacuoles

occupy a large part of the cell and help to store water and nutrients.

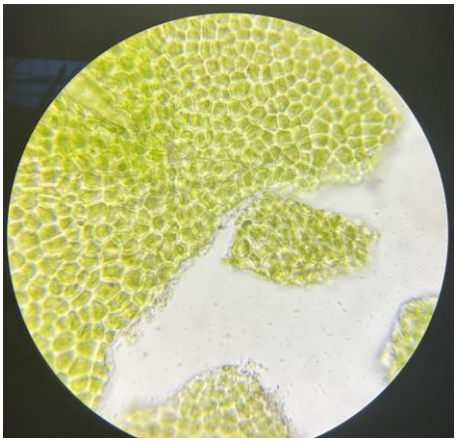

Figure 1. Microscopic observation *40 of *Ulva rigida*

3.2. *Thin layer chromatography*

As observed in figures 2 and 3, we obtained spots in the upper part of the plate (with a high retention factor, close to 1) corresponding to compounds with: Low polarity and high affinity to the mobile phase. These compounds interact little with the stationary phase and migrate rapidly with the solvent front. Red fluorescence is an indicator of the chemical nature of the compounds. Under UV light at 365 nm, this fluorescence is associated with substituted aromatic compounds: certain polyphenols or flavonoids have a red fluorescence in relation to their chemical structure.

Figure 2. TLC of *Ulva rigida* extract with naked eye

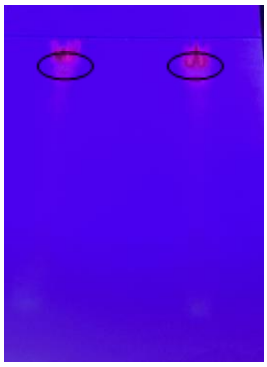

Figure 3. TLC of *Ulva rigida* extract under UV light 356 nm

3.3. *HPLC analysis of the extract*

A graph is produced (Figure 4) in which the peaks corresponding to the different compounds are separated as a function of their retention time (RT). The area under each peak is proportional to the concentration of the compound.

Each compound has a specific RT, which depends on its polarity, the mobile phase used, and interactions with the stationary phase. Two flavonoids (rutin and luteolin 7G) and one phenolic acid (gallic acid) were determined by HPLC analysis of the extract: phenolic acids are generally detected at RTs ranging from 2 to 12 minutes. Gallic acid was detected at 5.920 min. Flavonoids often appear later, between 15 and 20 minutes. In our chromatogram, rutin and luteolin 7G were detected at 17.998 and 18.434 minutes respectively.

The concentrations of the two flavonoids were 0.41 10^{-3} mg/mL for rutin and 0.2 10^{-4} mg/mL for luteolin 7G. The concentration of gallic acid was 0.432 10^{-2} mg/mL. The chromatogram also contained other unidentified peaks.

<< PDA >>

ID#1 Compound Name: Luteolin7G

Data File Name	Sample Name	Ret. Time	Area	Conc.	Unit
rutin stansard _rutin stansard _001	rutin stansard	19.349	31908	0.00018	mg/ml
OFT_Pg_23032019_004.lcd	Luteolin7g	18.533	34606246	0.19982	mg/ml
OFT_Pg_23032019_007.lcd	Gallic acid	0.000	0	0.00000	mg/ml
ulva _ulva_002.lcd	ulva	18.434	3481	0.00002	mg/ml

ID#2 Compound Name: Rutin

Data File Name	Sample Name	Ret. Time	Area	Conc.	Unit
rutin stansard _rutin stansard _001	rutin stansard	18.062	2276647	0.10000	mg/ml
OFT_Pg_23032019_004.lcd	Luteolin7g	0.000	0	0.00000	mg/ml
OFT_Pg_23032019_007.lcd	Gallic acid	0.000	0	0.00000	mg/ml
ulva _ulva_002.lcd	ulva	17.998	9402	0.00041	mg/ml

ID#3 Compound Name: Gallic acid

Data File Name	Sample Name	Ret. Time	Area	Conc.	Unit
rutin stansard _rutin stansard _001	rutin stansard	0.000	0	0.00000	mg/ml
OFT_Pg_23032019_004.lcd	Luteolin7g	0.000	0	0.00000	mg/ml
OFT_Pg_23032019_007.lcd	Gallic acid	5.688	287757	0.10000	mg/ml
ulva _ulva_002.lcd	ulva	5.920	12432	0.00432	mg/ml

Figure 4. Report of analyzing *Ulva rigida* with HPLC

3.4. Study of antioxidant activity

3.4.1. Study of antioxidant activity by ABTS

The results show significant antioxidant activity of the extract against ABTS, compared with a standard (vitamin C) to assess its relative efficacy. The percentage of ABTS inhibition was high at over 60%. The curve (Figure 5) was plotted using GraphPad Prism 10 software, and the IC50 and P value were determined

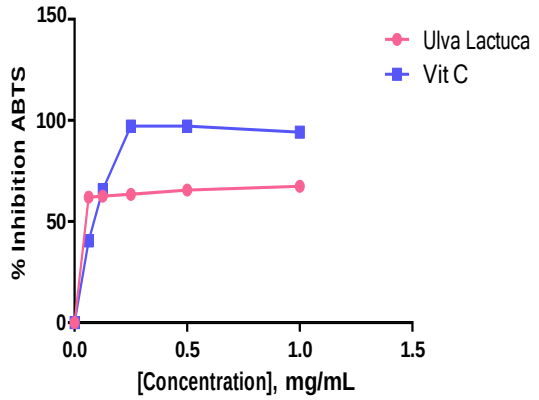

Figure 5. Variation curve for ABTS % inhibition as a function of extract concentration

3.4.2. Assay of antioxidant activity by DPPH

The results show significant antioxidant activity of the extract against DDPH, compared with a standard (vitamin C) to assess its relative efficacy. The percentage of DDPH inhibition was greater than 50%. The curve (Figure 6) was plotted using the same software: IC50 is 0.016 GraphPad Prism 10 software, and the IC50 and P value were determined using the same software: IC50 is 0.242 mg/mL and P value < 0.05.

These results are statistically significant according to GraphPad Pris2m 10 software mg/mL and P value < 0.05. These results are statistically significant according to GraphPad Pris2m 10 software.

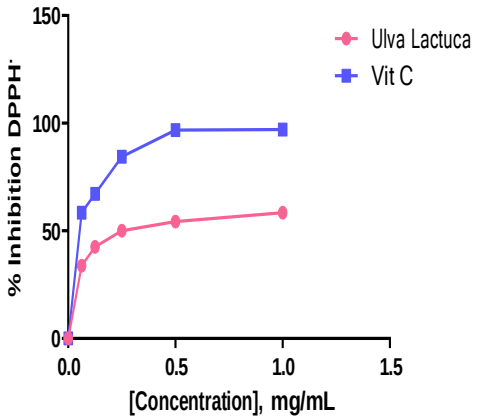

Figure 6. Variation curve for % DDPH inhibition as a function of extract concentration

3. Discussion

Microscopic observations of *Ulva rigida* reveal a simple yet functional structure, suited to its ecological role and ability to thrive in diverse marine environments. The abundance of chloroplasts indicates an efficient adaptation for light harvesting even in shallow waters, while the thickness of the cell wall confers resistance to environmental stresses such as salinity and desiccation.

Analysis of the antioxidant activity of *U. rigida* by ABTS and DPPH methods showed high activity, confirming its potential as a natural source of antioxidants. These results are consistent with several previous studies on *Ulva* spp. including *Ulva rigida*, which also showed high antioxidant activity due to its polyphenol content (Mezghani et al., 2016). However, while most research has attributed this activity primarily to polyphenols, our results suggest that other compounds may contribute, such as carotenoids, sulfated polysaccharides and certain proteins. These compounds have been shown to play a key role in various antioxidant assays: carotenoids, due to their hydrophobic character, mainly influence the DPPH assay, while water-soluble sulfated polysaccharides show remarkable activity in the ABTS assay (Olasehinde et al., 2019; Phomkaivon et al., 2024).

Compared to results obtained with other species of green algae, *Ulva rigida* appears to have competitive or even superior antioxidant activity, depending on the assay used (Altunkaya et al., 2009).

The consumption of harmful compounds can cause various chronic diseases in humans, including cancer, arteriosclerosis, age-related diabetes, inflammation, coronary heart disease, and neurological problems. To prevent food spoilage and protect consumers from these diseases, the lipid peroxidation of food can be suppressed using antioxidant chemicals or preservatives. As chemical components of biological materials, antioxidants can extend the shelf life of foods by delaying or preventing oxidation. Bioactive peptides are the most common antioxidant compounds found in food (Admassu et al., 2018; Pantoa et al., 2022).

Antioxidant activity makes *U. rigida* of great interest in a number of industrial sectors. In the agro-food sector, *Ulva rigida* could be used as a natural preservative to limit food oxidation. In the cosmetic industry, its inclusion in formulations would protect the skin from oxidative stress. Finally, its potential as an antioxidant dietary supplement is of growing interest in the pharmaceutical and human nutrition sectors.

Gallic acid is one of the most abundant phenolic acids and is often used to quantify the total phenol content of extracts. Its presence has already been reported in methanol, ethanol and aqueous extracts of *Ulva rigida* (Aslan et al., 2019; Farvin et al., 2013). In addition, two flavonoids are present in our extract HPLC results. Two flavonoids are present in our extract according to HPLC results.

These compounds are powerful antioxidants with various bioactivities, including antiviral, antimicrobial, antitumoral and anti-inflammatory properties, which are essential to human well-being (Pappou et al., 2022).

Thus, our results highlight the multifunctional interest of *Ulva rigida* and underline the need for further research to precisely identify the bioactive compounds responsible for its antioxidant activity and to optimize its exploitation in various industrial applications.

4. Conclusion

Ulva rigida is versatile algae with a wide range of applications in food, medicine and the environment. Its biochemical profile, rich in bioactive compounds,

makes it a promising resource for food, pharmacology and biomediation. Widespread in marine ecosystems, this green alga provides a unique combination of essential nutrients and secondary metabolites responsible for its beneficial biological effects. These findings encourage further research into this promising alga and maximize its potential in the areas of health, nutrition and environmental sustainability. The use of *Ulva rigida* in industrial products represents an important opportunity to promote the sustainable use of marine resources. As a fast-growing and easily cultivated seaweed, it offers an ecological and economical solution to meet the growing demand for natural antioxidants in the pharmaceutical, cosmetics and food sectors. *Ulva rigida* is thus emerging as a versatile marine resource that is both beneficial to human health and compatible with sustainable development objectives.

Despite these promising results, it must be stressed that the precise characterization of the bioactive compounds present in *Ulva rigida* remains a priority in order to better understand the mechanisms responsible for its antioxidant activity. Further studies involving more advanced chemical analyses such as LC-MS are needed. In vivo studies are also needed to validate the effects observed in vitro and to explore possible synergistic effects between the different compounds.

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