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Functional Properties and Biological Potentials of the Tunisian Green Seaweed Ulva lactuca

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Abstract

Recently, seaweeds are getting importance because of their numerous bioactive compounds and their utilisation as functional ingredients in various fields ranging from food to medical. In this respect, the green macro algae Ulva lactuca, known as « sea lettuce », has been studied. Hence, physico-chemical properties and biological potentials of Ulva lactuca, obtained from Cap Zebib collecting station (North of Tunisia) on July 2021, were investigated. It was found that the green seaweed species contained high level of moisture (12.75 ± 0.05 % dry weight (DW)), protein (10.63 ± 0.2 % DW), lipid (5.64 ± 0.11 % DW) and ash (17.25 ± 0.31 % DW). The study of the functional properties showed that WHC and OHC of this alga were 9.32 ± 0.42 g water/g DW and 1.67 ± 0.59 g oil/g, respectively. Antioxidant activity of the methanolic extract was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. Ulva lactuca exhibited a relatively high DPPH radical scavenging activity (54.46 ± 0.39 % of inhibition) with low IC_{50} 896.77 ± 0.31 µg/mL. Seaweed U. lactuca was screened for the potential bioactive natural substance against human pathogenic microorganisms. The methanolic extract showed the highest zone of inhibition against L. monocytogenes (17.04 ± 0.05 mm), followed by G. candicum (15.07 ± 0.09 mm), A. niger (14.02 ± 0.02 mm), S. aureus (13.01 ± 0.01 mm), E. coli (11.01 ± 0.01 mm) and S. typhimurium (10.06 ± 0.08 mm). The present study suggested that local seaweed Ulva lactuca could be potentially used as raw materials or additives to improve the nutritive value and functional properties of foods.

Keywords: Ulva lactuca; functional properties; antioxidant activity; antimicrobial avtivity.

1. Introduction

In the last years, natural bioactive compounds have obtained abundant concern in the world as functional ingredients in the diet. Especially, bioactive compounds derived from marine organisms have been considered as a rich source of components because of their potential as health-promoting [1].

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Macroalgae or seaweed-derived food products are developed in Asian countries, such as Japan and South Korea, since the ancient times as source of human and animal food, fertilizer, herbicides, as well as fungicides [2, 3]. However this is not the case for Tunisia yet. Recently, species of Ulva and several other algae species have been authorised for human consumption by France authorities [4]. Therefore, these marine organisms have been considered as an important functional foods since they provide a great variety of metabolites and natural bioactive compounds with potential therapeutic agents [3, 5]. Seaweeds are characterized by the presence of phenolic acids, flavonoids, phlorotannins, sterols, polyunsaturated fatty acids, carotenoids, and polysaccharides... [3]. These natural compounds contribute to several biological activities and potential health benefits, making them interesting potent for the application in pharmaceuticals, therapeutics, and regenerative medicine [6]. They protect the human against degenerative diseases such as diabetes, cardiovascular diseases, cancer, tissue damage by reactive oxygen species (ROS), and obesity… [3, 7]. Similarly, Ulva sp. (green algae) has been known for various natural bioactive compounds that have potential applications in several fields such as biomaterial science, nutraceuticals, functional foods and agriculture [8]. In genus Ulva, approximately 50 species have been identified and reported [9]. The biochemical composition of seaweeds is generally known to be highly influenced by species, environmental conditions, geographical location and maturity [2, 3]. Therefore, location of sampling sites should be taken in consideration for targeting certain seaweeds as sources of bioactive compounds [3].

Ulva species are still under-utilized because in Tunisia the knowledge about their nutritional composition, functional and biological properties is still limited. Our focus is Ulva lactuca, commonly known as « green laver » or « sea lettuce » The present study aimed to investigate the chemical composition, antioxidant properties of Ulva lactuca collected from different sites to see the potential for its valorisation for future applications in medicine, dietary supplements, cosmetics or food industries.

2. Materials and Methods

2.1 Sample collection and preparation

Samples of the green algae Ulva lactuca were obtained from Cap Zebib collecting station (North of Tunisia) on July 2021. This seaweed was selected because of the abundance in the study area and the availability throughout the year. Raw and fresh seaweeds were rinsed on the spot with seawater to remove salts, sands and epiphytes, and thoroughly placed in polyethylene bags. At the laboratory, the collected samples were rinsed again with distilled water to remove remaining impurities. The samples were cut into small pieces and air dried at room temperature until the constant weight obtained. Dried samples were ground to make fine and homogeneous powder and then were stored in hermetic bags at room temperature for further analysis.

2.2 Proximate composition

The moisture, protein and ash contents of the seaweed powder sample were determined according to the AOAC [10] standard method. The moisture content was determined by oven drying method at 105°C until their constant weight was obtained. Total protein was determined by the Kjeldahl method using a nitrogen

conversion factor of 6.25. Ash content was analyzed by incineration in a furnace at 525°C overnight. Crude lipid was extracted from the algal sample using Soxhlet apparatus with chloroform:methanol, 2:1, v/v according to the method of Bligh and Dyer [11]. The crude lipid content was determined gravimetrically after oven-drying at 80°C overnight.

2.3 Functional properties of algal powder

2.3.1 Water holding capacity (WHC)

The WHC of seaweed powder sample was measured as referred to Muraguri [12]. Algal powder (1 g) was weighed in a centrifuge tube and 60 mL of distilled water were added. The mixture was then vortexed in order to dissolve the powder before left at room temperature for 1 hour. After centrifugation (2060 rpm for 10 min at 25ºC; Beckman CS-6 centrifuge), the tube was weighed again after the supernatant was discarded. The WHC was expressed as gram of water held by 1 g of sample.

2.3.2 Oil holding capacity (OHC)

The OHC of seaweed samples were determined according to the method of Wong and Cheung [13]. About 10.5 g of corn oil was added to 3 grams of algal sample powder in a 50 mL centrifuge tube. The sample was stirred and left at room temperature for 30 min. After centrifugation (2500 × g for 30 min), the supernatant was removed and the OHC of seaweed sample was expressed as the number of grams of oil held by 1 g of sample.

2.4 Biological activities

2.4.1 Preparation of algal extract

The powdered sample (30 g) was extracted by maceration method using methanol (300 mL) [14]. The extraction was carried out at room temperature and in dark condition for seven days. To obtain its active compound, the extract was collected and concentrated under reduced pressure using a rotary vacuum evaporator at 40°C. The crude extract concentrated was stored at -20°C for the further experiments.

2.4.2 Antioxidant activity

The antioxidant activity of algal extract was estimated using The DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity according to the method of Prasedya and his colleagues [3] with slight modifications. Algal samples at different concentrations (50-1000 µg/mL) were mixed with 100 µL of DPPH solution (0.16 mM). The mixture was incubated in the dark at room temperature for 30 min and then, the absorbance was measured at 517 nm. The DPPH scavenging rate was calculated using the following equation :

$$\% \text{ Inhibition} = (A_{control} - (A_{sample} - A_{blank})/A_{control}) \times 100 \quad (1)$$

Where: $A_{control}$ is the absorbance of the control (DPPH without sample), A_{sample} is the absorbance of the test sample (the sample test and DPPH solution), A_{blank} is the absorbance of the sample blank (Sample without the DPPH solution).

The half-maximal inhibitory concentration (IC_{50}) was calculated by linier regression analysis and expressed as mean of three determinations. Ascorbic acid was used as positive control.

2.4.3 Antimicrobial activity

2.4.3.1 Microorganisms

Antimicrobial activities of algal extract were examined as the inhibitory effects against the growth of two Gram-positive bacteria (Staphylococcus aureus ATCC25923 and Listeria monocytogenes ATCC 070101121), two Gram-negative bacteria (Escherichia coli ATCC2124 and Salmonella typhimurium ATCC 25922) and two fungi (Geotrichum candidum ATCC, Aspergillus niger ATCC). All bacteria and fungi were obtained from culture collection of the Research Unit "Bio-Preservation and Valorization of Agro-Food Products" of the High Graduate School of Food Industry of Tunisia, in nutrient agar and stored at 4 °C.

2.4.3.2 Antimicrobial activity

Antimicrobial activity evaluation was carried out by the standard disc diffusion method as described by Güllüce and his colleagues [15]. To carry out the assay, a culture suspension of the indicator microorganism (10^6 cfu/ml) was spread on a Mueller–Hinton agar and Potato Dextrose agar, for antibacterial and antifungal activities, respectively.

The 6 mm diameter wells were punched in the inoculated agar medium with sterile Pasteur pipettes, and thereafter loaded with 100 µl of the algal crude extracts. Negative control was done using paper disc loaded with 100 µl of the methanol solvent. The plates were kept in the refrigerator at 4°C for 1 h to allow diffusion of the extracts into the media, and then incubated for 24 h to 48 h at 37°C for bactericidal and fungicidal activities. The antimicrobial activity was evaluated by measuring the diameter (mm) of bacterial-growth inhibition zone (clear zone around the well), in triplicate.

2.5 Statistical analysis

One-way analysis of variance (ANOVA) was performed, and the significance of each mean property valuewas determined ($p < 0.05$) with the Duncan's multiple range testusing the SPSS statistical analysis computer program for Windows (ver. 12.0, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Proximate composition

The results of proximate composition of U. lactuca are shown in table 1. The moisture content (12.75 ± 0.05 % dry weight (DW)) of the seaweed studied was significantly higher than that of other green seaweeds such as U. pertusa and U. intestinalis, collected in rainy and summer seasons from the Pattani Bay in Southern Thailand, which were within the range of 5.4 ± 0.9 % to 7.2 ± 0.2 % DW [2].

The protein content of U. lactuca obtained in this study (10.63 ± 0.2 % DW) was relatively high but significantly lower than that of U. pertusa (15.4 ± 0.9 % DW, Thailand), U. intestinalis (17.9 ± 1.7 % DW, Thailand), U. reticulata (13.5 % DW, India), E. compressa (12.3 % DW, India) and U. fasciata (12.3 % DW, Hawai) [2, 16, 17]. However, this result was in line with the findings of Fleurence [18] who reported that Ulva spp. had protein content within the range of 10-26 % DW. U. lactuca showed a high protein content thus justifying its importance in human and animal nutrition.

In this research, the studied U. lactuca contained 5.64 ± 0.11 % DW of crude lipids. According to McDermid and Stercke [16], most seaweeds contained low lipid contents (less than 4 %), however, some species had high level of crude lipid, such as Dictyota acutiloba (16.1 % DW) and D. sandvicenis (20.2 % DW). Benjama and Masniyom [2] found that the lipid contents of U. pertusa and U. intestinalis were within the range of 2.1 ± 0.0 %–7.4 ± 1.0 % DW and 7.3 ± 0.3 %–8.7 ± 0.6 % DW, respectively. The majority of seaweed lipids are polyunsaturated ω-3 and ω-6 fatty acids, which effectively reduce the risk of various diseases such as osteoporosis, cardiovascular diseases, and diabetes [19].

The ash content of sample species (17.25 ± 0.31 % DW) was lower than those reported in the literature for U. pertusa (27.2 ± 1.7 % DW), U. intestinalis (27.6 ± 0.9 % DW), E. flexuosa (23.2% DW), U. fasciata (25.4 % DW) and U. lactuca (21.3 % DW) [2, 13, 16] . The variation of chemical composition observed resulted from numerous factors including the geographical origins, seaweed species, seasons, environmental conditions as well as the method and the experimentation conditions [2].

Table 1: Proximate composition[a] (% dry weight), water holding capacity[a] (g water/g sample) and oil holding capacity[a] (g oil/g sample) of U. lactuca from Cap Zebib (North of Tunisia).

Components	**Values**
Teneur en eau	12.75 ± 0.05
Teneur en protéines	10.63 ± 0.2
Teneur en lipides	5.64 ± 0.11
Teneur en cendres	17.25 ± 0.31
WHC	9.32 ± 0.42
OHC	1.67 ± 0.59

[a]Average of three determinations ± SD.

3.2 Functional properties of algal powder

3.2.1 Water holding capacity (WHC)

To study the hydration property of U. lactuca, water holding capacity (WHC) was determined (table 1). WHC (9.32 ± 0.42 g water/g DW) of the seaweed studied was similar to that of U. pertusa (8.08 ± 0.52 g/g), however, it was lower than that of U. intestinalis (13.90 ± 1.29 g/g) [2]. For the same seaweed species, WHC observed in the present study was lower than previously reported (6.66 g /g) by Yaich and his colleagues [4] and higher than

results found (4.39 ± 0.07 g/g) by Udayangani and his colleagues [20]. It has been reported that it was difficult to compare the values of WHC between seaweed samples because this variation may be attributed to several factors which can influence the successful incorporation of fiber-enriched ingredients into foods. These factors were summarized into three types : chemical compositions, physical properties and experimental conditions. Chemical compositions factors were related to different protein conformations, the variations in the number and nature of the water binding sites on the protein molecules and the types of water associated with the fiber (1-water bound to the hydrophilic polysaccharides, 2-water held within the fiber matrix, 3-water trapped within the cell wall lumen). Physical properties, such as size and porosity of samples, density, types of ions in solutions, pH and ionic strength are important to well understand the different behaviors of samples during hydration. Experimental conditions were related to sample preparation, temperature, time, and centrifugation [2, 13, 20] . According to Cofrades and his colleagues [21], seaweeds have been shown to improve the water holding capacity of emulsions, thus having an influence on the rheological properties of cooked meat products (firmness, hardness and chewiness). Therefore, U. lactuca may be used as functional ingredient to improve properties of food products.

3.2.2 Oil holding capacity (OHC)

In this study, the OHC (1.67 ± 0.59 g oil/g) of U. lactuca was comparable to that of U. pertusa (1.53 ± 0.14 g oil/g DW) [2] and to the result found by Yaich and his colleagues [4] for the same species (1.46-1.68 g oil/g DW) (table 1). Moreover, according to Udayangani and his colleagues [20], the OHC of U. lactuca was higher than some terrestrial sources known as dietary fiber concentrates such as peach (1.02-1.11 g/g dry weight) and orange (0.86-1.28 g/g dry weight). OHC is an important parameter taken into consideration in both food and pharmaceutical industries. The importance of this OHC property is mainly due to the the physical entrapment of oil and the hydrophobicity of proteins [13]. According to [2] and Udayangani and his colleagues [20], the major role of seaweeds in fat absorption make them a good choice as stabilizers in formulate food products with high fat content and emulsions, as flavor retainer that increases the mouth-feel of foods and as natural remedies for obesity, coronary heart disease risk and blood lipid level. Therefore, U. lactuca powder could be a potential functional ingredient in formulate food products.

3.3 Biological activities

3.3.1 Antioxidant activity

Marine green algae contribute to the antioxidant capacity of organisms, by acting as exogenous antioxidants regulating the oxidative stress related diseases in human. In this regard, the antioxidant capacity of U. lactuca was assessed with in vitro assay using 1,1-diphenyl-2-picryl hydrazil (DPPH) radical scavenging. Different concentrations (50-1000 µg/mL) of the methanolic extract and standard antioxidant (ascorbic acid) were prepared. Figure 1 clearly point up that the antioxidant activity increased with increasing concentration of the algal extract concentration. The methanolic extract of seaweed powder exhibited a relatively high antioxidant potential with 54.46 ± 0.39 % of inhibition of the DPPH radical, at the concentration of 1000 µg/mL, and low IC_{50} value of 896.77 ± 0.31 µg/ml. This activity was comparable to that of the positive control, ascorbic acid (52.48 ± 1.2 %), at the concentration of 1.5 µg/mL. The antioxidant activity result agreed with that obtained by

Farasat and his colleagues [22], who found that the methanolic extract of U. intestinalis showed high DPPH scavenging activity (48 % inhibition) and a lower IC_{50} value of 2.32 mg/mL. In the present study, the IC_{50} observed (896.77 ± 0.31 µg/mL) was higher than previously reported by Prasedya and his colleagues [3] for the same seaweed species collected form different sampling sites in Indonesia (IC_{50} ranged from 522.23 ± 43 µg/mL to 682.23 ± 23 µg/mL). Previous studies have stated high scavenging activity of Ulva lactuca and demonstrated the promising antioxidant activity for Ulva species in general [3, 22].

Edible marine algae have attracted a special interest for their richness in sulfated polysaccharides (SPs). These chemically anionic SPs polymers have a significant effect on the antioxidant effect of macroalgae. In this regard, radical scavenging is higher for over-sulfated ulvan from U. linza compared to its native form. Moreover, it was reported that low molecular weight ulvan have shown potent antioxidant activity than high molecular weight [8]. Several researchers demonstrated the capacity of Ulvan to enhance antioxidant enzyme activity by activating the transcription enzymes (e.g., Nrf2, NF-kB, AP-1, AP-2, Sp1 and C/EBP) involved in the expression of antioxidant enzymes [8]. From these results, it can be concluded that U. lactuca could be a potential rich source of natural antioxidants.

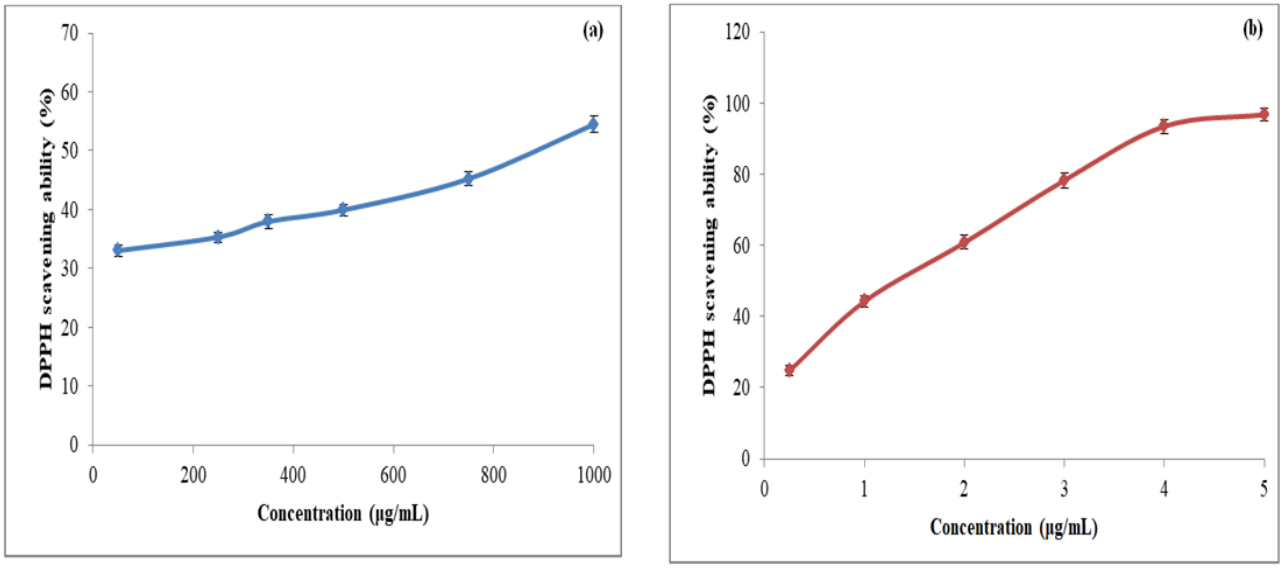

Figure 1: DPPH scavenging ability (%) of the methanolic extract of U. lactuca seaweed powder **(a)** and ascorbic acid **(b)** at different concentrations. Each value is presented as mean ± SD (n = 3).

3.3.2 Antimicrobial activity

The potential of antimicrobial activity of the U. lactuca extract was assessed using well agar diffusion method. The Figure 2 clearly depicts that the algal extract exhibited different antibacterial activities against four human bacterial pathogens. Both Gram-positive and Gram-negative bacteria showed high zones of inhibition ranged form 10.06 ± 0.08 mm to 17.04 ± 0.05 mm. The maximum inhibition zone was obtained against L. monocytogenes (17.04 ± 0.05 mm), followed by S. aureus (13.01 ± 0.01 mm), E. coli (11.01 ± 0.01 mm) and S.

typhimurium (10.06 ± 0.08 mm). Figure 2 reveals also that the methanolic extract of U. lactuca showed remarkable inhibitory activity against A. niger and G. candicum with inhibition zone recorded as 14.02 ± 0.02 mm and 15.07 ± 0.09 mm, respectively. Kandhasamy and Arunachalam [23] reported that C. racemosa, green algae were more active compared to other groups of algae screened for their antibacterial activity. The methanolic extract showed maximum zone of inhibition against K. pneumonia (15 ± 0.41 mm), P. aeruginosa (14 ± 0.26 mm) and S. aureus (16 ± 0.36 mm). According to Srikong and his colleagues [14], the organic extracts of U. intestinalis demonstrated antimicrobial activity against only Gram-positive bacteria, with inhibition zones that ranged from 6.85 ± 0.17 to 16.4 ± 2.4 mm, and were ineffective against Gram-negative bacteria. Devi and his colleagues [24] stated that the methanolic extract of brown seaweed Sargassum wightii showed highest zone of inhibition against Escherichia coli (17.3 ± 0.15mm) and Staphylococcus aureus (15.5 ± 0.25 mm) and a moderate zone of inhibition against Salmonella typhi (11.7 ± 0.51 mm). In order to confirm the antimicrobial potential of U. intestinalis, Srikong and his colleagues [14] performed morphological observations using scanning electron microscopy for the treated L. monocytogenes cells with the hexane extract. They reported that SEM images showed pore formation and extensive damage to the cell envelope, including the membrane and cell wall, that led to cell lysis.

Differences between results obtained in this study and in previous researchs were due to several factors. First, seasonal variations can affect the intraspecific variability and as consequence the production of secondary metabolites. Secondly, there may also be differences in assay methods protocols to extract active metabolites and that could result in different susceptibilities of the target strains. In addition, antibacterial activities of seaweeds varied with the species division. It has been demonstrated that brown seaweeds showed greater antibacterial activity than green and red ones [14, 24].

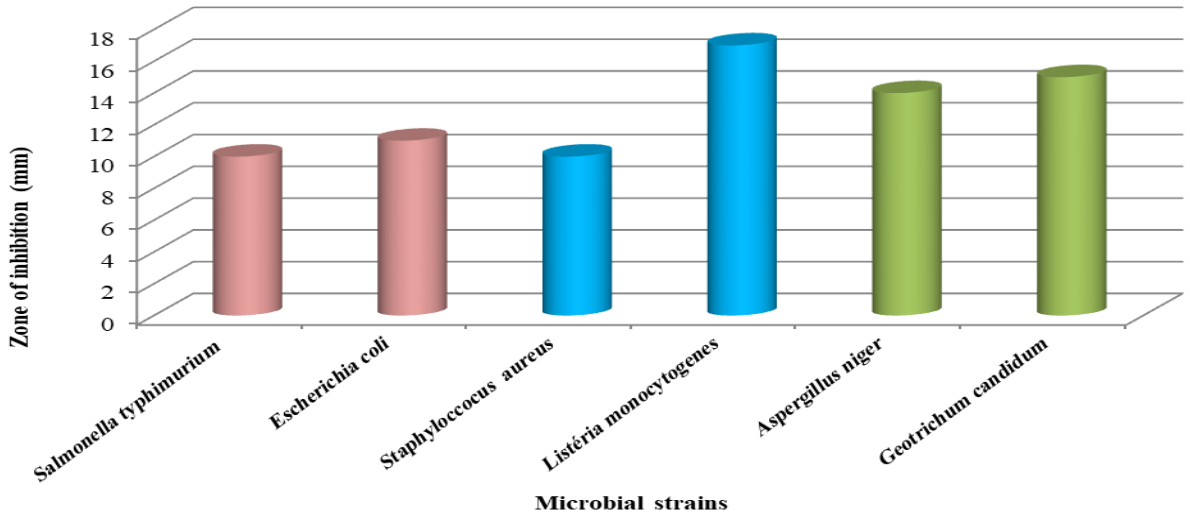

Figure 2: Antimicrobial activity of Ulva lactuca extract against human pathogens.

4. Conclusion

In conclusion, this study has revealed that U. lactuca seaweed was a potential source of bioactive compounds.

Its relatively high protein content make it an interesting source of plant food proteins. According to results of the functional properties, such as WHC, and especially the OHC, U. lactuca powder could be a potential functional ingredient to be incorporated in lipid-based foods. Moreover, investigation of the antioxidant activity showed that U. lactuca exhibited a relatively high DPPH radical scavenging activity with low IC_{50}. Hence, the search for antioxidants from natural sources such as seaweeds is important for the food industry to increase the quality and shelf-life of foods. Screening of U. lactuca for the potential bioactive natural substance against human pathogenic microorganisms showed that it had a considerable antimicrobial activity against all pathogenic micoorganisms tested. Further work concerned phytochemical investigation, mineral contents, fatty and amino acids compositions, vitamins and toxic elements is necessary (i) to provide more informations for safer and more versatile utilization of U. lactuca, and (ii) to better characterize the active components responsible for the antimicrobial and antioxidant potentials.

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