Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 22 July 2026
Revised 26 Aug 2026
Accepted 12 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (22s): 552–564

KEYWORDS:
antioxidant activity; dietary fiber; heavy metals; LC-HRMS; phytochemicals; *Ulva lactuca*

Nutrition, Phytochemicals, Antioxidant Capacity and Heavy Metal Safety of ULVA Lactuca from Nusa Lembongan, Bali

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ABSTRACT: *Ulva lactuca* is a green macroalga with potential nutritional and functional food applications, although its nutritional composition, phytochemical characteristics, antioxidant capacity, and contaminant levels require comprehensive evaluation. This study investigated these properties in *U. lactuca* collected from Nusa Lembongan, Bali, Indonesia. Proximate composition, dietary fiber, qualitative phytochemical characteristics, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, metabolite profiles, and heavy-metal concentrations were analyzed using standard analytical methods, spectrophotometric assays, liquid chromatography–high-resolution mass spectrometry (LC-HRMS), and atomic absorption spectrophotometry. The dried algal material contained 59.01% carbohydrates, 19.20% crude protein, 0.47% lipids, and 57.08% total dietary fiber, predominantly insoluble fiber. Phytochemical screening indicated the presence of flavonoids, tannins, steroids, and triterpenoids. The ethanolic extract exhibited a TPC of 81.87 mg gallic acid equivalents (GAE) g^{-1} and a TFC of 30.07 mg quercetin equivalents (QE) g^{-1}, together with DPPH radical-scavenging activity. LC-HRMS analysis tentatively annotated ten compounds, including amino acids, betaine, lipid-related compounds, and other metabolites requiring further confirmation. Concentrations of mercury, lead, cadmium, and arsenic were below the maximum limits established by Indonesian National Standard SNI 2690:2018, although arsenic approached 80% of its permitted limit. These findings highlight *U. lactuca* as a promising source of dietary fiber and phytochemical constituents for food-related applications. Nevertheless, further assessment of arsenic speciation, metabolite identities, nutritional quality, and constituent bioaccessibility is necessary to establish its suitability for food development.

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1. INTRODUCTION

Seaweeds are increasingly recognized as natural resources that can contribute to human nutrition and health, supplying dietary fiber, minerals, protein and a diverse set of bioactive secondary metabolites [1, 2]. Phenolic compounds, flavonoids, terpenoids, alkaloids and sulfated polysaccharides from macroalgae have been associated with antioxidant, anti-inflammatory and other biological activities [2, 3]. However, the nutritional and functional value of a given seaweed varies with species, geographical origin and season, and many reports of bioactivity rely on extracts of poorly defined composition, which limits their relevance for food use [1]. Because seaweeds also accumulate metals and metalloids from seawater, nutritional characterization must be coupled with food safety assessment [4, 5].

The green macroalga *Ulva lactuca* (sea lettuce) is widely distributed, grows rapidly and tolerates variable environmental conditions, which makes it an attractive candidate for sustainable production in tropical coastal waters. It is rich in carbohydrates and dietary fiber, including the sulfated polysaccharide ulvan, and contains proteins, minerals and phenolic compounds [6-8]. Extracts of *U. lactuca* have shown antioxidant and enzyme-inhibitory activities in vitro [9, 10], and a biscuit containing *Ulva* reduced blood glucose in sucrose-loaded rats [11]. Most detailed compositional data on *U. lactuca*, however, originate from temperate and subtropical regions, and its composition is known to differ among sites [8, 12].

Indonesia has extensive tropical coastlines and a long tradition of seaweed cultivation, yet integrated information that links the nutritional composition, phytochemistry, antioxidant capacity and contaminant load of wild *U. lactuca* from specific Indonesian habitats is limited. This gap is relevant for Nusa Lembongan, Bali, a small island exposed to both aquaculture and tourism-related coastal activities, where no such characterization has been reported. Without it, the suitability of local *U. lactuca* as a food or nutraceutical resource cannot be judged.

This study therefore asked whether *U. lactuca* from Nusa Lembongan combines favorable nutritional and antioxidant properties with heavy metal levels compliant with Indonesian food standards. We determined (i) proximate composition and dietary fiber fractions, (ii) Hg, Pb, Cd and As concentrations, (iii) the qualitative phytochemical profile of an ethanolic extract, (iv) its LC-HRMS metabolite profile, and (v) its total phenolic and flavonoid contents and DPPH radical-scavenging activity.

2. MATERIALS AND METHODS

2.1 Sample collection, identification, and preparation

Ulva lactuca was collected from coastal waters around Nusa Lembongan Island, Bali, Indonesia (08°30'43"–08°41'43" S; 115°25'36"–115°28'20" E). The identification of macroalgae species was conducted using reference guides by Al-Yamani et al. [13]. Additionally, the scientific names of the macroalgae were cross-checked with Guiry and Guiry [14] to ensure alignment with current taxonomic standards. The biomass was rinsed with clean seawater followed by freshwater to remove sand, epiphytes and debris, and was dried in a custom-built temperature-controlled cabinet at 50–60 °C to constant weight. The dried material was ground with a mechanical grinder and sieved through an 80-mesh stainless-steel sieve. The resulting powder was stored in airtight, light-protected containers at room temperature until analysis.

2.2 Proximate composition

Proximate composition analyses were performed at the National Research and Innovation Agency (BRIN) through its ELSA laboratory service followed the Indonesian National Standard for dried seaweed (SNI 2690:2018) and the specific SNI methods listed below. Moisture was determined according to SNI 2354.2:2015, ash according to SNI 2354.1:2010, lipid according to SNI 2354.3:2017, and protein according to SNI 01-2354.4-2006. Carbohydrate was determined according to SNI 01-2891-1992. The proximate composition values are expressed on an as-analysed basis.

2.3 Dietary fiber

Dietary fiber content was determined using the enzymatic–gravimetric method described by Asp et al. [15], following the procedure outlined in AOAC [16]. The analysis was performed at the Laboratory of Biotechnology, IPB University, Indonesia. Samples were sequentially hydrolyzed with α-amylase, protease and amyloglucosidase to simulate gastrointestinal digestion and to remove non-fiber components. Insoluble dietary fiber (IDF) was collected as the residue after filtration, and soluble dietary fiber (SDF) was recovered from the filtrate by ethanol precipitation. Total dietary fiber (TDF) was calculated as:

TDF = IDF + SDF (1)

2.4 Heavy metals

Mercury (Hg) was determined according to SNI 2354.6:2016, lead (Pb) and cadmium (Cd) according to SNI 2354.5:2011, and arsenic (As) according to SNI 2354.15:2017. Results were compared with the maximum limits in SNI 2690:2018. Heavy metal analyses were conducted at the laboratory of BBP3KP Jakarta, Ministry of Marine Affairs and Fisheries, Indonesia. All laboratories for proximate, dietary fiber, and heavy metals analyses employed routine analytical procedures, instrument calibration, and quality-control measures in accordance with the respective SNI methods and/or AOAC.

2.5 Preparation of ethanolic extract

The ethanolic extract was prepared by maceration following Rompas and Gasah [17] with minor modifications. Dried algal powder was macerated in 96% ethanol at a ratio of 1:4 (w/v) and stirred with a magnetic stirrer at room temperature without heating for three consecutive days, with filtration performed daily. The residue was re-extracted under the same conditions for three successive cycles. The combined filtrates were concentrated under reduced pressure in a rotary evaporator (Büchi R-300) at 40 °C to obtain the crude ethanolic extract, which was used for phytochemical screening, LC-HRMS, TPC, TFC and DPPH assays.

2.6. Qualitative phytochemical screening

The extract was screened for alkaloids, flavonoids, tannins, saponins, triterpenoids and steroids. Alkaloids were tested with Mayer and Wagner reagents [18]; white-to-yellowish and brown precipitates, respectively, were considered positive. Saponins, flavonoids, triterpenoids and steroids were tested according to Harborne [19]. Persistent stable foam indicated saponins, and a red, yellow or orange color indicated flavonoids. The Liebermann–Burchard test was used to distinguish triterpenoids (red, yellow or purple) from steroids (green or blue). Tannins were detected according to Ridwan [20], with a dark blue or greenish-black color considered positive.

2.7 Total phenolic content (TPC)

TPC was determined by the Folin–Ciocalteu method [21]. An extract aliquot (0.5 mL) was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and vortexed. After 5 min, 0.2 mL of 7.5% Na_2CO_3 was added, the mixture was vortexed again and incubated for 30 min at room temperature in the dark, and absorbance was read at 765 nm. A calibration curve was prepared with gallic acid (0, 20, 40, 60, 80 and 100 μg mL^{-1}), and TPC was expressed as mg gallic acid equivalents per g of sample (mg GAE g^{-1}).

2.8 Total flavonoid content (TFC)

TFC was determined by the aluminum chloride colorimetric method [22]. An extract aliquot (1.0 mL) was mixed with 1.0 mL of 2% $AlCl_3$, vortexed and incubated for 30 min at room temperature in the dark. A blank consisting of 1 mL of extract plus 1 mL of solvent without $AlCl_3$ was prepared, and absorbance was read at 415 nm. Quercetin (0, 20, 40, 60, 80 and 100 μg mL^{-1}) was used as the standard, and TFC was expressed as mg quercetin equivalents per g of sample (mg QE g^{-1}).

2.9 DPPH radical-scavenging activity

Antioxidant activity was determined by the DPPH free-radical scavenging assay following Tamokou's et al. [23] procedures with a slight modification in serial concentration, 15.6, 31.3, 62.5, 125.0, 250.0, 500.0 ppm. The percentage of inhibition was calculated as:

Inhibition (%) = [(A_control − A_sample) / A_control] × 100 (2)

The relationship between inhibition (Y) and extract concentration (x) was described by linear regression (Equation 3), and the IC_{50} (the concentration giving 50% inhibition) was calculated from the regression equation:

Y = a + bx (3)

2.10 LC-HRMS metabolite profiling

Untargeted metabolite profiling followed Windarsih et al. [24]. Analyses were performed at the National Research and Innovation Agency (BRIN) through its ELSA laboratory service using a Thermo Scientific Vanquish UHPLC system coupled to a high-resolution Orbitrap mass spectrometer [Q Exactive]. Separation was achieved on an Accucore Phenyl-Hexyl column (100 mm × 2.1 mm, 2.6 μm) at 40 °C, with a flow rate of 0.3 mL min^{-1} and an injection volume of 3 μL. The mobile phases were (A) MS-grade water and (B) methanol, each containing 0.1% formic acid. The gradient started at 5% B, increased linearly to 90% B over 16 min, was held for 4 min, and returned to initial conditions by 25 min. Data were acquired in full-MS/data-dependent MS^2 mode in both positive and negative electrospray ionization over m/z 66.7–1000, at resolutions of 70,000 (full MS) and 17,500 (dd-MS^2). Nitrogen served as sheath, auxiliary and sweep gas; the spray voltage was 3.30 kV, the capillary temperature 320 °C and the auxiliary gas heater temperature 30 °C. Compounds were annotated from accurate m/z values and compared with the Natural Product Library.

2.11 Statistical analysis

Results are presented as mean ± standard deviation (SD), based on triplicate measurements. Descriptive statistical analyses were performed using SPSS software.

3. RESULTS AND DISCUSSION

3.1 Proximate composition and dietary fiber

The dried *U. lactuca* was dominated by carbohydrate (59.01%), followed by protein (19.20%), ash (11.56%) and moisture (9.76%), whereas lipid was minor (0.47%) (Table 1). The low moisture content is favorable for storage stability, although effective moisture control after drying remains essential.

Table 1. Proximate composition and dietary fiber of dried *U. lactuca* from Nusa Lembongan, Bali (mean ± SD).

Parameter	Content (%)
Moisture	9.76 ± 0.01
Ash	11.56 ± 0.01
Lipid	0.47 ± 0.01
Protein	19.20 ± 0.01
Carbohydrate	59.01 ± 0.01
Soluble dietary fiber (SDF)	20.528 ± 0.032
Insoluble dietary fiber (IDF)	36.547 ± 0.098
Total dietary fiber (TDF = SDF + IDF)	57.075 ± 0.066

The ash content (11.56%) is lower, and the protein content (19.20%) higher, than reported for *U. lactuca* from Tunisia [8], namely ash 19.6% and protein 8.5%. Such differences are expected because seaweed composition depends on species, geographical region, season and post-harvest handling [1], and the variation in lipid (0.47% here versus 7.9% in the Tunisian sample [8] may additionally reflect differences in extraction methods. The protein value also depends on the nitrogen-to-protein conversion factor. If the conventional factor of 6.25 was applied to total nitrogen, protein may be overestimated by roughly 43% relative to the seaweed-specific factor of five proposed by Angell et al. [25], because seaweeds contain non-protein nitrogen [25, 26]. The protein content should therefore be interpreted as crude protein pending amino acid analysis.

The very low lipid content is typical of macroalgae and implies limited energy density, but also limited lipid oxidation during storage. The high carbohydrate value mainly represents structural polysaccharides rather than digestible carbohydrate: when carbohydrate (59.01%) and TDF (57.08%) are expressed on the same basis, only about 2% of the biomass would be non-fiber carbohydrate. This is consistent with the composition of the Ulva cell wall, which contains ulvan together with cellulose and hemicellulose [7, 27].

With a total dietary fiber content of 57.08%, the value is consistent with earlier findings for *U. lactuca*, such as the 53.09–54.9% observed in Tunisian specimens [8] and the 60.5% recorded in Chilean samples [6]. With insoluble fiber (36.55%) exceeding soluble fiber (20.53%) (Table 1), this pattern aligns with the results from Yaich et al. [27], who noted 34.37% insoluble fiber and 20.53% soluble fiber. Physiologically, insoluble fiber aids gastrointestinal passage, while soluble fiber, such as ulvan, can create viscous solutions that postpone gastric emptying and decelerate the absorption of glucose [28, 29]. Consequently, the substantial total dietary fiber content underscores the potential of *U. lactuca* to serve as a fiber-rich food ingredient. Nevertheless, the current research did not assess viscosity, sulfate content, fermentability, or glycemic response. As a result, any possible advantages for glycemic control are still theoretical and need more study.

The nutritional interpretation of the proximate composition requires consideration of the relationship between total carbohydrate and dietary fiber. Carbohydrate content (59.01%) was calculated by difference, whereas total dietary fiber (57.08%) was determined independently using an enzymatic–gravimetric method. Dietary fiber therefore represents a substantial fraction of the reported carbohydrate content and should not be interpreted as an additional component of the proximate composition. The predominance of insoluble dietary fiber (36.55%) over soluble dietary fiber (20.53%) further indicates that the carbohydrate fraction is largely associated with structural polysaccharides, although their specific composition and functional properties were not characterized in this study. The reported composition values represent the dried seaweed sample as analyzed; therefore, unless corrected for residual moisture, they should not be interpreted as strictly moisture-free dry-weight values.

3.2 Heavy metals and food safety

Levels of each of the four metals remained under the maximum thresholds specified by SNI 2690:2018 (Table 2), arranged in descending order as: As > Cd > Pb > Hg. The levels of lead, cadmium, and mercury were quite low, representing at most 7% of their allowed limits. Conversely, arsenic levels hit 2.41 mg kg^{-1}, which is nearly 80% of the 3 mg kg^{-1} regulatory ceiling; this suggests a slim safety buffer and necessitates diligent oversight of possible seasonal and batch-to-batch fluctuations. It is worth noting that the standard deviations for Pb and Hg were greater than their mean values, which signifies considerable variation in the recorded concentrations. Even so, notwithstanding this variation, the total concentrations stayed significantly underneath the peak thresholds defined by SNI 2690:2018.

Table 2. Heavy metal concentrations in dried *Ulva lactuca* from Nusa Lembongan, Bali, compared with SNI 2690:2018.

Metal	Concentration (mg kg^{-1})	SNI 2690:2018 limit (mg kg^{-1})	% of limit
Arsenic (As)	2.4104 ± 0.72	3	80.3
Cadmium (Cd)	0.1295 ± 0.06	2	6.5
Lead (Pb)	0.0325 ± 0.22	5	0.7
Mercury (Hg)	0.0033 ± 0.02	1	0.3

Total arsenic is a conservative indicator, because arsenic in seaweeds occurs largely as organic arsenic species such as arsenosugars, while inorganic arsenic, the more toxic form, is usually a smaller fraction, although occasionally high inorganic concentrations have been found in some seaweeds [1, 30]. Speciation analysis is therefore needed to refine the risk assessment of this biomass. The values found here also contrast with the notable lead content reported in *U. lactuca* fiber concentrates from Tunisia, which was considered to limit their use as a food ingredient [27], indicating that the Nusa Lembongan material is not constrained by lead.

Metal content in macroalgae reflects ambient concentrations and the metal-binding capacity of cell wall polysaccharides and functional groups, which makes seaweeds useful biomonitors but also potential vectors of exposure [4, 5, 31]. Because

chronic exposure to As, Cd, Pb and Hg is associated with adverse health outcomes [32, 33] and coastal metal contamination continues to be a concern [34], we recommend that the compliance observed in one sampling be treated as provisional. Monitoring across seasons and sites, arsenic speciation, and exposure estimates based on realistic portion sizes are necessary before the material is promoted for regular or concentrated consumption.

3.3 Phytochemical screening

The ethanolic extract was positive for flavonoids, tannins, steroids and triterpenoids, and gave a positive reaction for alkaloids with Wagner's reagent but not with Mayer's reagent; saponins were not detected (Table 3). The presence of flavonoids and tannins is consistent with the phenolic content found in the extract (Section 3.6) and with earlier reports of phenolic compounds in *U. lactuca* [2, 9].

Table 3. Qualitative phytochemical screening of the ethanolic extract of *U. lactuca*.

Metabolite class	Test/reagent	Positive reaction	Result
Alkaloids	Mayer	White to yellowish precipitate	−
Alkaloids	Wagner	Brown precipitate	+
Flavonoids	Color test [17]	Red, yellow or orange	+
Tannins	Ridwan [18]	Dark blue or greenish-black	+
Saponins	Foam test	Stable foam	−
Triterpenoids	Liebermann–Burchard	Red, yellow or purple	+
Steroids	Liebermann–Burchard	Green or blue	+

+, detected; −, not detected.

These results indicate that the ethanol extract of *U. lactuca* is composed of various types of potentially bioactive secondary metabolites. Nevertheless, these findings must be viewed carefully, as initial phytochemical screening is qualitative and possesses natural constraints regarding specificity. Specifically, identifying alkaloids is still uncertain because a positive result was only obtained with Wagner's reagent, whereas utilizing several different reagents would offer more robust proof of their existence. Similarly, a positive Liebermann–Burchard reaction suggests that steroids or triterpenoids may be present, though it cannot reliably differentiate between these two categories. Furthermore, qualitative screening does not establish the precise identities, concentrations, or biological activities of the detected compounds. Therefore, these findings should be regarded as an initial characterization of the extract, providing a basis for more detailed investigations using chromatographic and spectrometric techniques to identify and characterize individual metabolites.

3.4 LC-HRMS metabolite profile

Table 4 lists ten primary features tentatively identified in the extract: betaine, the aromatic amino acids phenylalanine and tyrosine, the alkylresorcinol 5-pentylresorcinol, the phenolic compound 6-shogaol, the monoacylglycerol monolinolenin, three fatty acid amides, and a citrate ester. The stated m/z values correspond to the calculated $[M+H]^+$ ions for the suggested molecular formulas. Nevertheless, one should approach these annotations carefully, as they rely largely on comparisons with libraries and accurate-mass measurements. Based on the identification confidence framework established by Schymanski et al. [35], these assignments typically represent tentative candidates (Level 3), or Level 2 when corroborated by matching MS/MS spectra. Therefore, it is not possible to exclude the possibility of structural isomers.

Table 4. Metabolites tentatively annotated in the ethanolic extract of *U. lactuca* by LC-HRMS.

No.	Compound	Class	Formula	m/z $[M+H]^+$	RT (min)	Peak area
1	5-Pentylresorcinol	Alkylresorcinol	$C_{11}H_{16}O_2$	181.12230	10.11	4.1×10^7
2	Betaine	Quaternary ammonium compound	$C_5H_{11}NO_2$	118.08627	0.80	6.1×10^7
3	L-Phenylalanine	Amino acid	$C_9H_{11}NO_2$	166.08626	2.50	2.9×10^7
4	L-Tyrosine	Amino acid	$C_9H_{11}NO_3$	182.08125	1.29	9.6×10^6
5	6-Shogaol	Phenolic compound	$C_{17}H_{24}O_3$	277.17987	10.71	2.4×10^7
6	Monolinolenin	Monoacylglycerol	$C_{21}H_{36}O_4$	353.26859	13.86	9.7×10^6
7	Hexadecanamide	Fatty acid amide	$C_{16}H_{33}NO$	256.26358	16.81	2.1×10^8
8	Myristamide	Fatty acid amide	$C_{14}H_{29}NO$	228.23216	14.99	6.6×10^7
9	Stearamide	Fatty acid amide	$C_{18}H_{37}NO$	284.29504	18.54	2.5×10^7
10	Citroflex 4 (tributyl citrate)	Citrate ester	$C_{18}H_{32}O_7$	361.22211	14.87	1.6×10^7

Specifically, two sets of annotations require more detailed examination. To begin with, 6-shogaol is a recognized compound found in ginger instead of being an anticipated metabolite from green macroalgae. Consequently, its existence within the extract must be verified by comparing it to a genuine reference standard or by utilizing supporting MS/MS fragmentation data. Additionally, fatty acid amides frequently serve as slip agents in plastics and can leach from lab consumables into extraction solvents [36]. In the same way, tributyl citrate is utilized commercially as a plasticizer. Consequently, the presence of hexadecanamide, myristamide, stearamide, and the citrate ester in the annotations might indicate procedural contamination instead of being endogenous compounds of *U. lactuca*. To ensure they are authentic algal metabolites or to evaluate their possible biological activities, their presence must be validated through suitable procedural and solvent blanks.

Notwithstanding these uncertainties, several of the leftover annotations remain chemically plausible regarding the metabolism of green macroalgae. One example is betaine, a quaternary ammonium compound frequently linked to osmotic regulation and the ability to tolerate cellular stress. The aromatic proteinogenic amino acids phenylalanine and tyrosine are present, which aligns with the extract's protein levels (Table 1). However, because only a few features were annotated and their identification remains uncertain, these results should be viewed as a preliminary chemical fingerprint instead of a full characterization of the metabolite profile in *U. lactuca*. Consequently, the prior assumption that the majority of identified compounds have antidiabetic properties was excluded, as this study failed to provide enough compound-specific evidence to support it. To gain a deeper understanding of the extract's chemical makeup and its antioxidant properties, more research is required through MS/MS-supported identification, quantitative metabolite profiling, and the targeted characterization of specific phenolic compounds that make up the total phenolic content (TPC).

3.5 Total phenolic and flavonoid contents

The total phenolic content (TPC) of the 96% ethanol extract from *U. lactuca* was 81.865 mg GAE g^{-1}, while the total flavonoid content (TFC) was 30.066 mg QE g^{-1} (Table 5). However, the reported concentrations of phenolics and flavonoids

in *U. lactuca* vary considerably among studies, depending on the extraction solvent, extraction technique, and calibration standards used. For instance, Ouahabi et al. [9] reported substantially higher values in extracts obtained from Moroccan *U. lactuca*, with the aqueous maceration extract showing maximum TPC and TFC values of 379.67 mg GAE g^{-1} and 212.11 mg QE g^{-1}, respectively, while the ethyl acetate extract contained 198.09 mg GAE g^{-1} of TPC. Compared with these reported values, the TPC and TFC obtained in the present study were relatively lower. Nevertheless, direct comparisons should be made cautiously because differences in extraction conditions and solvents can influence the recovery of phenolic and flavonoid compounds. Additionally, it is necessary to account for how concentration is expressed (either per gram of extract or per gram of dry biomass) when comparing findings from different studies. Furthermore, one must acknowledge the methodological constraints inherent in these colorimetric assays. The Folin–Ciocalteu method lacks complete specificity for phenolic compounds, as other reducing agents like specific sugars and amino acids can affect the recorded response. In a similar way, the aluminum chloride colorimetric method lacks equal sensitivity for all flavonoid subclasses. Consequently, the TPC and TFC values ought to be viewed as practical assessments of the extract's total reducing and aluminum-binding activities, instead of precise measurements of the actual concentrations of specific phenolic and flavonoid molecules.

Table 5. Total phenolic content, total flavonoid content and DPPH radical-scavenging activity of the ethanolic extract of *U. lactuca*.

Parameter	Value
Total phenolic content (mg GAE g^{-1})	81.865
Total flavonoid content (mg QE g^{-1})	30.066
DPPH regression equation (Y = a + bx)	Y = 26.876 + 5.890x
R^2	> 0.8953
DPPH IC_{50} (μg mL^{-1})	3.93

GAE, gallic acid equivalents; QE, quercetin equivalents.

3.6 DPPH radical-scavenging activity

The antioxidant capacity of the extract, measured via DPPH radical scavenging, rose alongside higher extract concentrations, demonstrating a concentration-dependent effect. Through linear regression analysis (Y = 26.876 + 5.900x; R^2 > 0.8953), an IC_{50} of 3.93 μg mL^{-1} was determined (Table 5).According to standard antioxidant activity classifications, where IC_{50} values under 50 μg mL^{-1} signify very strong activity, the extract showed exceptional radical-scavenging capacity. This result can be compared with the findings of Ouahabi et al. [9], who reported an IC_{50} of 0.095 mg mL^{-1} (approximately 95 μg mL^{-1}) for their most active *U. lactuca* extract. Although the extract examined in the present study exhibited a lower IC_{50} value, suggesting greater DPPH radical-scavenging activity, direct comparisons should be interpreted cautiously because differences in extraction solvents, sample characteristics, and experimental conditions may influence the measured antioxidant capacity.

While it shows a robust DPPH-scavenging response, this assay mainly evaluates how well compounds provide hydrogen atoms or electrons to neutralize free radicals in certain in vitro settings, which may not accurately represent antioxidant performance within living biological systems. Even though the extract possessed significant levels of phenolics and flavonoids (Table 5), it is impossible to statistically determine their separate roles in the measured antioxidant activity, given that only a single extract was tested. However, the phytochemical screening results in Table 3 reveal the presence of tannins and flavonoids, implying that these specific groups could aid in radical scavenging, which aligns with their known antioxidant qualities [2]. Different components might also play a part in the recorded activity, though their specific functions have yet to be determined. Consequently, conducting additional studies with supplementary antioxidant tests, including ABTS radical-scavenging and ferric reducing antioxidant power (FRAP), would allow for a more thorough evaluation of the extract's antioxidant capacity. Furthermore, cell-based and in vivo investigations must be conducted to ascertain if the demonstrated in vitro activity results in biologically significant antioxidant properties prior to assuming any possible health advantages [1].

3.8 Implications for human health and limitations

Altogether, the results show that *U. lactuca* from Nusa Lembongan is a fiber-dense biomass with low lipid and moderate protein content that possesses extractable antioxidants, and its heavy metal levels stay below the relevant Indonesian regulatory thresholds. Such properties indicate its possible use as a food additive, especially for increasing dietary fiber and creating functional food items. However, the findings regarding nutritional content and in vitro antioxidant capacity presented here should not be viewed as conclusive proof of health advantages. A few constraints must also be noted, such as the use of one sampling period that ignores seasonal changes; the lack of testing for iodine, minerals, sulfate, and amino acids; the qualitative approach to phytochemical screening; the uncertain metabolite identification produced without solvent or procedural blank controls; the reliance on just one extract and one assay for antioxidant activity; and the measurement of total arsenic instead of distinguishing between organic and inorganic forms. Of these constraints, the somewhat elevated arsenic levels, which are nearing the established regulatory threshold, deserve special consideration. Further investigations should therefore prioritize seasonal monitoring, more comprehensive nutritional and phytochemical characterization, validation of metabolite annotations, and arsenic speciation to establish the nutritional value and long-term safety of *U. lactuca* as a potential food and functional ingredient.

3.7 Discussion

The analysis of nutritional, phytochemical, antioxidant, and heavy metal contents reveals different perspectives on using *Ulva lactuca* from Nusa Lembongan, Bali, as a food component. Instead of acting as isolated traits, these properties work together to define its nutritional value, functional capabilities, and fitness for use in food products. Specifically, the makeup of its cell wall polysaccharides makes it a possible source of dietary fiber, and its extractable phytochemicals might offer further functional benefits. Nevertheless, these possible advantages should be weighed against the nutritional deficiencies and food safety issues linked to eating marine-sourced biomass.

The nutritional profile of *U. lactuca* indicates that its primary value is in providing dietary fiber, rather than serving as a typical source of protein or energy. The prevalence of structural carbohydrates aligns with the intricate polysaccharide makeup of the *Ulva* cell wall, specifically consisting of ulvan, cellulose, and hemicellulose [7, 27]. These elements form a structural foundation for the high fiber levels and could present chances to create fiber-rich food products. However, one cannot judge the nutritional value of these polysaccharides based only on their overall amount, since their physiological effects might be influenced by molecular structure, solubility, viscosity, and fermentability [28, 29]. Likewise, the protein content necessitates additional assessment, as traditional nitrogen-based methods might exaggerate true protein levels in seaweed because of non-protein nitrogen [25]. Therefore, accurately determining the nutritional potential of *U. lactuca* requires analyzing its amino acid profile, polysaccharide fractions, and digestibility instead of relying solely on proximate composition.

Aside from its nutritional value, the phytochemical composition indicates that *U. lactuca* might also offer extractable substances with possible functional benefits. The presence of flavonoids and tannins, alongside the quantifiable phenolic and flavonoid levels, offers a credible chemical explanation for the antioxidant properties found in the ethanolic extract. Nonetheless, the connection among these traits is still tentative, as colorimetric assays offer general approximations instead of measurements for specific compounds, and qualitative phytochemical screening is unable to determine the identity or concentration of particular metabolites. While the LC-HRMS data provide further insight into the extract's chemical makeup, the provisional nature of the annotations constrains their interpretation. Specifically, the potential for contamination from lab materials and the unforeseen annotation of 6-shogaol underscore the necessity of proper analytical controls and structural verification. As a result, the recorded antioxidant activity has not yet been linked to particular metabolites; therefore, additional focused investigation is required to determine which specific components are primarily responsible for the extract's chemical characteristics.

The extract's antioxidant capacity could provide further prospects for food uses, especially in instances where natural antioxidant components are preferred. However, the potent DPPH radical-scavenging activity must be viewed through the lens of the assay's constraints, as it measures chemical radical-neutralizing potential in a controlled in vitro setting instead of true physiological antioxidant efficacy. In addition, evaluating phenolic compounds through extraction methods may not accurately reflect how available or stable they are inside a food matrix or after undergoing gastrointestinal digestion. When developing functional foods based on *U. lactuca*, it is crucial to differentiate between chemical antioxidant capacity and

biological activity. To better assess their potential nutritional importance, it would be beneficial to use complementary antioxidant assays, characterize specific phenolic compounds, and study how their stability and bioaccessibility change during digestion.

Ensuring food safety is just as critical a factor when evaluating whether *U. lactuca* is practically suitable for human consumption. Even though the recorded heavy metal levels met the relevant Indonesian regulatory standards, the slim safety margin for arsenic deserves special consideration. This point is particularly significant since the ability of seaweed cell wall polysaccharides to capture and concentrate environmental substances, a trait that makes them valuable ecological biomonitors, may also affect their levels of contaminants [4, 5, 31]. Furthermore, the toxic effects of arsenic are heavily influenced by its chemical form; therefore, measuring only total arsenic levels is inadequate for determining true nutritional risks [1, 30]. Consequently, the possible nutritional and functional benefits of *U. lactuca* must be weighed alongside its profile of contaminants. To prove it is suitable for food use, one must look beyond regulatory adherence to include arsenic speciation, fluctuations caused by harvest timing and location, and possible human exposure during actual consumption.

Regarding practical applications, these results indicate that *U. lactuca* might be best studied first as a source of dietary fiber for food production, while its extractable phytochemicals offer a further subject for potential investigation. This method would permit the study of its nutritional value while avoiding the early assignment of unconfirmed health advantages to its antioxidant components. Nevertheless, effectively integrating seaweed biomass into food items relies on more than just its chemical makeup; it also involves technical traits like solubility, water-holding capacity, texture, flavor, and how well it is accepted by consumers. Additionally, how stable and bioaccessible its possible beneficial components are throughout food processing and digestion stays a key point of interest. Whether the nutritional and phytochemical profiles identified in this research can be applied to real-world food products will depend on these factors, alongside the requirement for stable contaminant levels.

In general, these results provide an initial scientific foundation for viewing *U. lactuca* from Nusa Lembongan as a possible origin of dietary fiber and extractable antioxidant components. However, utilizing it as a food ingredient in practice necessitates a comprehensive evaluation that goes further than just examining its composition. Upcoming research should focus on detailing polysaccharide structure and function, assessing protein quality and mineral content, verifying metabolite identities with suitable analytical controls, and measuring antioxidant activity using diverse assays and bioaccessibility tests. It is also vital to monitor arsenic speciation across various harvesting sites and seasons to verify the biomass's safety and consistency. Combining these aspects of nutrition, function, technology, and safety will create a stronger foundation for assessing how well *U. lactuca* can be utilized to create fiber-rich and possibly functional food products.

4. CONCLUSION

Ulva lactuca collected from Nusa Lembongan, Bali, exhibited promising nutritional characteristics, particularly its high carbohydrate (59.01%) and total dietary fiber (57.08%) contents, predominantly comprising the insoluble fraction. It also contained a relatively high crude protein content (19.20%) and a low lipid content (0.47%). Phytochemical screening of the ethanolic extract indicated the presence of flavonoids, tannins, steroids, and triterpenoids, while quantitative analysis revealed a total phenolic content of 81.87 mg GAE g^{-1} and DPPH radical-scavenging activity (IC_{50} = 3.93 μg mL^{-1}). The concentrations of Hg, Pb, Cd, and As were below the maximum limits established by SNI 2690:2018, although the relatively high proportion of the permitted arsenic limit warrants particular attention. Collectively, these findings highlight the potential of *U. lactuca* as a source of dietary fiber and antioxidant-containing extracts for food-related applications. However, its suitability for food development should be evaluated in conjunction with its nutritional quality and contaminant profile.

Further research is needed to establish the consistency and practical value of these properties across different harvesting seasons and locations. In particular, arsenic speciation is necessary to provide a more meaningful assessment of food safety, while detailed characterization of amino acids, minerals, and sulfates would strengthen the evaluation of nutritional quality. Confirmation of the tentatively identified metabolites using appropriate analytical standards and blanks, together with complementary antioxidant assays, would also improve the reliability of the phytochemical findings. Ultimately, evaluating the bioaccessibility of relevant constituents through in vitro digestion and assessing the sensory acceptability of prototype food products will be important steps toward determining the feasibility of incorporating *U. lactuca* into food products.

ACKNOWLEDGEMENTS

The authors acknowledge the facilities, scientific, and technical support from the Research Organization of Energy and Manufacture, National Research and Innovation Agency (BRIN). This research was funded by the Degree by Research DBR-BRIN through the Doctor by Research program and the APC fee as supported by the PUTI Q1, Directorate of Funding and Research Ecosystem (DPER), Universitas Indonesia, 2025 (Contract No. PKS-179/UN2.RST/HKP.05.00/2025).

AUTHOR CONTRIBUTIONS

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Irshan Zainuddin: Data Curation, Formal Analysis.

Agus Setiawan: Data Curation, Formal Analysis.,

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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