

Evaluation of Antioxidant and Photoprotective Activities of Extract and Fractions from *Ulva lactuca*

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Abstract

Background. Exposure to sunlight, the main component of which is ultraviolet radiation, can cause various damage to the skin, including sunburn, premature aging, and more serious conditions such as increased symptoms of autoimmune diseases and skin cancer. Therefore, there is currently a growing interest in developing more effective photoprotective agents in protecting the skin from UV exposure as well as antioxidants that can overcome the oxidative stress it causes. This study aims to evaluate the potential photoprotective and antioxidant activities of various *Ulva lactuca* (*U.lactuca*) fractions and determine the relationship between the parameters tested.

Methods. *U. lactuca* samples were extracted using 70% ethanol solvent, then fractionated in stages using *n*-hexane, ethyl acetate, and water solvents. Analysis was carried out to determine the yield, total phenols, photoprotective activity, and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, and ferric reducing antioxidant power (FRAP) methods on the extract and each fraction of *U. lactuca*.

Results. The ethyl acetate fraction showed the highest photoprotective activity with an SPF value of 42.38 ± 0.87 , followed by the ethanol extract (32.60 ± 1.10), aqueous fraction (16.26 ± 0.21), and hexane fraction (9.00 ± 0.17). Consistently, the ethyl acetate fraction also exhibited the strongest antioxidant activity in all assays, with IC₅₀ values of 17.23 ± 0.27 µg/mL in the DPPH assay, 4.15 ± 0.02 µg/mL in the ABTS assay, and 2.15 ± 0.02 µg/mL in the FRAP assay. The ethanol extract showed moderate antioxidant activity with IC₅₀ values of 19.38 ± 0.23 µg/mL (DPPH), 6.31 ± 0.12 µg/mL (ABTS), and 6.88 ± 0.75 µg/mL (FRAP), followed by the aqueous fraction with IC₅₀ values of 27.79 ± 0.88 µg/mL (DPPH), 9.13 ± 0.07 µg/mL (ABTS), and 6.83 ± 0.07 µg/mL (FRAP). In contrast, the hexane fraction exhibited the lowest antioxidant activity, with IC₅₀ values of 38.91 ± 0.43 µg/mL (DPPH), 42.55 ± 0.63 µg/mL (ABTS), and 32.33 ± 0.14 µg/mL (FRAP). Correlation analysis showed a positive relationship between yield, phenolic compound content, antioxidant activity, and SPF value.

Conclusion. Bioactive compounds of *U. lactuca* as a potential source of natural photoprotective and antioxidant ingredients for cosmetic formulations.

Keywords: Antioxidants, Marine bioactives; Sun protection factor; *Ulva lactuca*.

1. Introduction

Exposure of human skin to ultraviolet radiation can trigger a variety of short-term and long-term biological responses (Wu *et al.*, 2024). Common short-term effects include skin redness (erythema), pigmentation changes, and decreased immune function. Meanwhile, prolonged exposure can lead to photocarcinogenesis and premature skin aging (Rubio *et al.*, 2025). These mechanisms are related to changes at the molecular and cellular levels, including DNA damage, increased formation of reactive oxygen species (ROS), release of inflammatory mediators, activation of melanogenesis, and apoptosis (Menakh *et al.*,

2024). Antioxidants play a vital role in maintaining human health. These compounds work by neutralizing ROS produced by both exogenous sources such as UV radiation and endogenous cellular processes (Russo *et al.*, 2008). In recent years, increasing attention has been directed toward natural photoprotective agents derived from biological sources, as these compounds may provide both antioxidant and UV-protective effects with fewer adverse reactions compared to synthetic ingredients (Schneider *et al.*, 2022). As skin cancer cases increase globally each year, dermatologists emphasize the importance of regular sunscreen use to reduce the harmful effects of the sun's ultraviolet radiation (Hanif *et al.*, 2025). The level of sunscreen protection against erythema or sunburn is

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measured using the Sun Protection Factor (SPF). The SPF value represents the ratio of the minimum UVR dose required to cause erythema on protected and unprotected skin under artificial sunlight conditions.

Demand for sunscreen products continues to rise as consumers become more aware of the importance of facial skincare. However, one major issue is the use of synthetic ingredients in many over-the-counter cosmetic products on the market, which can potentially have detrimental effects on skin health. An alternative to address the damage caused by UV radiation is to look for molecules that have antioxidant and photoprotective potential. Therefore, the development of natural sunscreen ingredients is a promising alternative, especially from *Ulva lactuca* which is rich in bioactive compounds with natural photoprotective abilities.

The green seaweed *Ulva lactuca* (Chlorophyta), widely known as “sea lettuce.” These algae have a thin, wavy leaf structure, reaching 20–30 cm in length, and are easily recognized by their bright green color. *U. lactuca* grows in coastal areas, especially in tidal zones, attached to rocks or coral, and is often found in tropical and temperate climates, thus playing an important role in maintaining the balance of aquatic ecosystems. As a photosynthetic organism, *U. lactuca* is an important part of the food chain in marine ecosystems and is known to have various biological activities (Kaur *et al.*, 2025).

U. lactuca contains various commercially valuable compounds that have potential applications in the cosmetics, pharmaceutical, food, chemical and bioenergy industries (Kusmiyati *et al.*, 2020). The composition consists of carbohydrates up to 60%, protein 10–47%, lipid 1–3%, and mineral ash 7–38% (Ambarsari *et al.*, 2018). The main bioactive compounds that have industrial value include phenolics, pigments (chlorophyll and carotenoids), and polysaccharides (Merdekawati *et al.*, 2019). Recent studies have shown that *U. lactuca* has antioxidant, antimicrobial, and anticancer activities (Pradhan *et al.*, 2023). Therefore, *U. lactuca* is increasingly receiving attention both from a health perspective and for its industrial potential (Putra *et al.*, 2024). Several studies have reported the antioxidant potential of *U. lactuca* extracts. Methanolic extracts were shown to exhibit strong DPPH radical scavenging activity, with an IC₅₀ value of 0.45 mg/mL (Korkmaz, 2025). In addition, ethanol extracts demonstrated antioxidant activity with IC₅₀ values of 0.88 mg/mL in the DPPH assay and 1.12 mg/mL in the ABTS assay, along with a total phenolic content of 20.5 mg GAE/g (Putu *et al.*, 2024). However, most previous investigations have primarily focused on single crude extracts, with limited attention given to solvent-partitioned fractions and their association with photoprotective efficacy. Therefore, systematic evaluation of different solvent fractions and their relationship with both antioxidant capacity and photoprotective activity remains limited. The aim of this study was to evaluate the potential photoprotective and antioxidant activities of various fractions of *U. lactuca* obtained from 70% ethanol extract, and to determine the relationship between the parameters tested to assess the potential of this alga as a source of natural active ingredients for sunscreen formulations and skin care products.

2. Methods

2.1. Material

U. lactuca L. samples were obtained from Teluk Kode, West Pemenang Village, Pemenang District, North Lombok Regency, Indonesia with coordinate points 8°26'27.6"S 116°6'7.2"E. The samples were then placed in plastic containers filled with sterile seawater and stored in a coolbox to maintain the freshness (Kusmita *et al.*, 2021; Nugraheni *et al.*, 2010). Botanical determination was performed to ensure the authenticity of the materials used in this study. The determination process was carried out at the Indonesian Institute of Sciences (LIPI), Marine Bioindustry Center, North Lombok Regency, and the results were stored as authentic specimens with collection number B-164/III/DI.02/9/2021.

After collection, the samples were washed with seawater to remove epiphytes, sand, and dirt, then rinsed with distilled water. The cleaned samples were air-dried in a shaded area at room temperature, then dried in a circulating air oven at 40°C for 72 hours. The dried material was then ground into a fine powder and stored in a tightly closed container for further extraction and analysis.

The chemicals used were 100% ethanol (Merck), *n*-hexane (Merck), ethyl acetate (Merck), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), FeCl₃, ammonium acetate; K₂S₂O₈, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); ascorbic acid, trolox; dimethyl sulfoxide (Merck).

2.2. Extraction of *U. lactuca*

500 grams of dried *U. lactuca* powder was weighed and extracted using 70% ethanol, each volume of 5 L. The method used was maceration for 72 hours with occasional stirring. The filtrate was filtered using Whatman No. 1 filter paper and evaporated with a rotary evaporator at 40–50°C until a thick extract was obtained.

2.3. Preparation of *U. lactuca* Fraction

The dried resulting extract was then fractionated using the liquid-liquid partitioning method with solvents of increasing polarity (*n*-hexane, ethyl acetate, and water) according to the protocol described by Mfopa (Mfopa *et al.*, 2017). In brief, 50 g of crude extract was dissolved in 100 mL of distilled water (water fraction) and placed in a separating funnel. 100 mL of *n*-hexane was then added, the mixture was shaken vigorously for 3 minutes, then left for 5 minutes until two layers were formed. The water fraction (lower layer) and the *n*-hexane fraction (upper layer) were separated and collected separately. The water fraction obtained was then fractionated again with ethyl acetate (1:1) to obtain the ethyl acetate fraction and the water fraction.

2.4. Determination of Total Phenolic Content (TPC)

The total phenolic content in *U. lactuca* extract was measured spectrophotometrically using the Folin-Ciocalteu reagent, as described by Mutmainah *et al.* (2019). Specifically, 7.9 mL of distilled water and 0.1 mL of sample were placed in a glass vial and homogenized by vortexing. 0.5 mL of Folin-Ciocalteu reagent was added and homogenized again, followed by the addition of 1.5 mL of 20% (w/v) Na₂CO₃ solution. The final mixture was

then incubated for 30 minutes in a water bath at 40 °C, and the absorbance was measured at 765 nm using a UV-VIS spectrophotometer (SHIMADZU), then compared with the gallic acid calibration curve. Total phenol value was expressed as gallic acid equivalents (mg/g dry weight). Measurements were carried out three times (triplicate).

2.5. Photoprotection Activity Test

The Sun Protection Factor (SPF) value of *U. lactuca* extract was determined using a Shimadzu UV-Vis spectrophotometer (Aparicio-álvarez *et al.*, 2023). The extract and its fraction were dissolved separately in ethanol at a concentration of 100 mg/mL and their absorbance was measured at a UV-B wavelength (290–320 nm) at 5 nm intervals. Ethanol p.a. (pro analysis grade) was used as a blank solution. The SPF value was calculated using the equation:

$$SPF = CF \times \sum_{\lambda=290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where CF represents the correction factor, EE (λ) denotes the erythral effectiveness of radiation at a specific wavelength λ , I(λ) is the solar intensity spectrum (considered constant), and Abs (λ) corresponds to the absorbance value of the sample at wavelength λ .

2.6. Antioxidant activity test using the DPPH method

Antioxidant activity test used the DPPH method (Pramesti *et al.*, 2025). The test was performed by dissolving the sample in methanol, then making serial dilutions. A 2 mL sample solution was reacted with 2 mL of 0.1 mM DPPH solution and incubated for 30 minutes in the dark. Absorbance was then measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

2.7. Antioxidant activity test using the ABTS method

The radical scavenging potential of *U. lactuca* extracts and fractions against ABTS radicals was determined using a spectrophotometer (Untea *et al.*, 2018). ABTS radicals were produced by mixing two stock solutions, namely 7 mM ABTS and 2.4 mM potassium persulfate, in equal proportions, then incubated in the dark for 12 hours at room temperature. The ABTS⁺ solution that had been formed was then diluted with ethanol until the absorbance value reached 0.70 at a wavelength of 734 nm. A total of 6 mL of ABTS⁺ solution was reacted with 40 μ L of sample, then the absorbance was measured at a wavelength of 734 nm using a UV-Vis spectrophotometer with ethanol as a blank. The ABTS radical scavenging activity of the sample was stated based on the calibration curve.

2.8. Antioxidant activity test using the FRAP method

The ferric reducing antioxidant capacity (FRAP) of *U. lactuca* extract was determined based on the method described by Wijayanti *et al.* with some modifications (Wijayanti *et al.*, 2024). Fresh reaction solution was prepared by mixing 10 mM TPTZ (2,4,6-Tripyridyl-s-

triazine) in 40 mM HCl and 20 mM ferric chloride in 300 mM acetate buffer (pH 3.6) at a ratio of 5:5:25 (v/v), then stored at 37 °C for 0.5 h. A 0.15 g sample was mixed with 2850 μ L of FRAP reagent. The mixture was incubated in the dark for 0.5 h, then the absorbance was measured at a wavelength of 539 nm. Trolox was used as a positive control. The FRAP activity of each sample was determined at different concentrations, and the IC₅₀ value (μ g/mL) was calculated from the concentration–response curve. All tests were performed in triplicate.

2.9. Data analysis

Statistics were calculated using GraphPad Prism version 9.0. Results are presented as mean $\pm$ SD (standard deviation).

3. Results

With the aim of evaluating the effect of the fractionation process on the bioactive potential of *U. lactuca*, this study focused on determining the antioxidant and photoprotective activities of the ethanol extract and the resulting solvent fractions. Furthermore, this study also aimed to determine the relationship between the tested parameters to understand the contribution of each fraction to the overall biological activity of *U. lactuca*.

3.1. Yield

The use of solvents with different levels of polarity aims to extract bioactive compounds from *U. lactuca* based on their polarity properties. The yield of *U. lactuca* extracts and fractions is presented in Table 1.

Table 1. Extraction and fractionation Results of *U. lactuca*

Sample	Average extract weight (g) $\pm$ SD	Average extract yield (%) $\pm$ SD
Ethanol Extract	58.86 $\pm$ 0.88	11.77 $\pm$ 0.18
Hexane Fraction	5.43 $\pm$ 0.69	10.85 $\pm$ 1.38
Ethyl Acetate Fraction	28.28 $\pm$ 0.53	56.55 $\pm$ 1.07
Aqueous Fraction	16.30 $\pm$ 0.98	32.60 $\pm$ 1.95

3.2. Total Phenolic Content (TPC)

Differences in solvent polarity produce extracts with varying photoprotective and antioxidant activities (Bagiana *et al.*, 2025). This is due to the selective ability of each solvent in extracting chemical compounds, especially phenolic compounds that have high antioxidant capacity. In this study, extracts and fractions of *U. lactuca* obtained using three different types of solvents - *n*-hexane, ethyl acetate, and water - were evaluated to assess their effectiveness in extracting bioactive compounds that have the potential to provide natural photoprotective and antioxidant activities (Figure 1).

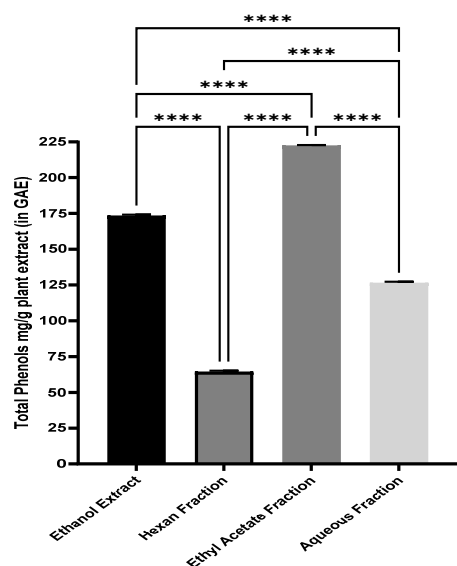


Figure 1. Total amount of plant phenolic compound of extract and fractions of *U. lactuca*. The data were mean $\pm$ SD ($n=3$). The data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$).

3.3. Photoprotection Activity Test

The crude extract, *n*-hexane, ethyl acetate, and water fractions of *U. lactuca* were tested for their Sun Protection Factor (SPF) activity using UV-Vis spectrophotometry (Figure 2).

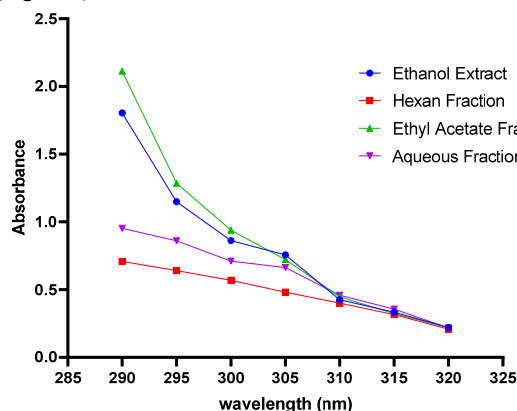


Figure 2. Spectrophotometric absorption spectra (290-320 nm) of ethanol extract, hexane, ethyl acetate, and aqueous fractions of *U. lactuca* (100 mg/mL).

The chemical ability of a compound to absorb UV radiation is greatly influenced by the nature of the solvent used. The photoprotective ability of *U. lactuca* extracts and fractions is expressed in SPF values (Table 2).

Table 2. Sun protection factor values of extract and fractions of *U. lactuca*

Sample	Sun protection factor	Category
Ethanol Extract	32.60 ± 1.10	High protection
Hexane Fraction	9.00 ± 0.17	Minimal protection
Ethyl Acetate Fraction	42.38 ± 0.87	High protection
Aqueous Fraction	16.26 ± 0.21	Moderate protection

3.4. Antioxidant test

The antioxidant activity of *U. lactuca*, including ethanol extract and *n*-hexane, ethyl acetate, and water fractions, was evaluated using three assay methods: DPPH (Figure 3), ABTS (Figure 4), and FRAP (Figure 5). Ascorbic acid was used as a positive control for the DPPH method, while Trolox was used as a positive control for the ABTS and FRAP methods.

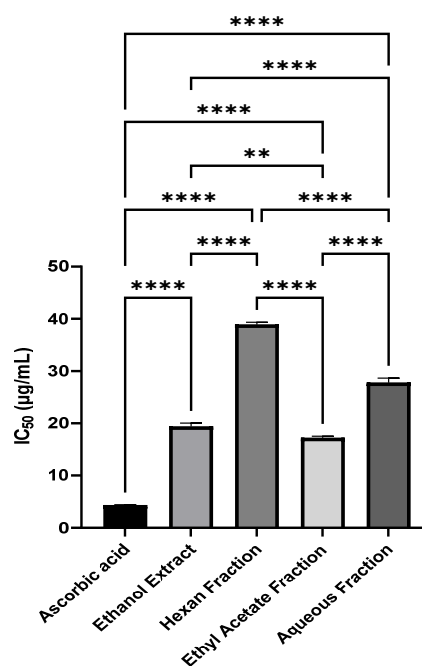


Figure 3. Free radical scavenging activity results of extract and fractions of *U. lactuca* against DPPH. The data were mean $\pm$ SD ($n=3$). The data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$).

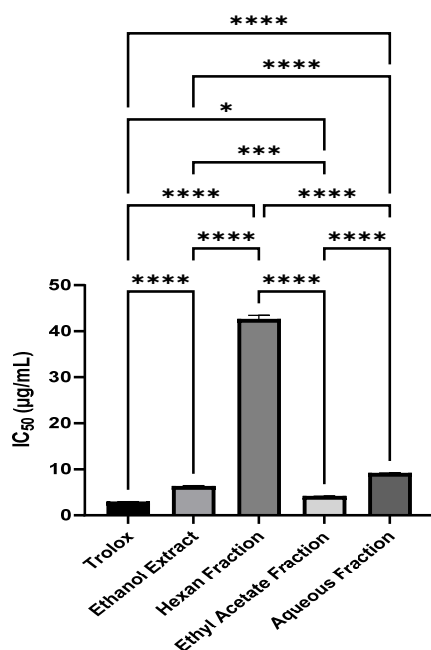


Figure 4. Free radical scavenging activity results of extract and fractions of *U. lactuca* against ABTS. The data were mean $\pm$ SD (n =3). The data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's test (*p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001).

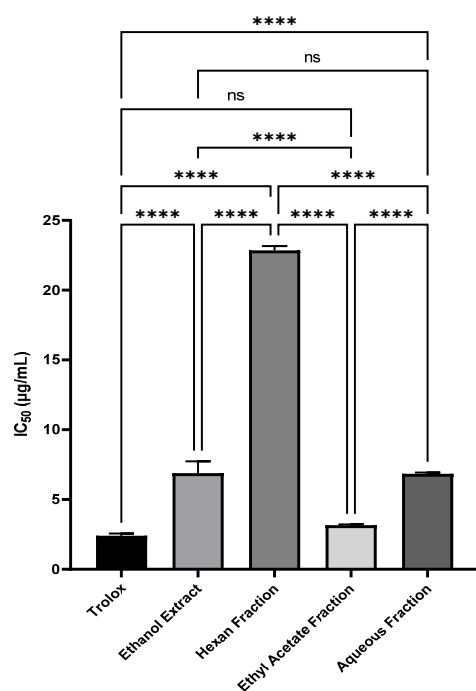


Figure 5. Free radical scavenging activity results of extract and fractions of *U. lactuca* against FRAP. The data were mean $\pm$ SD (n =3). The data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's test (ns= not significant; *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001).

3.5. Statistical study

To evaluate the strength and direction of the relationship between the variables studied, a correlation analysis was conducted using the Pearson linear correlation coefficient test. This analysis produced the correlation coefficient (R^2) values presented in Table 3.

Table 3. Pearson correlation coefficients among yield, total phenolic compound, SPF values and antioxidant capacities of fractions of *U. lactuca*.

Parameters	Parameters					
	Yield	TPC	SPF	DPPH	ABTS	FRAP
Yield	1.00	1.00	0.96	-1.00	-0.91	-0.93
TPC	1.00	1.00	0.98	-0.99	-0.86	-0.89
SPF	0.96	0.98	1.00	-0.95	-0.75	-0.79
DPPH	-1.00	-0.99	-0.95	1.00	0.93	0.95
ABTS	-0.91	-0.86	-0.75	0.93	1.00	1.00
FRAP	-0.93	-0.89	-0.79	0.95	1.00	1.00

4. Discussion

In this study, *U. lactuca* was selected as a potential source of natural compounds with photoprotective and antioxidant activities that can be utilized in the field of dermocosmetics, particularly as active ingredients in sunscreen formulations. The yield of *U. lactuca* fractions varied depending on the type of solvent used (Table 1). Ethanol extraction produced a yield of $11.77 \pm 0.18\%$, demonstrating the effectiveness of ethanol as a solvent capable of extracting active compounds of various polarity levels. The fractionation results showed that the ethyl acetate fraction had the highest yield followed by the water fraction and the *n*-hexane fraction. The high yield of the ethyl acetate fraction indicates the dominance of semi-polar compounds, such as flavonoids and phenolic compounds, which have the potential to provide the main biological activity (Zaatout *et al.*, 2019). Conversely, the low yield of the *n*-hexane fraction indicates that it contains relatively few nonpolar compounds. Overall, this distribution pattern reflects the diversity of secondary metabolites based on the polarity of the solvent used.

The extraction procedure and the type of solvent used play an important role in dissolving endogenous compounds found in plants (Siddhuraju & Becker, 2003). Plant components can be polar or non-polar. Phenolic compounds are more soluble in polar organic solvents due to the presence of hydroxyl groups (Alara *et al.*, 2021). Figure 2 shows the total phenol content (expressed in mg GAE/g plant extract) of the ethanol extract and three fractions of *U. lactuca* obtained using solvents with different polarities, namely *n*-hexane, ethyl acetate, and water. The analysis results showed a significant difference (****, p < 0.0001) between all groups. The ethyl acetate fraction had the highest total phenol content (~220 mg GAE/g), followed by the ethanol extract (~175 mg GAE/g), the water fraction (~125 mg GAE/g), and the *n*-hexane fraction (~65 mg GAE/g). The ethanol extract also showed high phenol content due to its amphiphilic nature, capable of dissolving both polar and semi-polar compounds. Ethanol extraction of natural products is effective because it has a wide solubility range as a neutral solvent, capable of extracting various compounds, including water-soluble hydrophilic components and lipophilic components such as fats (Lee *et al.*, 2024). The high phenol content in the ethyl acetate fraction indicates that most of the phenolic compounds in *U. lactuca* are semi-polar, making them more easily extracted with ethyl

acetate. In contrast, the non-polar *n*-hexane fraction is only able to dissolve a small amount of phenolic compounds.

The photoprotective activity of *U. lactuca* extracts and fractions was evaluated by calculating the Sun Protection Factor (SPF) value using a spectrophotometric method. Figure 3 shows the spectrophotometric absorption profiles of *U. lactuca* extracts and fractions. It was observed that both the extracts and fractions displayed characteristic absorption bands in the UVB region, indicating a potential photoprotective effect. Measurements were made in the UV-B region, which is the region with the highest intensity during the day and the longest exposure to human skin (Putri *et al.*, 2025).

Based on the SPF value classification, the SPF range of 2–12 is included in the minimal protection category, SPF 12–30 is included in moderate protection, and SPF ≥ 30 is categorized as high protection (Mansur *et al.*, 1986). The results in Figure 3 show that the ethyl acetate fraction has the highest SPF value (42.38 ± 0.87), followed by the ethanol extract (32.60 ± 1.10), both of which are included in the category of high protection against UV radiation. These values indicate that both samples have the potential as candidates for natural sunscreen active ingredients. Meanwhile, the water fraction shows an SPF value of 16.26 ± 0.21 which is included in the category of moderate protection, while the *n*-hexane fraction has the lowest SPF value (9.00 ± 0.17) and is included in the category of minimal protection. This difference in SPF values is likely caused by variations in the content of active compounds that play an important role in photoprotective activity through their ability to absorb UV radiation and neutralize free radicals.

Antioxidants are compounds that help the body fight oxidative stress associated with tissue damage and the activity of highly reactive free radicals (Abu-Lubad *et al.*, 2024). Exogenous antioxidant intake from food can effectively enhance the body's natural antioxidant system by stimulating the production of antioxidant enzymes and playing a role in protecting the body from excessive free radical accumulation (Anam *et al.*, 2023). Antioxidant activity strength is generally categorized as very strong (<50 ppm), strong (50–100 ppm), moderate (101–150 ppm), and weak (>150 ppm). Based on this classification, MKE's antioxidant activity is included in the very strong category because it has an IC_{50} value of <50 mg/L (Flieger *et al.*, 2021). In this study, extracts and fractions of *U. lactuca* were used as research subjects whose antioxidant activity will be determined through in vitro free radical scavenging methods using several tests, namely DPPH, ABTS and FRAP.

The antioxidant activity of *U. lactuca* extracts, including ethanol extract, *n*-hexane fraction, ethyl acetate, and water, was tested using the DPPH free radical scavenging method. Ascorbic acid was used as a positive control. The DPPH method is based on the ability of antioxidant compounds to donate hydrogen atoms or electrons to stabilize DPPH free radicals (Nuraini *et al.*, 2025). This reaction causes a decrease in the absorbance value accompanied by a change in the color of the solution from dark purple to light yellow or pale purple, which can then be measured quantitatively using a spectrophotometer (Tue *et al.*, 2024).

The results of the antioxidant activity test of *U. lactuca* extracts and fractions using the DPPH method showed

variations in potential influenced by the type of solvent used (Figure 4). Ascorbic acid as a positive control showed very strong antioxidant activity with an IC_{50} value of 4.34 ± 0.05 $\mu\text{g/mL}$. Among the test samples, the ethyl acetate fraction showed the highest antioxidant activity with an IC_{50} value of 17.23 ± 0.27 $\mu\text{g/mL}$, followed by the ethanol extract (19.38 ± 0.23 $\mu\text{g/mL}$), the water fraction (27.79 ± 0.88 $\mu\text{g/mL}$), and the *n*-hexane fraction (38.91 ± 0.43 $\mu\text{g/mL}$). Statistical analysis showed that all extracts and fractions had significant differences in IC_{50} values compared to ascorbic acid. In addition, significant differences were also observed between fractions (Figure 4), which indicates that solvent variations affect the antioxidant capacity of *U. lactuca* fractions.

The results of the antioxidant activity test of *U. lactuca* extracts and fractions using the ABTS method showed varying abilities in neutralizing ABTS⁺ radicals (Figure 5). Trolox was used as a positive control and showed the highest activity with an IC_{50} value of 2.94 ± 0.02 $\mu\text{g/mL}$. Among the test samples, the ethyl acetate fraction had the strongest antioxidant activity ($IC_{50} = 4.15 \pm 0.02$ $\mu\text{g/mL}$), followed by the ethanol extract (6.31 ± 0.12 $\mu\text{g/mL}$), the water fraction (9.13 ± 0.07 $\mu\text{g/mL}$), and the *n*-hexane fraction (42.55 ± 0.63 $\mu\text{g/mL}$). Based on the classification of antioxidant potential, all extracts and fractions except the *n*-hexane fraction are classified as having very strong antioxidant activity ($IC_{50} < 50$ $\mu\text{g/mL}$). The ethyl acetate fraction showed an ability close to the positive control, indicating that the active compounds contained therein have high effectiveness in neutralizing ABTS⁺ radicals. Statistical analysis using one-way ANOVA followed by Tukey's test showed a significant difference between samples ($****p < 0.0001$). The strong ABTS radical scavenging activity in the ethyl acetate fraction indicates that compounds with medium polarity play a dominant role in the antioxidant activity of *U. lactuca*.

The antioxidant activity test of *U. lactuca* using the FRAP method showed variations in the Fe^{3+} ion reduction ability between fractions (Figure 5). Trolox as a positive control had the highest activity ($IC_{50} = 0.23 \pm 0.02$ $\mu\text{g/mL}$). Among the samples, the ethyl acetate fraction showed the strongest activity ($IC_{50} = 2.15 \pm 0.02$ $\mu\text{g/mL}$), followed by the water fraction (6.83 ± 0.07 $\mu\text{g/mL}$) and the ethanol extract (6.88 ± 0.75 $\mu\text{g/mL}$). The *n*-hexane fraction had the lowest activity ($IC_{50} = 32.33 \pm 0.14$ $\mu\text{g/mL}$), but was still considered strong. These results indicate that the dominant active compounds in *U. lactuca* are polar to semi-polar, such as flavonoids and phenolics, which play a major role in the electron transfer mechanism. Statistical analysis using one-way ANOVA followed by Tukey's test showed significant differences ($****p < 0.0001$) between most samples, except between the ethanol extract and the water fraction (ns) which showed similar activity. The antioxidant activity of the ethyl acetate fraction and Trolox as a positive control was also not significantly different, indicating comparable radical scavenging abilities. The high FRAP reduction activity in the ethyl acetate fraction indicates that *U. lactuca* contains compounds with moderate polarity that contribute predominantly to its antioxidant capacity. The photoprotective and antioxidant effects of *U. lactuca* can be attributed to the presence of specific phenolic acids such as gallic acid and vanillic acid, which act through electron and hydrogen donation mechanisms to neutralize free radicals, as well as through

UV absorption. Total phenolics, flavonoids, and saponins extracted by subcritical water hydrolysis have been shown to enhance radical scavenging and reducing power, which supports the observed high SPF and antioxidant activities. Additionally, sulfated polysaccharides such as ulvan may contribute synergistically to protection against UV-induced oxidative damage (Pangestuti *et al.*, 2021).

Pearson correlation analysis showed a very strong relationship between the parameters tested in the *U. lactuca* fraction. The correlation matrix between the extract yield, total phenols (TPC), SPF value, and antioxidant parameters (DPPH, ABTS, and FRAP) showed a strong relationship between these variables (Table 3). The Pearson correlation coefficient (r), which ranges from -1 to $+1$, was used to evaluate the strength and direction of these relationships, where positive values indicate direct correlations and negative values indicate inverse correlations. A significant positive correlation was found between the yield, TPC, and SPF ($r = 0.96$ – 1.00), indicating that the higher the extract yield and the concentration of phenolic compounds, the higher the photoprotective activity was. This relationship indicates that phenolic compounds play an important role in absorbing UV rays and providing a protective effect, thus contributing to the SPF value of the *U. lactuca* extract.

In contrast, a strong negative correlation was recorded between TPC and antioxidant parameters (DPPH, ABTS, and FRAP) with r values ranging from -0.75 to -1.00 . This inverse relationship reflects the fact that antioxidant capacity is expressed as IC_{50} values, where lower IC_{50} values correspond to stronger radical scavenging activity. This inverse relationship arises because lower IC_{50} values in antioxidant assays indicate stronger radical scavenging activity. Thus, extracts rich in phenolic compounds exhibit higher antioxidant capacity. Among the three antioxidant assays, the DPPH assay showed the strongest negative correlation with TPC ($r = -0.99$) and yield ($r = -1.00$), confirming the sensitivity of this method in detecting phenolic-based antioxidant potential. In addition, high positive correlations were also found between antioxidant methods (DPPH vs. ABTS = 0.93 ; DPPH vs. FRAP = 0.95 ; ABTS vs. FRAP = 1.00), indicating that all three assays consistently measure the redox capacity of phenolic compounds in *U. lactuca*. The consistency of the correlation between these tests strengthens the reliability of the research results and confirms the dominant contribution of phenolic compounds to antioxidant and photoprotective activities.

The evaluation of the photoprotective and antioxidant activities of *U. lactuca* in this study provides strong evidence for its potential as a potential UV filter and natural antioxidant source. The results showed that the polarity of the extraction solvent significantly influenced the photoprotective activity and antioxidant effectiveness. The results of the activity test showed a consistent pattern, where the ethyl acetate fraction of *U. lactuca* had the highest activity, followed by the ethanol extract and the water fraction, while the *n*-hexane fraction showed the lowest activity. This solvent-dependent pattern is in line with the basic principles of phytochemistry, where solvents with medium polarity are able to optimally extract various secondary metabolites, such as phenolic acids, flavonoids, and their derivatives in the form of glycosides (Hidayati *et al.*, 2020).

5. Conclusion

Fractionation using solvents with increasing polarity demonstrated a clear linear trend toward higher extract yield, antioxidant activity, and SPF values. Among the tested solvents, ethyl acetate was identified as the most effective in enhancing both antioxidant capacity and photoprotective activity of *U. lactuca*, indicating the predominance of semi-polar bioactive compounds. These findings highlight the significance of solvent polarity in maximizing the biological potential of *U. lactuca* and support its promising application as a natural source of photoprotective and antioxidant agents in the health and cosmetic industries. The results provide practical implications for the selection of optimal fractionation strategies as a foundation for the isolation and further development of active compounds. However, this study is limited to *in vitro* evaluations; therefore, further studies involving compound identification, formulation development, and *in vivo* or clinical assessments are required to confirm efficacy and safety.

Conflict of Interest

The authors have no conflict of interests regarding this investigation.

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