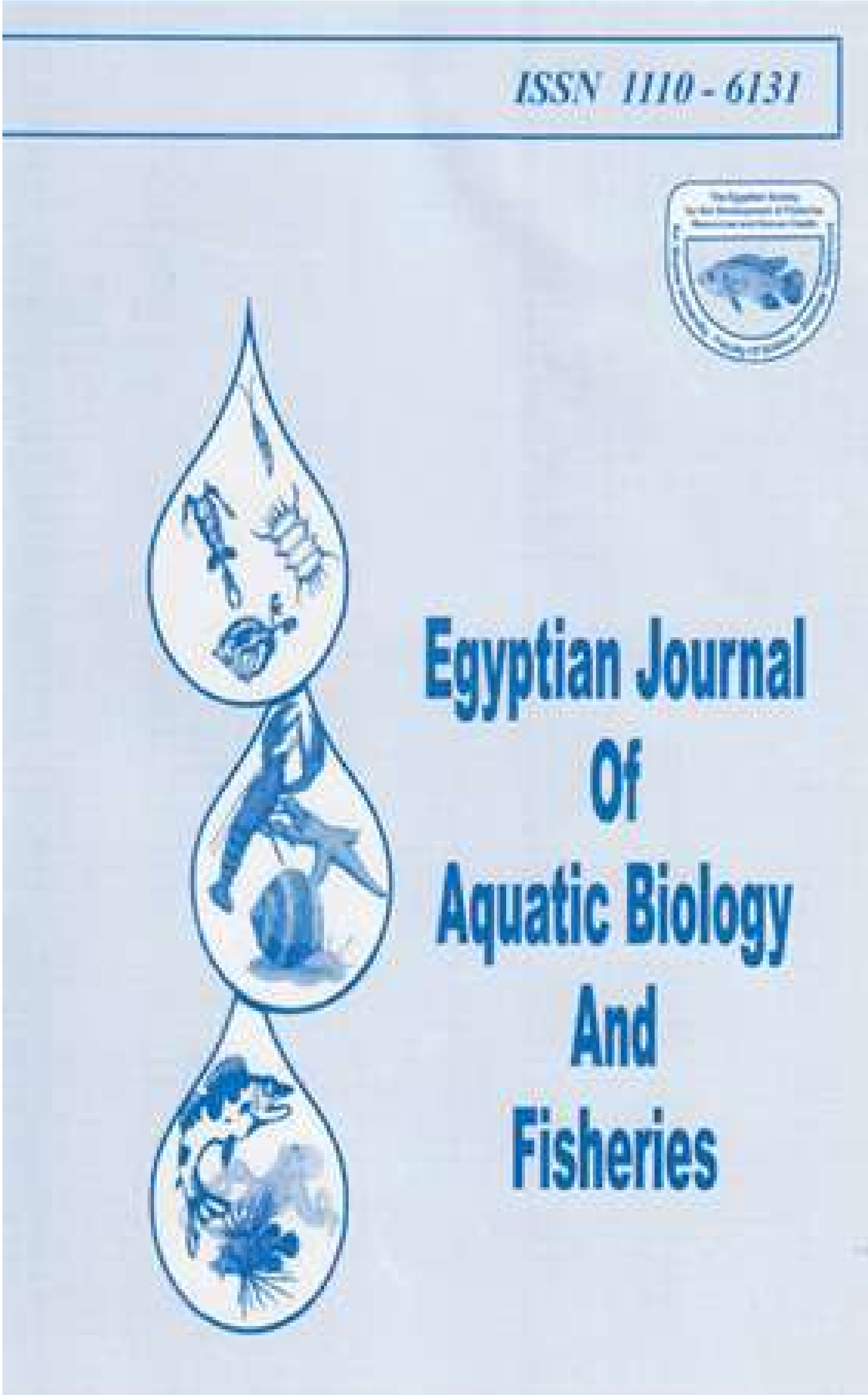

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Bioactivity of Ultrasound-Assisted Ethanolic Extract of *Ulva lactuca*: Antioxidant, Anti-Inflammatory, and Antibacterial Evaluation

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ABSTRACT

Green seaweed contains many bioactive compounds, including phenols, saponins, flavonoids, and terpenoids. These compounds are considered to produce strong antioxidant activity, and it is hoped that they can provide health benefits and play a role in disease prevention. The aim of this study is to evaluate the antioxidant, anti-inflammatory, and antibacterial activities of *Ulva lactuca* ethanolic extract obtained using ultrasound-assisted extraction (UAE). *Ulva lactuca* powder was extracted using UAE with 96% ethanol at a 1:5 ratio in a closed container. The extract was then subjected to phytochemical screening for phenolic, flavonoid, alkaloid, saponin, tannin, and terpenoid compounds. Antioxidant activity was assessed using the DPPH, FRAP, and ABTS methods, with ascorbic acid as the standard. Anti-inflammatory activity was evaluated using the BSA method, with diclofenac sodium as the standard. Antibacterial activity was tested using the agar diffusion method against *E. coli* and *S. aureus*. The yield from ethanol extraction using the UAE method was 16.56%. Phytochemical screening showed that the extract contained phenols, flavonoids, alkaloids, saponins, and terpenoids, while tannins were absent. The IC50 values for antioxidant activity using DPPH, FRAP, and ABTS methods were 52.65 ± 0.46, 47.48 ± 0.52, and 41.17 ± 1.09µg/ mL, respectively. All extract results were significantly different compared to ascorbic acid as the standard. The anti-inflammatory activity of the extract (IC50 = 23.15 ± 1.61µg/ mL) was not significantly different from diclofenac sodium. Higher antibacterial activity against *S. aureus* indicates that the antibacterial compounds in *Ulva lactuca* are more effective against Gram-positive bacteria. The antibacterial activity against both *S. aureus* and *E. coli* was significantly different from K+ (amoxicillin), except for the 20% concentration extract, which did not significantly inhibit *E. coli*. In conclusion, extraction of *Ulva lactuca* using UAE can enhance its antioxidant, anti-inflammatory, and antibacterial activities.

INTRODUCTION

Advances in pharmaceutical science and food technology have driven innovation in functional products based on natural ingredients. Natural products are designed not only as food and beverages but also as delivery vehicles for bioactive compounds with high biological activity, such as antioxidants, anti-inflammatory agents, and antibacterial agents. These three activities are interconnected in maintaining the body's physiological balance. Antioxidants play a role in preventing oxidative stress that can trigger inflammatory responses, while anti-inflammatory activity inhibits the release of inflammatory mediators and suppresses tissue damage caused by excess oxidants **(López-Valverde *et al.*, 2023)**. On the other hand, antibacterial activity serves to prevent microbial infections, which often trigger secondary inflammation. Therefore, the development of herbal remedies containing bioactive compounds with these three activities is expected to provide comprehensive protective effects against oxidative stress, inflammation, and microbial infections simultaneously, while increasing the bioavailability and stability of the active compounds in the body **(Liang *et al.*, 2025)**.

Most compounds that contain antioxidants are found in plants, one of the plants that contains potential antioxidant activity is green seaweed **(Rompas & Gasah, 2022)**. GC–MS analysis of *Ulva lactuca* revealed the fatty acid composition of the samples, showing that palmitic acid, eicosenoic acid, and linoleic acid were the most abundant fatty acids. LC-MS analysis indicated that naringin, rutin, sinapic acid, quercetin, salicylic acid, apigenin, cinnamic acid, flavone, and flavanone were the predominant phenolic compounds. The aqueous extract obtained by maceration showed a total phenolic content of 379.67 ± 0.09 mg GAE/g and a total flavonoid content of 212.11 ± 0.11 mg QE/g **(Ouahabi *et al.*, 2024)**. *Ulva lactuca* collected from Kukup Beach, Gunung Kidul regency, Central Java had 5.17% of fat, 17.43% of protein, 62.93% of carbohydrate, 11.53% of water content, and 2.94% of ash content. Antioxidant test using DPPH found that inhibition concentration (IC_{50}) of *Ulva lactuca* was 88890.55 ppm (weak category). *Ulva lactuca* contains chlorophyll a, b, and c, neoxanthin, anteraxanthin, dinoxanthin, flavoxanthin, micronone, and vaucheriaxanthin **(Franzisca da Costa *et al.*, 2018)**. Ethanol extract *Ulva lactuca* obtained by maceration has antioxidant activity with a percentage value of 51.63% at a concentration of 100 mg/L with DPPH method **(Aprilia *et al.*, 2019)**. Recent antioxidant research shows that methanolic extract *Ulva lactuca* obtained by maceration also exhibited an impressive ability to scavenge DPPH radicals, as indicated by its IC_{50} value of 0.095 ± 0.12mg/ mL, while the methanolic extract obtained using the soxhlet method demonstrated antioxidant properties by preventing *β*-carotene discoloration, with an IC_{50} of 0.087 ± 0.14mg/ mL **(Ouahabi *et al.*, 2024)**. Hydrocolloid sulfate from *Ulva lactuca*, better known as ulvan, has also been identified as a weak antioxidant with an IC_{50} value of 469 ppm **(Jacoeb *et al.*, 2024)**. Chlorophyll extraction on *Ulva* sp. was performed using the ultrasound assisted extraction (UAE) method with acetone solvent and obtained antioxidant inhibition of 45.32% with the DPPH method **(Humaidi *et al.*, 2024)**. The role of ultrasound assisted extraction (UAE) is also proven in optimizing the function of ulvan hydrocolloids for food applications **(Istiqlaal *et al.*, 2025)**.

Ulva lactuca has also been reported to have antibacterial activity. Ethanolic 96% extract of *Ulva lactuca* obtained by maceration was able to inhibit the growth of *Staphylococcus aureus* bacteria with strong categories at concentrations of 20, 40, and 60% **(Emelda *et al.*, 2021)**. Furthermore, this extract has antibacterial activity against three different bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) with an MIC at 12.5% **(Panjaitan *et al.*, 2022)**. Methanol and ethyl acetate extracts also have potential as antibacterial agents for *Pseudomonas aeruginosa* and *Staphylococcus aureus* **(Lembang, 2023)**. Various active compounds in the *Ulva lactuca* extract are thought to have multiple antibacterial and anti-inflammatory properties that can overcome the MRSA antimicrobial resistance and accelerate tissue growth in the wound healing process **(Ardita *et al.,* 2021)**. Methanolic extract also has the power to aid antibiotics in reducing the growth of pathogenic *Klebsiella pneumoniae* **(EL-Sayed *et al.*, 2023)**. However, *Ulva lactuca* ethanolic extract did not show the ability to inhibit *Streptococcus agalactiae* bacteria **(Mubayyinah *et al.*, 2024)**.

In silico prediction of ulvan using the protein structure database cyclooxygenase-2 enzyme (4COX) showed a best binding affinity energy of gluconic acid ulvan -7.62 kcal/mol similar to the control drug diclofenac sodium (-7.81 kcal/mol), followed by iduronic acid ulvan -7.57 kcal/mol, fucoidan (-6.11 kcal/mol), alpha carrageenan (-6.93 kcal/mol), and lambda carrageenan (-5.38 kcal/mol) **(Utami *et al.*, 2023)**. The anti-inflammatory activity of each extract/fraction was assessed by the human red blood corpuscles (HRBCs) membrane stabilizing method on methanol extract, light petroleum fraction, dichlormethane, ethyl acetate, butanol, and water, showing hemolysis-prevention (%). The highest hemolysis-prevention was found in the light petroleum fraction (29%) and the lowest was found in the ethyl acetate fraction (12%) **(Zaatout *et al.*, 2019)**. *Ulva lactuca* as a food source and medicinal ingredient has never been subjected to ultrasound assisted extraction (UAE) extraction to test antioxidant activity using methods other than DPPH, antibacterial, and anti-inflammatory with the BSA (Bovine serum albumin) method.

MATERIALS AND METHODS

Collection and preparation of samples

Ulva lactuca were collected from Krakal sea Gunung Kidul, Yogyakarta, Indonesia, with coordinate points S8°8'43" E110°35'59". The *Ulva lactuca* samples were gathered at sea level and rinsed with sterilized seawater on-site to remove any sand and sediments. The samples were then placed in a cool box and transported to the laboratory for further processing **(Nugraheni *et al.*, 2010; Sabdaningsih *et al.*, 2017)**.

Extraction with ultrasound-assisted extraction (UAE)

One hundred grams of *Ulva lactuca* powder was carefully weighed. The *Ulva lactuca* powder was extracted using ultrasonic-assisted extraction (UAE) using 96% ethanol solvent (smartlab, Indonesia) at a ratio of 1:5 in a closed container **(Pertiwi *et al.,* 2025)**. The powder was soaked for 60 minutes with occasional stirring in UAE. The extraction process was carried out at 30°C, and the frequency of 20 KHz. Extraction was replicated 5 times. Ethanolic extract was evaporated using a rotary evaporator.

Phytochemical screening

Phytochemical tests were conducted to identify secondary metabolite compounds in ethanol extracts. Phenolic tests were performed by adding 5% $FeCl_3$ (Sigma-Aldrich, USA) which produced a blue-black color **(Jemal *et al.*, 2022)**, while flavonoids were detected by adding Mg powder (Supelco, Germany), concentrated HCl (bratachem, Indonesia), and amyl alcohol (Merck, Germany), which produced a red, yellow, or orange color in the amyl alcohol layer **(Faramayuda *et al.*, 2021)**. Alkaloids were tested using Mayer, Bouchardat, and Dragendorff reagents (Nitrakimia, Indonesia), which produced white, yellow, or brick-red precipitates, respectively **(Dilshad & Batool, 2022; Jemal *et al.*, 2022)**. Tannins were identified by adding 10% NaCl (Brtachem, Indonesia) and 0.5% gelatin (Sigma-Aldrich, USA) solution, which formed a white precipitate **(Bonetti *et al.*, 2020)**. Saponins were identified by the formation of a stable foam after shaking and adding 2N HCl (bratachem, Indonesia) **(Dilshad & Batool, 2022; Jemal *et al.*, 2022)**, while terpenoids and steroids were distinguished using the Libermann-Burchard reagent (Nitrakimia, Indonesia), which produced a brown color for triterpenoids and blue or green for steroids **(Dilshad & Batool, 2022; Jemal *et al.*, 2022)**.

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Antioxidant activity test

a. DPPH method

Extract samples at various concentrations (3mL each) were mixed with 1mL of 0.1 mM DPPH (Sigma Aldrich, USA) solution. The tube was covered with aluminum foil and homogenized. The mixture was incubated for 30 minutes at 37°C, and the absorbance was measured at 517nm using a UV-Vis spectrophotometer (Shimadzu, Japan) **(Wahyuono *et al.,* 2024)**.

b. FRAP method

A total of 10mg of extract was dissolved in 10mL of 96% ethanol. Then, 1mL of the solution was mixed with 1mL of 0.2 M phosphate buffer (pH 6.6) and mL of 1% $K_3Fe(CN)_6$ (Sigma Aldrich, USA), followed by incubation at 50°C for 20 minutes. After incubation, 1mL of TCA (Supelco, Germany) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. One milliliter of the supernatant was pipetted into a test tube, mixed with 1mL of distilled water and 0.5mL of 0.1% $FeCl_3$ (Sigma Aldrich, USA), and left for 50 minutes. Absorbance was measured at 695nm. A blank consisting of oxalate solution was used, and a calibration curve was prepared using ascorbic acid solutions at various concentrations **(Wahyuono *et al.*, 2024)**.

c. ABTS method

A total of 0.1mL of extract was added to 2mL of ABTS (Sigma Aldrich, USA) stock solution and vortexed. The mixture was incubated for 6 minutes, and absorbance was measured at 734nm using a UV-Vis spectrophotometer. Antioxidant activity was expressed as the percentage inhibition of ABTS radicals **(Wahyuono *et al.*, 2024)**.

Anti-inflammatory activity test

To evaluate anti-inflammatory activity, 500µL of each sample, positive control, and negative control solution was added to 0.2% BSA (Sigma Aldrich, USA) in TBS (Sigma Aldrich, USA) to a final volume of 5mL. The mixtures were incubated at 25°C for 30 minutes, then heated at 70°C for 5 minutes, followed by cooling in water for 10 minutes. After cooling, solutions were vortexed, and absorbance was measured at 660nm using a UV-Vis spectrophotometer **(Laksmitawati & Tiffani, 2020)**.

Antibacterial activity test

The antibacterial activity was assessed using the agar diffusion method. Mannitol salt agar (MSA, Merck, Germany) was used for *Staphylococcus aureus* and nutrient agar (NA, Merck, Germany) for *Escherichia coli*. Wells were formed using cylinder cups. Bacterial suspensions adjusted to ½ McFarland standard were added at 10µL per 100mL of media. Each plate contained 10, 15, and 20% *Ulva lactuca* extract, 0.005% amoxicillin (positive control, Kimia Farma, Indonesia), and 4% DMSO (negative control, Merck, Germany), with 50µL pipetted into each well. Plates were incubated at 37°C for 24 hours (Binder, Germany), and the diameter of the inhibition zones was measured **(Pertiwi *et al.*, 2025)**.

Data analysis

All experiments were performed in triplicate. Data are presented as mean ± standard deviation. Statistical analyses were performed using GraphPad Prism (version 9.1.2; GraphPad Inc., San Diego, CA, USA). IC_{50} values represent the concentration of the test sample causing 50% inhibition. Significance levels were defined as $P < 0.05$ (), < 0.005 (), < 0.0005 (), and < 0.0001 (****) **(Suharsanti *et al.*, 2023)**. Two-way ANOVA was used for antioxidant and antibacterial tests, while t-test was applied for the anti-inflammatory test.

RESULTS

1. Extraction and phytochemical screening

The extraction method used in this study was UAE (Ultrasound-assisted extraction). This method was chosen because it is more efficient than conventional methods and can shorten extraction time, reduce the amount of solvent, and increase the yield **(Shen *et al.*, 2023)**. The yield from ethanol extraction using the UAE method was 12.65%. The 96% ethanol used in this study refers to the results of previous research that 96% ethanolic extract of *Ulva Lactuca* obtained by maceration was able to inhibit the growth of *Staphylococcus aureus* bacteria in the strong category **(Emelda *et al.*, 2021)**. The extraction results were then phytochemically screened to determine the compound content in the extract. The results of the phytochemical screening are shown in Table (1).

Table 1. Phytochemical screening of ethanol extract using UAE method

Compound	Literature	Research	Result
Phenolic	dark bluish	dark bluish	Positive
Flavonoids	yellow, red solution in amyl alcohol	Red solution in amyl alcohol	Positive
Tannin	White precipitate	Green solution	Negative
Alkaloid	Mayer: white precipitate Dragendroff: orange precipitate	Mayer: white precipitate Dragendroff: orange precipitate	Positive
Saponin	The solution forms a stable foam	The solution forms a stable foam	Positive
Terpenoid	The solution forms a red or purple color	The solution forms a red or purple color	Positive

Based on Table (1), the ethanol extract of *Ulva lactuca* is positive for containing phenols, flavonoids, alkaloids, saponins, and terpenoids but negative for tannins. Each compound component contained in the *Ulva lactuca* extract has the potential to produce certain biological activities. The biological activities that can be tested in *Ulva lactuca* extracted using the UAE method are antioxidant, anti-inflammatory, and antibacterial.

2. Antioxidant activity

The antioxidant activity of ethanol extract of *Ulva lactuca* using the UAE method was carried out using 3 methods, namely DPPH, FRAP, and ABTS. The results of the antioxidant activity test of ethanol extract of *Ulva lactuca* using the UAE method are illustrated in Fig. (1).

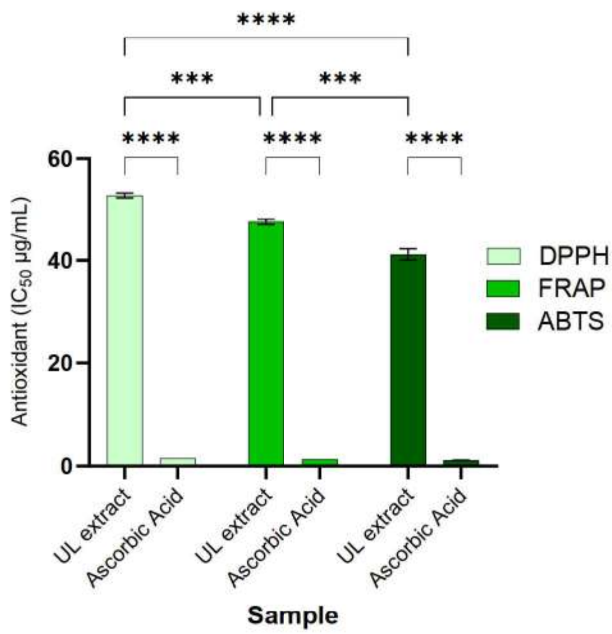

Fig. 1. Antioxidant activity of *Ulva Lactuca* (LC) ethanolic extracted using UAE with DPPH, FRAP, and ABTS method

= significant, *P value* <0.0005 and *= significant, *P value* <0.0001, n=3, analyzed by two way ANOVA).

Based on the analysis of antioxidant activity test data using three methods, significant differences were found in all groups using the two-way ANOVA. Clearly significant differences were only observed between the *Ulva lactuca* extract group and the ascorbic acid standard for each method (DPPH, FRAP, and ABTS).

3. Anti-inflammatory activity

To determine the anti-inflammatory ability of the ethanol extract of *Ulva lactuca*, a protein denaturation method was used to observe the IC_{50} value. The anti-inflammatory effect was tested by adding BSA (Bovine serum albumin), which causes denaturation when heated. The results of the ethanol extract test of *Ulva lactuca* are shown in Fig. (2).

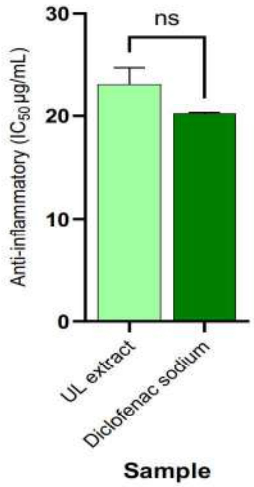

Fig. 2. Anti-inflammatory activity of *Ulva Lactuca* (UL) extract and diclofenac sodium as positive control

ns=no significant, *p value* >0.05, n=3, analyzed by oneway ANOVA.

Based on the analysis of anti-inflammatory activity test data using t-test, there was no difference between the *Ulva lactuca* extract group and the diclofenac sodium standard. This indicates that the extract's effectiveness is equivalent to that of the diclofenac sodium standard.

4. Antibactery activity

Ethanol extract of *Ulva lactuca* using UAE method showed antibacterial activity against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria with inhibition zones formed at various extract concentrations (Figs. 3, 4).

Fig. 3. Antibacterial activity of *Ulva lactuca* against ethanolic extract of (a) *E. coli* and (b) *S. aureus*

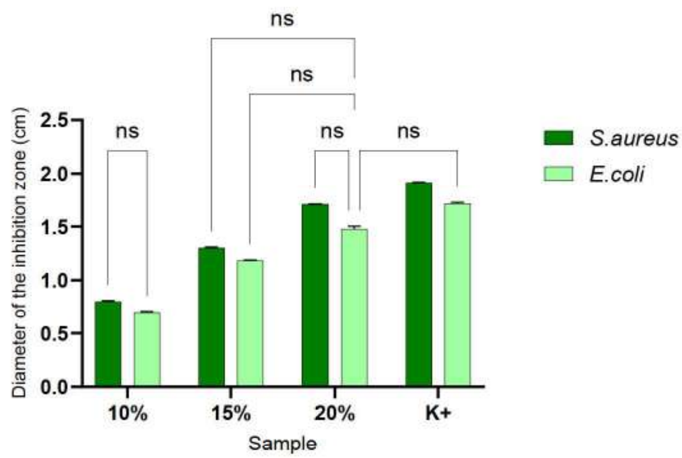

Fig. 4. Diameter of the inhibition zone (cm) against *S.aureus* and *E.coli* compared with K+ (Amoxicilin)

ns = no significant, *P*- value <0.05, n=3, analyzed by two- way ANOVA.

Based on the analysis of antibacterial activity test data processed with two-way ANOVA, all groups showed significant differences except the group marked with ns in Fig. (2). In Fig. (2), ns means that there is no significant difference between the groups. *Ulva lactuca* extract tested on *E. coli* bacteria has no significant difference at a concentration of 20% when compared to the positive control amoxicillin, while the extract when tested on *S. aureus* bacteria has better activity compared to *E. coli*.

DISCUSSION

The yield from ethanol extraction using the UAE method was 12.65%. The yield obtained was remarkably greater than extraction using the maceration method. In terms of maceration extraction, the aqueous extract demonstrated the highest extraction yield at 9.45 ± 0.05%, followed by the methanolic extract at 1.18 ± 0.09%. Furthermore, the methanolic extract obtained through Soxhlet extraction had the highest polyphenolic yield with 4.21 ± 0.04%, followed by the hexanic extract with 2.23 ± 0.07%. The ethyl acetate extract had the lowest yield at 1.34 ± 0.06% **(Ouahabi *et al.*, 2024)**. The UAE method is more optimal in extracting compounds because it is assisted by electromagnetic waves **(Carreira-Casais *et al.*, 2021)**. Screening results showed that the ethanol extract of *Ulva lactuca* using the UAE method contained flavonoids, alkaloids, saponins, and terpenoids. The results of the extract identification are the same as in previous research which were positive for tannin **(Mubayyinah *et al.*, 2024)**, phenolic, flavonoids and terpenoids **(Ouahabi *et al.*, 2024; Pertiwi *et al.*, 2025)**, and alkaloid **(Lailaturramadhini *et al.*, 2025)**. These results are consistent with the study of **Rompas**

and Gasah (2022), which showed that the ethanol extract of *Ulva Lactuca* contained all four compounds. Similarly, **Lailaturramadhini *et al.* (2025)** studied the comparative phytochemical composition of flavonoids, alkaloids, and tannins from Sukabumi and Lombok. The compounds contained in *Ulva lactuca* have the potential to possess bioactivity, such as antioxidant, anti-inflammatory, and antibacterial properties.

The antioxidant activity of ethanol extract of *Ulva Lactuca* using the UAE method was carried out using 3 methods, namely DPPH, FRAP, and ABTS. All three methods used spectrophotometry to measure antioxidant activity based on the principle of radical or reduction reactions. The difference lies in the mechanism of action of the antioxidant. The DPPH method has a hydrogen donor mechanism that will pair with the DPPH radical, causing the loss of purple color and producing a bright yellow color. FRAP has a mechanism where the antioxidant compound will donate electrons to the ferri-tripyridyltriazine ion, thus converting it into a blue ferri-tripyridyltriazine. Meanwhile, the ABTS mechanism is to transfer electrons or hydrogen to the $ABTS^{\cdot+}$ radical, resulting in color fading **(Irianti *et al.*, 2021)**.

The ability of antioxidant activity is seen from the IC_{50} value (the ability of a compound to inhibit by 50%). The IC_{50} value indicates the strength or weakness of antioxidant activity. A compound is said to have a very strong antioxidant if the IC_{50} value is >50 ppm, strong antioxidant activity with an IC_{50} value of around 50-100 ppm, moderate antioxidant activity if the IC_{50} is 100-150 ppm and weak antioxidant activity if the IC_{50} is 151-200 ppm **(Molyneux, 2004; Kedare & Singh, 2011)**. To obtain a comprehensive picture of the antioxidant capacity of a sample, it is recommended to use more than one method because each method measures different aspects of the mechanism of antioxidant activity. Of the three methods, the antioxidant activity of *Ulva Lactuca* ethanol extract has quite high activity in the DPPH IC_{50} method obtained at 52.66±0.47 µg/mL in the strong category, FRAP at 47.49±0.52 µg/mL in the very strong category, and ABTS 41.17±1.09 µg/mL in the very strong category. The DPPH method will produce higher IC_{50} values (low activity) if the compound is not completely soluble or the reaction does not proceed optimally in the organic solvent **(Csepregi *et al.*, 2016)**. When compared with previous research, *Ulva lactuca* extracted with 70% ethanol using the maceration method had an IC_{50} of 46.68 ppm, which was better in activity when compared to the extract produced in this study using the UAE method **(Rompas & Gasah, 2022)**. Whereas in other research, *Ulva lactuca* Algae had antioxidant activity with a percentage value of 51.63% at a concentration of 100 mg/L which showed that the UAE method was better **(Aprilia *et al.*, 2019)** The antioxidant test using DPPH obtained an inhibitory concentration (IC_{50}) of wet *Ulva lactuca* L. extract macerated with methanol of 88890.55 ppm.**(Franzisca da Costa *et al.*, 2018)**. The ABTS method often provides higher antioxidant activity values than DPPH or FRAP, especially for complex extracts or polar-nonpolar mixtures, due to its broader mechanism **(Thaipong *et al.*, 2006; Shah & Modi, 2015)**. Meanwhile, FRAP provides high activity values for compounds with strong reducing ability, but does not consider the ability of compounds that only react through radical scavenging mechanisms **(Kotha *et al.*, 2022)**. In an optimization study of *Ulva lactuca* L. extract using two optimization methods: the response surface method (RSM) and the artificial neural network-genetic algorithm (ANN-GA), the antioxidant activity obtained using the FRAP method was 95.36±1.55 mg Trolox Equi/g and 106.14±0.82 mg Trolox Equi/g, respectively **(Korkmaz, 2025).**

The antioxidant activity of a sample is also influenced by the extraction method. **Arbi *et al.* (2016)** tested the antioxidant activity with DPPH from the ethanol extract of *Ulva lactuca* using the maceration extraction method. The results of the activity test showed that the IC_{50} value obtained was 60.97μg/ mL, a result obtained that was greater. **Ghasemzadeh *et al.* (2015)** found that ultrasound extraction with ethanol-water solvents produced the highest phenolic content and antioxidant activity compared to the maceration method or extraction with a single solvent. On the other hand, the testing method (assay) can also affect the results due to differences in reactivity to the radicals tested.

To determine the anti-inflammatory ability of the ethanol extract of *Ulva lactuca*, a protein denaturation method was used to observe the IC_{50} value. The anti-inflammatory effect was tested by adding BSA (Bovine serum albumin), which causes denaturation when heated. Secondary metabolites that are able to maintain protein stability from denaturation have potential as anti-inflammatory agents. The interaction of BSA with secondary metabolites such as tyrosine, lysine, and threonine can increase the bond between active substances, thus preventing protein denaturation.

Based on the measurement results, the IC_{50} value of the ethanol extract of *Ulva lactuca* was 23.15±1.61 μg/mL and as a control diclofenac sodium had an IC_{50} value of 20.22±0.11 μg/mL. Based on these results, it can be said that the ethanol extract of *Ulva lactuca* with the UAE method has anti-inflammatory activity that is comparable *in vitro*. The anti-inflammatory activity of the ethanol extract is influenced by the secondary metabolite compounds present in the extract. Secondary metabolites have the potential as anti-inflammatories by inhibiting denatured proteins in the body, which can occur because the formation of free radicals causes inflammation by stimulating inflammatory mediators **(Purba *et al.*, 2019)**. Flavonoid compounds can inhibit protein denaturation because the structure of the hydroxyl group and aromatic ring interact with albumin through hydrogen bonds, resulting in a more stable protein structure and less prone to denaturation **(Panche *et al.*, 2016)**. Alkaloids inhibit targets (COX-2 and prostaglandins) and also act on endothelial cells, neutrophils, and leukocytes, reducing inflammation in these cells **(Nurjanah *et al.*, 2020)**. Terpenoids inhibit the production of inflammatory factors nitric oxide and IL-6 induced by lipopolysaccharide **(Yang *et al.*, 2020)**. Meanwhile, diclofenac sodium, a comparator, acts as a COX inhibitor for anti-inflammatory effects, demonstrating its ability to inhibit the formation of prostaglandins, which act as inflammatory mediators. *Ulva lactuca* ethanol extract enhances wound healing properties *in vivo*. These results suggest that bioactive compounds derived from *Ulva lactuca* extract are beneficial for wound healing and anti-inflammatory therapies **(Wang *et al.*, 2025)**.

Ulvan sulfate polysaccharide isolated from *Ulva lactuca* has been widely reported to possess physiologically interrelated antidiabetic and anti-inflammatory activities. Ulvan isolated from *Ulva lactuca* is able to lower blood glucose levels by increasing insulin sensitivity, improving pancreatic β-cell function, and inhibiting intestinal glucose absorption through the regulation of the α-glucosidase enzyme. This antidiabetic activity is inseparable from ulvan's ability as a natural anti-inflammatory agent, which works by suppressing the expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1*β* through inhibition of the NF-κB and MAPK transduction pathways. This anti-inflammatory role is important because in type 2 diabetes mellitus, low-grade inflammation occurs that contributes to insulin resistance and metabolic dysfunction. Furthermore, ulvan's antioxidant properties help reduce oxidative stress that triggers chronic inflammation, thereby enhancing its antidiabetic effects. Thus, ulvan has the potential to be developed as a candidate for multifunctional phytotherapeutic agents that simultaneously target the inflammatory-oxidative-metabolic pathways in diabetes mellitus **(Flórez-Fernández *et al.*, 2023)**.

Based on the results above, the antibacterial activity of ethanol extract of *Ulva lactuca* using the UAE method was higher against *S. aureus* bacteria. The difference in sensitivity between *E. coli* and *S. aureus* is generally caused by differences in the structure of their cell walls. *E. coli* is a Gram-negative bacterium that has a lipopolysaccharide (LPS) layer on the outer membrane that functions as a barrier against hydrophobic compounds. Meanwhile, *S. aureus* as a Gram-positive bacterium has a thicker cell wall consisting of peptidoglycan, but does not have an outer layer of LPS, so antibacterial compounds can more easily penetrate and work on target cells **(Ardita *et al.*, 2021; Panjaitan *et al.*, 2022)**.

The antibacterial activity of the ethanol extract of *Ulva lactuca* is thought to be due to the presence of secondary metabolites such as phenolics, flavonoids, alkaloids, saponins, and terpenoids reported in this species. These compounds work through several mechanisms, including damaging cell membrane integrity, inhibiting protein and nucleic acid synthesis, and causing leakage of intracellular components **(Ardita *et al.*, 2021)**. Flavonoids and phenolics, in particular, play an important role as inhibitors of bacterial growth by forming complexes with extracellular proteins and cell walls, thereby disrupting membrane permeability **(Evans & Cowan, 2016)**.

Furthermore, the use of ethanol as a solvent in the extraction process significantly impacts the extract's ability to dissolve polar and semi-polar compounds such as phenols and flavonoids. This explains why ethanol extracts often yield higher antibacterial activity compared to non-polar solvents such as n-hexane **(Liswandari *et al.*, 2018)**. Previous research supports these findings. According to **Hamed *et al.* (2015)**, ethanol extract of *Ulva lactuca* showed inhibition zones of 14–18mm against *S. aureus* and 10–15mm against *E. coli* at a concentration of 100mg/ mL. Higher activity against *S. aureus* indicates that the antibacterial compounds in *Ulva lactuca* are more effective against Gram-positive bacteria. The results of other the study showed that the methanol extract from maceration was active against *S. aureus* with a clear zone of 8.20mm so that extraction with UAE in this study is better with a larger clear zone diameter **(Lembang, 2023)**.

The UAE extraction method used in this study also affected the resulting antibacterial activity. **Panjaitan *et al.* (2022)** studied the antibacterial activity of ethanol extract of *Ulva lactuca* against *E. coli* and *S. aureus* bacteria, resulting in lower activity than the UAE method. The UAE method increases the efficiency of active compound extraction through ultrasonic cavitation, which damages cell walls and accelerates the release of secondary metabolites, so the resulting ethanol extract has the potential to have a higher antibacterial content than conventional extraction with the same time. Although the results of this study show promising potential, further research is needed to address limitations such as the lack of *in vivo* testing and more in-depth isolation of active compounds.

CONCLUSION

Ultrasonic-assisted ethanol extracts of *Ulva lactuca* exhibited potent antioxidant and anti-inflammatory activities, as well as moderate antibacterial potential, particularly against *Staphylococcus aureus*. These findings support the potential use of *Ulva lactuca* as a source of marine bioactives for future functional product development.

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