Antioxidant and antibacterial activity of ethanolic extract from *Ulva sp.*

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**Abstract.** The study aimed to investigate the antioxidant and antibacterial activity of ethanolic extracts from *Ulva sp.* The antioxidant effects of the extracts were evaluated by the DPPH scavenging assay. Agar well diffusion method was performed to determine the antibacterial activity of the extracts against *Escherichia coli* FNCC 194. The chemical constituents of the extracts were analyzed by FTIR spectroscopy. The result exhibited that the ethanolic extract of *Ulva sp.* 2 possessed higher antioxidant activity compared to the ethanolic extract of *Ulva sp.* 1. At a concentration of 0.8 mg/mL, the radical scavenging activities from ethanolic extract of *Ulva sp.* 1 and *Ulva sp.* 2 were 22.34±9.71% and 32.67±4.23%, respectively. The ethanolic extract of *Ulva sp.* 2 showed higher antibacterial activity against *E. coli* FNCC 194 compared to the ethanolic extract of *Ulva sp.* 1. The FTIR spectroscopy analyzed that both ethanolic extracts have the same functional groups as follows O-H alcohols, C-H alkanes, C=C aromatic, and C-O alcohols. It indicated that the ethanolic extracts possibly contained phenolic compounds. From the study, it was concluded that the ethanolic extracts of *Ulva sp.* can be explored as antioxidant and antibacterial agent candidates.

1. **Introduction**

Marine macroalgae, also known as seaweeds, are one of the most significant marine resources. The metabolites of marine macroalgae exhibit many benefits for nutritional and health promotion [1]. The bioactive compounds of marine macroalgae are extensively studied for therapeutic use, such as pigments, phenolic, polysaccharides, carotenoids, and flavonoids [2-5]. Marine macroalgae also contain various components of amino acids, essential fatty acids, minerals, and carbohydrates that fulfill human nutrition need [1,6].

According to nutrient and chemical components, marine macroalgae are classified into three groups as red algae (Rhodophyta), green algae (Chlorophyta), and brown algae (Phaeophyta) [7]. *Ulva sp.* is a member of green algae (Chlorophyta). The studies of biological activities of metabolites from *Ulva sp.* exhibit various pharmacological effects, such as anti inflammation, immunostimulant, anticancer, cardioprotective, antihyperlipidemic, anticoagulant, and antiviral [8-12]. *Ulva sp.* is also known as potential source of the antioxidant agent [2]. The phenolic compounds, as well as metabolites such as flavonoids, carotenoids, ascorbic acid, and sulfated polysaccharides are antioxidant molecules discovered in *Ulva sp.* [1,12]. It was also reported that *Ulva sp.* possesses antimicrobial activity against...
Gram positive bacteria e.g. *Staphylococcus aureus*, *S. epidermidis, Enterococcus faecalis, Bacillus subtilis, Streptococcus pyogenes*; Gram negative bacteria e.g. *Escherichia coli, Salmonella typhimurium, Vibrio cholerae, Klebsesella pneumoniae*; and fungi e.g. *Aspergillus flaurus, A. niger* [13-15].

The samples of seaweed *Ulva sp.* were collected from Sepanjang Coast, Gunungkidul, DI. Yogyakarta province, Indonesia. The nutritional components of *Ulva sp.* have been studied by Jatmiko et al. (2019) by proximate analysis method, but the antioxidant and antibacterial activities of the sample have not yet been studied [16]. The objective of present study was to investigate the antioxidant and antibacterial activity of ethanolic extract from two species of *Ulva sp.*

2. Materials and methods

2.1. Samples collection

The fresh *Ulva sp.* were collected from Sepanjang Coast, Gunungkidul, DI. Yogyakarta. The samples were *Ulva sp.* 1 with small leaves and *Ulva sp.* 2 with big leaves (Figure 1). The samples were rinsed off with distilled water to remove the impurities, salts, and sand particles. The samples were then dried in the shade of the sun (not direct exposure of sunlight) for two days. The dried samples were ground to be powder.

![Ulva sp. 1 and Ulva sp. 2](image)

Figure 1. *Ulva sp.* 1 and *Ulva sp.* 2.

2.2. Preparation of ethanolic extracts

The extracts were obtained by maceration the samples powder in ethanol 95% with the ratio of 1:8 w/v for three days at room temperature, and then were filtered. The filtrate was evaporated in a rotary evaporator Buchi Rotavapor R-200.

2.3. Fourier-transform infrared (FTIR) spectroscopy analysis

The samples were prepared in KBr pellets by mixing samples with KBr as a 0.5-1 mm thick film. FTIR analysis was carried out using FTIR spectrometer Shimadzu 8201 PC. The FTIR spectra were recorded in the range between 4000 to 500 cm⁻¹.

2.4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay

Antioxidant activity was evaluated by the DPPH method according to Darsih et al. (2019) with modification [17]. Several concentrations of *Ulva sp.* extracts were dissolved in methanol, and then reacted with DPPH 1.01 mM at room temperature. The reaction was incubated in the dark condition for 30 min. Absorbance was measured using a UV-Vis spectrophotometer at 517 nm. The scavenging activity was calculated by the equation as follows:

\[
\text{DPPH radical scavenging activity (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]
where $A_n$ is absorbance of the control and $A_1$ is absorbance of the sample. The assays were carried out in triplicates. The positive control was 0.025 mg/mL ascorbic acid.

2.5. Antibacterial activity assay

The antibacterial activities of the samples against *E. coli* FNCC 194 were evaluated using the agar well diffusion method [18]. The samples were dissolved in DMSO. About 100 µL of bacterial suspension were inoculated in nutrient agar plates. The wells were punched in the solid media. Several concentrations of the ethanolic extracts were added to wells, and then incubated at 37°C for 24 hours. The positive control was ampicillin, while the negative control was DMSO. After incubation, the inhibition zone diameter was measured in millimeter (mm).

3. Results and discussions

In this study, *Ulva sp.* extract was acquired by the maceration process in ethanol. Extraction *Ulva sp.* with ethanol generated functional phytochemicals, such as phenolics and pigments, which were contributing to antioxidant, anti inflammatory, and antibacterial activities [5,14]. The result of extraction showed that the yield of *Ulva sp.* 1 extract was higher than the yield of *Ulva sp.* 2 extract (table 1.)

| Sample       | Dry weight (g) | Extract weight (g) | Yield (%) w/w |
|--------------|----------------|--------------------|---------------|
| *Ulva sp.* 1 | 4.496          | 1.173              | 26.08         |
| *Ulva sp.* 2 | 2.087          | 0.360              | 17.24         |

The antioxidant activity of the samples was assessed by radical scavenging method. The DPPH method is the most extensively used to evaluate the radical scavenging activity of extracts from natural substances. Based on the DPPH scavenging activity assay, *Ulva sp.* 2 extract showed a higher antioxidant activity compared to *Ulva sp.* 1 extract. At the highest concentration of 0.8 mg/mL, *Ulva sp.* 2 extract presented the DPPH radical scavenging as 32.67±4.23 %, while *Ulva sp.* 1 extract showed 22.34±9.71% radical scavenging activity (Figure 2). Ascorbic acid as a positive control had a radical scavenging activity as of 76.67±1.20 % at 0.025 mg/mL.

Several studies had explored the potential of antioxidant activity of *Ulva sp.* Research by Bourguiba et al. (2017) showed that *Ulva rigida* had the scavenging activity as of 92.5% at 1 µg/mL on the DPPH test [19]. It also revealed that *U. rigida* exhibited a preventive action against hydrogen peroxide on yeast cell and zebrafish embryo from death. Another study also evaluated that marine macroalgae *Ulva sp.* and *Gracilaria sp.* showed high antioxidant activity due to its polyphenolic compounds and its protein content [20]. A tubular green seaweed *Ulva intestinalis* that rich in polysaccharides content also showed good antioxidant activity [21]. The polysaccharides were extracted by using extraction method with different solvents, those were distilled water, 0.1N HCl, and 0.1N NaOH.

In the present study, agar well diffusion method is used to investigate antibacterial activity of the sample. The size of inhibition zone on agar plate is one of the parameter to assess the antibacterial activity. *Ulva sp.* 1 and *Ulva sp.* 2 extract were assessed for the antibacterial activity against *E. coli* FNCC 194. Ampicillin was used as a positive control, while DMSO was a negative control. The results are showed in Table 2. At the concentration of 0.5 mg/mL, *Ulva sp.* 1 extract did not show inhibition activity of bacterial growth. At concentration 1.0 mg/mL, *Ulva sp.* 1 extract inhibited *E. coli* growth with inhibition zone as 5.40 mm. Compared to *Ulva sp.* 1 extract, *Ulva sp.* 2 extract possessed higher antibacterial activity against *E. coli*. *Ulva sp.* 2 extract with concentration 0.5 mg/mL inhibited bacterial growth with inhibition zone as 22.70 mm, while DMSO that used as solvent showed no inhibition against the bacteria.
Figure 2. The comparison of radical scavenging activities from two types of Ulva sp. extract. Data shown as mean±SD, n=3. Ulva sp. 2 extract exhibited more radical scavenging activity compared to Ulva sp. 1 extract.

Table 2. Inhibition zone of Ulva sp. extract against E. coli FNCC 194 growth.

| Samples     | Concentration (mg/mL) | Inhibition zone (mm) | Mean |
|-------------|------------------------|----------------------|------|
| Ulva sp. 1  | 0.5                    | -                    | -    |
|             | 1.0                    | 5.40                 | 5.40 |
| Ulva sp. 2  | 0.5                    | 22.70                | 18.20| 20.45|
|             | 0.5                    | 32.95                | 22.65| 27.80|
| Ampicillin  |                        |                      |      |

(-) : no inhibition

The study by Chakraborty et al. (2010) also showed the antibacterial activity of seaweed Ulva fasciata [22]. Their antibacterial activity resulted from the sesquiterpene derivatives compound from U. fasciata were guai-2-en-10α-ol and guai-2-en-10α-methanol that was isolated from CHCl₃/CH₃OH soluble fraction. Composite of silver nanoparticles AgNPs with algae extract of Ulva compressa and Cladophora glomerata also performed outstanding antimicrobial activity against several bacteria P. aeruginosa, E. coli, K. pneumonia and S. aureus [23].

The chemical components of both ethanolic extract were analyzed using FTIR spectroscopy. The FTIR spectra between 4000 to 500 cm⁻¹ represent functional groups of Ulva sp. extracts (figure 3; table 3.). For Ulva sp. 1 extract, a broad peak observed at 3425.58 cm⁻¹ represented O-H stretching vibrations of the alcohol groups. Peaks at 2924.09 and 2854.65 cm⁻¹ were C-H asymmetric and symmetric stretching vibration of the alkanes, respectively. The sharp peaks at 1635.64 and 1427.32 cm⁻¹ indicated the C=C aromatic groups. A peak at 1103.28 cm⁻¹ was due to C-O stretching vibration of alcohol groups.

The FTIR spectra of Ulva sp. 2 extract was similar to spectra of Ulva sp. 1 extract. The functional groups for IR absorption of Ulva sp. 2 extract based on the spectra were as follows: 3387.00 cm⁻¹ (O-H stretching of alcohol), 2924.09 cm⁻¹ (C-H asymmetric stretching of alkanes), 1635.64 and 1427.32 cm⁻¹ (C=C stretching of aromatic groups), and 1095.57 cm⁻¹ (C-O stretching of alcohol groups).
Figure 3. FTIR spectra of *Ulva* sp. 1 dan *Ulva* sp. 2 extract.

Table 3. Interpretation of functional groups of *Ulva* sp. extract by FTIR spectroscopy

| Sample     | Functional groups                  | Wave number (cm⁻¹) | Peak       |
|------------|------------------------------------|--------------------|------------|
| *Ulva* sp. 1 | O-H alcohols, phenols               | 3425.58            | Broad      |
|            | C-H alkanes asymmetric stretch     | 2924.09            | Sharp      |
|            | C-H alkanes symmetric stretch      | 2854.65            | Sharp      |
|            | C=C aromatic                       | 1635.64 and 1427.32| Sharp      |
|            | C-O alcohols                       | 1103.28            | Sharp      |
| *Ulva* sp. 2 | O-H alcohols, phenols               | 3387.00            | Broad      |
|            | C-H alkanes asymmetric stretch     | 2924.09            | Sharp      |
|            | C=C aromatic                       | 1635.64 and 1427.32| Sharp      |
|            | C-O alcohols                       | 1095.57            | Sharp      |

FTIR spectroscopy analysis indicated that *Ulva* sp. extract is likely contained of phenolic compounds, in accordance with previous studies [14,24]. The antioxidant and antibacterial activity of the samples possibly was due to the phenolic compounds. The recent study stated that the phenolic compounds contributed to the antioxidant and antibacterial activity of *Ulva* sp. [5,14,24].

4. Conclusion

In conclusion, *Ulva* sp. collected from Sepanjang Coast, Gunungkidul, Indonesia exhibit antioxidant and antibacterial activity. Ethanolic extract of *Ulva* sp. 2 possessed higher antioxidant and antibacterial
activity against *E. coli* compared to ethanolic extract of *Ulva sp.* 1. It is likely that the antioxidant and antibacterial activity were attributed by the phenolic compounds of the two *Ulva sp.* extract.

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