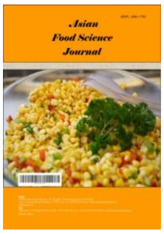

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Antioxidant and Antibacterial Activity of Caulerpa Flour (*Caulerpa lentifera*) as Functional Food Raw Material

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This research aims to study the process of making Caulerpa seaweed flour and extract; analyzed the antioxidant activity and inhibitory antibacterial activity of Caulerpa flour.
Study Design: The experimental design used in the first stage is the T-test by comparing the two washing treatments (3 times and 5 times), and in the second stage using a Completely Randomized Design.
Place and Duration of Study: The present study was conducted in Department of Aquatic Product Processing and Storage, Pangkep State Polytechnic of Agriculture. The duration of research is 6 month i.e from April, 2022 – September, 2022.
Methodology: The drying method is carried out by weighing fresh *Caulerpa lentifera*. samples and spread them on a baking sheet and in an oven to dry at 50°C. *Caulerpa lentifera*, which has been

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in powder form, will be analyzed for its antioxidant and antimicrobial properties. The extraction process will also be carried out on *Caulerpa lentifera* flour and analyzed for its antioxidant and antimicrobial properties.

Results: The results of the first phase of the study showed that the 5 times washing process on Caulerpa seaweed resulted in better quality than 3 times washing with an antioxidant value of 115.36 ppm, the water content of 9.9%; ash content of 23.65%; Ca levels of 152.4 mg/100 g; fiber 41.24%; Iodine 31.08 g/g and 4.16% yield. Caulerpa flour had the highest antioxidant and antibacterial activity compared to ethyl acetate and methanol with a value of 109.40 mg/L and an inhibitory diameter of 6.88 mm against *Escherichia coli.*

Conclusion: The antioxidant activity of seaweed flour is in the medium category and has an inhibitory power against pathogenic bacteria *E.coli*, so it has the potential as a functional food.

Keywords: Antibacterial; antioxidant; caulerpa; flour; sea grape.

1. INTRODUCTION

Green seaweed has a very high abundance in Indonesia, especially *Caulerpa* sp., *Halimeda* sp., and *Ulva* sp. *C. serrulata* extract had a moderate antioxidant activity with IC50 of 136.89 ppm [1]. The carotenoid pigment extract of *H. discoidea* has potent antioxidant activity with an IC50 value of 99.65 ppm [2]. *U. lactuca L*. extract has low antioxidant activity with an IC50 value of 4921.79 ppm [3]. The antioxidant activity of seaweed has been widely applied, including for sunscreen cosmetic ingredients [4-6]. One type of green seaweed that is quite potential in South Sulawesi is the type of seaweed *Caulerpa lentilifera*., commonly known as sea grape, is a green alga commonly found on sand and coral substrates. Sea grapes are commonly consumed as vegetables or fresh vegetables by people in the tropics.

Sea grapes (*Caulerpa* sp.) is one type of green seaweed that grows in the waters of the Makassar Strait. Local people know *Caulerpa* sp. as "Lawi-Lawi" which is used as fresh vegetables and has even become one of the favorite menus of South Sulawesi. Fresh *Caulerpa lentilifera* is dominated by water and is very susceptible to damage. The chemical composition *Caulerpa lentilifera*. is important to know so that its utilization and development as food can be carried out properly without losing its nutritional content. Several studies have been carried out to determine the chemical composition of fresh and dried sea grapes (*Caulerpa lentilifera*) from several researchs [7-13].

Antioxidant properties of *Caulerpa lentilifera* Fresh water from Makassar Strait has been reported, but information about flour's antioxidant and antimicrobial properties and the extraction of *Caulerpa lentilifera* from these waters is still minimal. Based on the description above, it is necessary to characterize Caulerpa flour from Makassar Strait before further utilization as a raw material for functional and non-food foods. Information on the characterization and antioxidant and antimicrobial potential of green seaweed originating from the Makassar Strait has not been widely studied, so it is necessary to research to determine the characteristics of green seaweed species, especially *Caulerpa lentilifera* This study aimed to determine the antioxidant and antimicrobial properties of *Caulerpa lentilifera*’s flour and the antioxidant potential of green seaweed extract.

2. MATERIALS AND METHODS

2.1 Materials

The main ingredient used in making Caulerpa flour (*Caulerpa lentilifera*) obtained from the waters of the Makassar Strait. Materials used in the analysis include n-Hexane and Ethyl acetate, 0.2% *2.2-Diphenyl-1-Picrylhydrazyl* (DPPH) solution to test antioxidant activity, Potato Dextrose Agar (PDA), and Natrium Agar (NA) to test antibacterial activity.

Equipment used includes oven, basin, and drain. Laboratory equipment used for analysis includes an oven, desiccator, iron grinder, wood grinder, distillation flask, Erlenmeyer, burette, glass funnel, beaker, dropper, measuring flask, Whatman paper, spray bottle, test tube, hot plate, and condenser.

2.2 Methods

Caulerpa lentilifera cleaned of still attached dirt, then weighed to determine the initial weight of the sample. The washing was carried out 3 times and 5 times to reduce the salt content of the seaweed. The drying method is carried out by weighing fresh *Caulerpa lentilifera* samples and spread them on a baking sheet and in an oven to dry at 50°C. *Caulerpa lentilifera* which has been in powder form, will be analyzed for its antioxidant and antimicrobial properties. The extraction process will also be carried out on *Caulerpa lentilifera*. flour and analyzed for its antioxidant and antimicrobial properties. Extraction was done by maceration with different solvents, namely 90% ethanol, 90% methanol, and 90% ethyl acetate. The maceration process is carried out by immersing the sample, which is added with ethyl acetate solvent in a ratio of 1:5 (v/w) or until the piece is completely submerged, tightly closed, and stored in a dark place away from light. This is done to avoid damage to the compounds in the sample. Maceration is carried out for 2x24 hours, and once a day, stirring is carried out to balance the solvent and extractive ingredients. The filtrate was filtered using Whatman paper. The filtrate was evaporated using a rotary evaporator at a temperature below 60°C.

2.3 Experimental Design

The experimental design used in the first stage of this study was the T-test by comparing the two washing treatments and in the second stage using a completely randomized design with different extracting types, namely ethanol, methanol, and ethyl acetate.

2.4 Analysis Data

The research data were analyzed using analysis of variance using Statistical Package for the Social Sciences (SPSS) software

3. RESULTS AND DISCUSSION

3.1 Charateristic Caulerpa Flour

Caulerpa lentilifera, commonly known as sea grape, is a green alga commonly found on sand and coral substrates. Sea grapes are commonly consumed as vegetables or fresh vegetables by people in the tropics. The distribution of marine algae *Caulerpa lentilifera* and its density in waters depends on the type of substrate, season, and species composition [14]. The making of seaweed powder is done by washing, soaking, draining, chopping, drying, and mashing using a blender until it becomes powder.

Caulerpa lentilifera, which grows in Indonesian waters and is widely consumed by the public, has not been widely reported on the profile of the chemical composition of its fiber and minerals [11]. This study performed comparative testing to determine the composition of the *Caulerpa lentilifera* powder used. Tests were carried out on several parameters, namely water content, ash content, calcium content, fiber, and iodine content. The results of the characteristics of *Caulerpa lentilifera* are as follows in Table 1.

The water content in this study was found to be lower when compared to the results of other research [15], which was 15.37%. This is due to the drying treatment of seaweed samples so that the water content will decrease with the length of time and the high temperature in drying. This is in line with the statement from [17] that the drying of Caulerpa type seaweed will affect its water content due to the high water content of this type of seaweed so that the shrinkage value will be high. The water content of *Caulerpa lentilifera* obtained in this study has the lowest value among other kinds of literature. This is because the drying process used is different. The *Caulerpa racemosa* powder in this study was dried using an oven at 45°C for 72 hours, while in the study conducted by [15], *Caulerpa racemosa* powder was dried using a freeze dryer at -20°C for 48 hours. Flour products must be low in water content because the higher the free water content they contain in flour will be damaged due to microorganisms.

The ash content of *Caulerpa racemosa* powder is 23.56%. Ash content is a parameter to indicate the value of inorganic (mineral) content in a material or product. The higher the ash content, the more inorganic material is contained in the product. [11] stated that *Caulerpa racemosa* seaweed contains minerals such as calcium 0.013, sodium 1.9 and potassium 0.195 in dry weight. [18] Explained that the ash content in seaweed ranged from 10-50%. The proportion of ash content in a food material is influenced by species, non-nutrient soil conditions, plant maturity, climate, growing area, and planting. [11] Explained that the chemical composition of seaweed, as an organism that carries out the process of photosynthesis, is not determined by the concentration of water nutrients but also by water temperature and water depth which is influenced by seasonal variations and geographical location.

Table 1. Characteristics of *Caulerpa lentilifera*

Component	*C. lentilifera*	*C. lentilifera [7]*	*C. Racemosa [15]*	*C. Racemosa [16]*
Moisture	9.9 %	-	15.37%	-
Ash	23.56 %	49.67 %	12.15%	17.94%
Calcium	152.4 mg/100g	119.2 mg/100g	-	17.95 mg/100g
Dietary Fiber	41.24 %	3.42 %	48.97%	23.02%
Iodium	31.08 µg/g	-	-	-
Antioxidant	109.40 mg/L	47.61 ppm	-	43.53 ppm
Yield	4.16 %.	4.46 %	-	0.84%

C. lentillifera were analyzed using the AOAC method, in the biochemical testing laboratory and in 2022

The content of calcium (Ca) in this study was higher than the study conducted by [7] and [16], with calcium (Ca) content of 119.2 mg/100g and 17.95 mg/100g, respectively, the high mineral Ca content is in line with the high ash content in seaweed. Nasrudin and Kusumaningrum [19] stated that the more mineral content, the higher the ash content. The Ca content of *C. lentillifera* obtained from the Pattani coast of Thailand was 187.4 mg/g [20]. Calcium (Ca) is one of the essential nutrients needed for various body functions. Calcium plays a role in forming and maintaining bones and teeth [21]. Calcium also has a structural role in strengthening the body's skeleton [22].

The crude fiber content in this study was quite high compared to other caulerpa flours. The crude fiber content of *C. racemosa* obtained from the waters of Jepara was 8.43% [11]. The amount of crude fiber is the amount of dietary and functional fiber. Crude fiber will be fermented in the digestive tract and produce bioactive components such as short-chain fatty acids (Short Chain Fatty Acids), which can play an essential role in digestive tract health [23]. Consuming fiber benefits people with obesity, cancer, and cardiovascular disease [24].

3.2 Antioxidant Activity

Antioxidant activity testing was carried out on the green algae extract *C. lentillifera* with the aim of determining the potential antioxidant activity of compounds in the extract using the radical 2,2-diphenyl1 picrylhydrazyl (DPPH). According to [25], a compound is categorized as very strong if the IC value is <50 mg/L, strong is 50-100 mg/L, moderate is 101-150 mg/L, and weak is >150 mg/L. The results of the antioxidant activity test in this study showed that the crude extract of *C. lentillifera* had an IC50 value of 109.40 mg/L. The results of the antioxidant activity test showed that the *C. lentillifera* extract had moderate antioxidant activity. The smaller IC value indicates the higher the antioxidant activity and the less use of extracts in inhibiting 50% of free radical activity.

The DPPH method is the most widely used method in antioxidant testing. This method was chosen because it has a high level of sensitivity, requires not too many samples in a relatively short time, and does not require a lot of reagents. However, this method can only measure antioxidant compounds that are soluble in organic solvents, especially alcohol [26].

The antioxidant activity of the DPPH method is based on the absorption of DPPH radicals by antioxidant compounds in seaweed extracts. DPPH is a free radical which is stable in solution. [1] reported the antioxidant activity of green algae *C. serrulata* with an IC50 of 136.89 mg/L. The antioxidant activity of *H. opuntia* obtained from the coast of the Red Sea, Egypt, has an IC50 of 192.3 mg/L [27]. Basir et al. [28] reported the antioxidant activity of the whole and cut *H. gracilis* fraction with IC50 of 289.34 mg/L and 278.41 mg/L, respectively. [3] reported the antioxidant activity of *U. lactuca* obtained from Ujung Genteng beach, Sukabumi Regency, with stratified extraction treatment using n-hexane, ethyl acetate, and ethanol, each had IC values of 11213.07 mg/L, 9770.28 mg/L, and 4921.79 mg/L. Chew et al. [29] stated that the IC50 value of natural material is closely related to the bioactive compounds it contains, so the selection of the extraction method used greatly affects the IC50 value produced.

3.3 Antibacterial Activity

The diameter of the inhibition zone of *C. lentilifera* against *E. Coli* was indicated by the formation of a clear zone around the agar pieces and measured with a size of 6mm. [30] Stated that the inhibition response in seaweed is due to the presence of active compounds such as tannins, flavonoids, steroids/terpenoids, alkaloids, and saponins that act as antibacterials.

The antibacterial activity of *C. lentilifera* was in the moderate category. Surjowardojo et al. [31] Described the antibacterial inhibition zone diameter category as <5mm weak inhibitory power, 6-10mm medium category, 11-20 mm strong category, and > 21 mm very strong category.

The test was carried out by measuring the diameter of the inhibition zone (zone of inhibition) of the test bacteria, namely *Escherichia Coli*. The first step is to drip the sample using a 50 L micropipette. The antibacterial test method was carried out using the Well (Agar Diffusion) technique. This test was conducted to determine the antibacterial activity of *Caulerpa lentilifera* Caulerpa flour by measuring the diameter of the growth inhibition area (clear zone) of the test bacteria, namely *E. coli* bacteria. This pitting method was chosen because the method of operation is quite simple as well because the results of the inhibition zone obtained are larger with other agar diffusion methods, namely disk agar diffusion and cylinder agar diffusion. This is because, in the good method, the sample extract does not only work on the surface of the media but also goes to the bottom so that the working power of the sample extract will be more effective.

4. CONCLUSION

The research showed that the five times washing process on Caulerpa flour resulted in better quality than 3 times washing with an antioxidant value of 115.36 ppm, the water content of 9.9%; ash content of 23.65%; Ca levels of 152.4 mg/100 g; fiber 41.24%; Iodine 31.08 g/g and 4.16% yield. Caulerpa flour had the antioxidant and antibacterial activity with a value of 109.40 mg/L and an inhibitory diameter of 6.88 mm against *E.coli*. The antioxidant activity of Caulerpa flour is in the medium category and has an inhibitory power against pathogenic bacteria *E.coli*, so it has the potential as a functional food.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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