



Antioxidant Activity of the Sea Grape (*Caulerpa racemosa*) Used As an Antioxidant Lotion

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ABSTRACT

Caulerpa racemosa is one type of seaweed that has the greatest potential in Indonesia. *Caulerpa racemosa* has attracted many attentions as an antioxidant agent which is potential for cosmetic industries. The study aims to analyze the antioxidant content of *Caulerpa racemosa* and determining its effectiveness as an anti-aging lotion. Testing the antioxidant activity of *Caulerpa racemosa* using the DPPH method. *Caulerpa racemosa* contains phenol compounds that function as antioxidants. The results showed that value antioxidant activity of *Caulerpa racemosa* lotion is very higher, value IC_{50} reach 0,58 ppm (a compound is called a very strong antioxidant if the value of IC_{50} is <50 ppm) and the results test of *Caulerpa racemosa* lotion was appropriate with quality standard SNI 16-4399-1996 about the standards sunscreen preparations for the skin. This paper presents the first-time production lotion from *Caulerpa racemosa*.

Keywords: Anti-aging lotion, *Caulerpa racemosa*, Antioxidant, DPPH.

INTRODUCTION

One of the seaweed type in Indonesia that is quite promising is *Caulerpa racemosa* or sea grape. *Caulerpa racemosa* production is 10.3 million tons per year and increases every year¹. This species in South Sulawesi is commonly known as lawi-lawi or bu'ne while in Java it is known as latoh.

Caulerpa racemosa has high nutritional content such as vegetable protein, minerals and vitamins². Moreover, *Caulerpa racemosa* has bioactive compound like tannin, phenol, flavonoid,

alkaloid that can inhibit the growth of bacteria and fungi, so that can be used in medical and cosmetic fields^{3,4}. Phenol compounds found in *Caulerpa racemosa* act to protect body cells from free radical damage by binding to free radicals so as to prevent inflammation and inflammation in the body's cells⁵.

Caulerpa racemosa is very common in coastal areas and coral reefs. In its utilization, this type of seaweed. *Caulerpa racemosa* is still very rarely processed⁶. One of the promising products of *Caulerpa racemosa* is the manufacture of lotions because they are rich in antioxidants which are good for the skin.



A lotion is a form of an emulsion that is defined as a mixture of two or more phases, namely water and fat, which is stabilized with an emulsion system and if placed at room temperature will be a liquid⁷. The function of skin lotion is that it can maintain skin moisture, soften skin, prevent water loss and cleanse skin⁸. The study aims to analyze the antioxidant content of *Caulerpa racemosa* and determining its effectiveness as an anti-aging lotion. The manufacture of lotions from *Caulerpa racemosa* is one of a promising process that can be done to increase the selling value of *Caulerpa racemosa*.

MATERIAL AND METHODS

The equipment used in the research is analytical scale, mortar and pestle, hotplate stirrer, oven, Ostwald viscometer, drop pipette, beaker, measuring flask, pycnometer, pH meter, petri dish and spectrophotometer UV-Vis US-110PC. The materials used in the research are *Caulerpa racemosa*, nipagin and nipasol, propylene glycol, stearic acid, triethanolamine, DPPH, vitamin C pa, aquades, aqubides, filter paper, pH buffer and label paper.

Production of *Caulerpa racemosa* lotion

Production of *Caulerpa racemosa* lotion and control composed of two phases namely oil phase and an aqueous phase. For Oil phase, dissolve *Caulerpa racemosa* and stearic acid in a hot mortar and added TEA. For Aqueous phase, dissolve nipagin and nipasol with propylene glycol and added aquades. Then mix the two phases to form a lotion⁹. For control lotion the production without the addition of *Caulerpa racemosa*.

Tabel 1: Formulation of *Caulerpa racemosa* lotion⁹

Materials	Control	<i>Caulerpa racemosa</i>
<i>Caulerpa racemosa</i>	x	10
Stearic Acid	14,2	14,2
Triethanolamine	1	1
Nipagin	0,1	0,1
Nipasol	0,05	0,05
Propilenglikol	5	5
Aquades	100	100

The *Caulerpa racemosa* lotion then tested according to SNI 16-4399-1996 standard that is an organoleptic test, pH, density, viscosity and microbial contamination.



Fig. 1. *Caulerpa racemosa* lotion and control lotion

Testing of *Caulerpa racemosa* lotion DPPH solution

Weighed as much as 10.0 mg of DPPH (2,2-diphenyl-1-picryl hydrazine) then dissolved in ethanol p. a. using a volumetric flask 100 mL, as a stock solution. For a concentration of 0.125 ppm, DPPH solution was pipetted as much as 1.50 ml then the volume was filled with ethanol p.a to 10.0 ml and the absorbance was observed in a spectrophotometer. Performed again for each concentration of 0.25 ppm, 0.5 ppm, and 0.75 ppm. The solutions is then transferred to a brown glass and allowed to stand for 30 min then the absorption is measured at the maximum DPPH wavelength of 515 nm.

Testing of *Caulerpa racemosa* lotion Antioxidant activity using DPPH method

As much as 2.5 g of a lotion is put into a mortar, then added 5.0 mL ethanol p. a. Then centrifuged for 10 min then filtered with filter paper until the solution is clear. The filtrate was put into a volumetric flask, added 2.0 mL of DPPH solution and the volume was filled with ethanol p. a. up to 10 mL, incubated at 37°C for 30 min put in a cuvette, then absorbed using a Spectrophotometer UV-Vis with a wavelength of 515 nm. Antioxidant activity is expressed by% inhibition.

$$\% \text{ inhibition} = \frac{\text{Abscontrol} - \text{Abssample}}{\text{Abscontrol}} \times 100\%$$

Information :

Abs Kontrol : Solution+ DPPH

Abs Sample : Solution + DPPH + Sample

Specifically, a compound is called a very strong antioxidant if the IC_{50} value is < 50 ppm, it is stated strongly if the IC_{50} value is 50-100 ppm, expressed as a medium if the IC_{50} value is 101-150 ppm, and is declared weak if the IC_{50} value is > 150 ppm.¹⁰

To find out the lotion quality of *Caulerpa racemosa*, measurement of pH value, viscosity,

density and total microbial contamination need to be considered so as to get a quality lotion.

RESULT AND DISCUSSION

Antioxidant activity of *Caulerpa racemose* lotion

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free radical that can react with compounds that can donate hydrogen or proton atoms, thus forming non-radical compounds that are non-radical DPPH used in testing antioxidant activity in an ingredient/ extract. Due to of unpaired electrons, DPPH gives a strong absorption at an absorbance of about 517 nm. When the electrons become paired by the presence of free radical catchers the absorbance decreases stoichiometrically according to the number of electrons taken. The presence of antioxidant compounds can change the color of DPPH solution from purple to yellow.¹¹

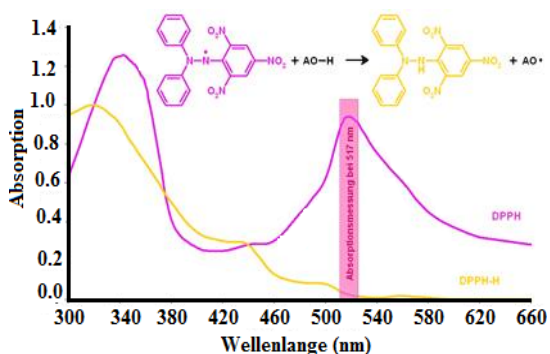


Fig. 2. Discoloration of the solution in DPPH radical reaction with antioxidants¹²

Determination of antioxidant activity from lotions by graphing the relationship between concentration (ppm) and % inhibition. The equation of the regression obtained from the graph is used to calculate the value of Inhibition Concentration (IC₅₀).

Calculation of IC₅₀ value for control lotion based on graph results:

$$\begin{aligned}
 a &= 26,808 \\
 b &= 25,685x \\
 r^2 &= 0,9911 \\
 y &= a + bx \\
 y &= 26,808 + 25,685x \\
 IC_{50} (X) &= \frac{(y - 26,808)}{25,685} = \frac{(50 - 26,808)}{25,685} = 0,90
 \end{aligned}$$

Calculation of IC₅₀ value for *Caulerpa racemose* lotion based on graph results:

$$\begin{aligned}
 a &= 31,496 \\
 b &= 32,011x \\
 r^2 &= 0,9954 \\
 y &= a + bx \\
 y &= 31,496 + 32,011x \\
 IC_{50} (X) &= \frac{(y - 31,496)}{32,011} = \frac{(50 - 31,496)}{32,011} = 0,58
 \end{aligned}$$

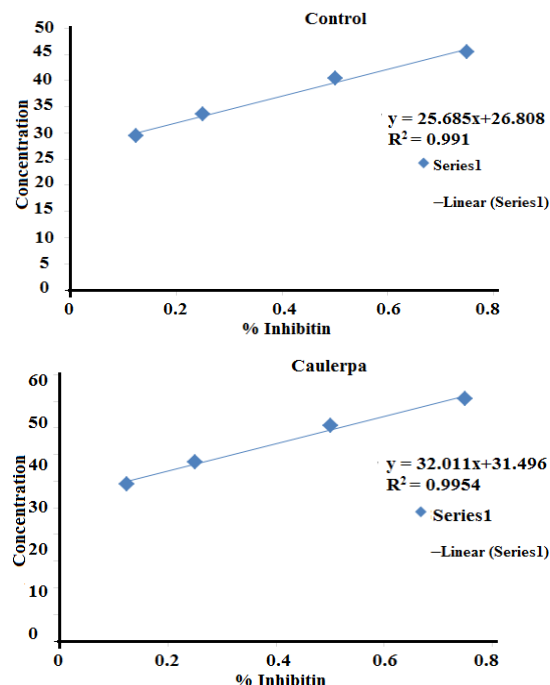


Fig. 3. Graph of measurements of control lotion and *Caulerpa racemose* lotion

Table 2: The result of Antioxidant activity from *Caulerpa racemosa* lotion and control lotion

No	Sample	Sample weight	Concentration	Absorbance Sample	Absorbance DPPH	% antioxidant	IC ₅₀
1	Control	1.425	0.125	0.525	0.905	29.47	0.90
		1.425	0.250	0.473	0.905	33.50	
		1.425	0.500	0.383	0.905	40.48	
		1.425	0.750	0.318	0.905	45.52	
2	<i>Caulerpa racemosa</i>	1.095	0.125	0.557	0.905	35.12	0.58
		1.095	0.250	0.506	0.905	40.26	
		1.095	0.500	0.440	0.905	46.92	
		1.095	0.750	0.353	0.905	55.70	

Caulerpa racemosa lotion has strong antioxidant activity compared to control lotions or without the addition of *Caulerpa racemosa*. IC_{50} values less than 0.05 mg/mL or 50 ppm identify that the lotion has very strong antioxidant activity. The high value of anti-oxidant activity in the lotion *Caulerpa racemosa* due to *Caulerpa racemosa* has a bioactive component that is the content of polyphenol compounds which have hydroxy groups substituted in ortho positions and para to -OH and -OR groups so that the compounds have antioxidant activity.

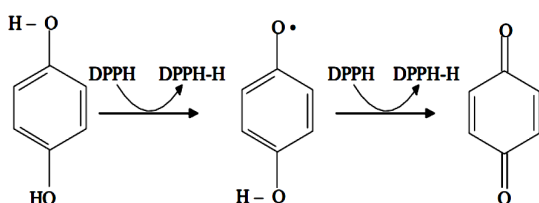


Fig. 4. The reaction of phenolic compounds with DPPH¹³

pH Value

pH lotion testing was carried out at the beginning of manufacture and for 4 weeks storage using a pH meter. Lotion with too acidic pH can irritate the skin while lotions that are too alkaline can make the skin dry so the preparation must have a pH that is suitable for the skin.

Table 3: pH value of *Caulerpa racemosa* lotion and control lotion

Sample	Initial pH	pH after 4 weeks
Control	8,02	8,15
<i>Caulerpa racemosa</i>	6,12	5,74

The results of pH measurements showed that the pH value of *Caulerpa racemosa* lotion was 6.12 and after 4 weeks of storage the pH value decreased to 5.74. Decreasing the pH of the lotion *Caulerpa racemosa* can be caused by the hydrolysis of acidic compounds that can be triggered by an increase in temperature during storage. However, based on Sunscreen Quality Standards (SNI 16-4399-1996) the pH value requirements are safe for the skin, namely pH 4.5 - 8.0 so that the pH of this lotion has fulfilled these requirements. While the control lotion had an initial pH of 8.02 and after 4 weeks of storage, the pH increased to 8.15, due to the water phase and oil phase on the control lotion begins to decompose so that the pH value of the control lotion increases.

Viscosity

Viscosity test is done to determine the thickness of a sample from the resistance of a substance to flow. The higher the flow viscosity, the greater the resistance. Viscosity affects the rate of absorption of lotion on the skin, the thicker the longer the absorption of the drug will be. The lotion measured its initial viscosity and after being stored for 4 weeks at room temperature.

Table 4: Viscosity value of *Caulerpa racemosa* lotion and control lotion

Sample	Initial viscosity (Cps)	viscosity after 4 weeks (Cps)
Control	53,4	73,40
<i>Caulerpa racemosa</i>	900	1100

Note : The fluidity unit is the centipoise (cps)

Viscosity is influenced by the selection of lotion ingredients, such as surfactants, disperse particles and the proportion of both water and oil phases. The viscosity value for control lotions is very low compared to lotions with the addition of *Caulerpa racemosa*. Viscosity value increases after 4 weeks of storage. This increase occurred due to evaporation of ethanol in the lotion so that the lotion thickened.

Density

Density is used to determine the absorption standard of a preparation. The smallest density identifies that the lotion has good homogeneity. The density of lotion types *Caulerpa racemosa* and control lotions are still in accordance with the quality standards of SNI 16-4399-1996, namely 0.95-1.05 g/mL.

Table 5: The density value of *Caulerpa racemosa* lotion and control lotion

Sample	Initial density (g/mL)	density after 4 weeks (g/mL)
Control	0,9862	0,9793
<i>Caulerpa racemosa</i>	1,0255	1,0394

Total Microbial Contamination

Based on the Regulation of the Head of the Indonesian Drug and Food Control Agency No. HK.00.06.1.52.4011 in 2009, concerning the determination of the maximum limits of microbial and chemical contamination. Microbial contamination is contamination from microbes that can be harmful one of which is *Escherichia coli*.¹⁴

The results of the total microbial contamination test on the lotion in accordance with the sunscreen quality standards SNI 16-4399-1996 that is a maximum of 102 colonies/gram.

Tabel 6: Total microbial contamination

Sample	Value of SPC (CFU)	<i>Escherichia coli</i>
<i>Caulerpa</i>	10 x 10 ³	-
Kontrol	25 x 10 ³	-

Note : CFU is Colony Forming Unit
SPC is Standard Plate Count

Antimicrobial test results showed that both lactic acid bacteria isolates from *Caulerpa racemosa* lotion and control lotions were able to inhibit *E.coli*. Organic acids produced by lactic acid bacteria are lactic acid and acetic acid. Lactic acid is the main metabolite of lactic acid bacteria. The inhibitory effect occurs because organic acid molecules enter the cell membrane and reduce the cytoplasmic pH. Hydrogen peroxide produced by the activity of lactic acid bacteria can inhibit pathogenic bacteria¹⁵.

In addition, other acids are produced by lactic acid bacteria such as acetic acid, propionate and formiate, although the production is small, but has stronger antimicrobial power than lactic acid. Lactic acid and a little acetic acid formed have an inhibitory effect on pathogenic microbes, apart from being caused by a decrease in pH below the optimum pH of pathogenic microbial growth, the presence of undissociated acid molecules that can penetrate the cell wall and disrupt the metabolic process and genetic mechanisms of pathogenic bacterial cells¹⁶

Profile of *Caulerpa racemosa* lotion

The results test of *Caulerpa racemosa* lotion was appropriate with quality standard SNI 16-4399-1996 about the standards of good cosmetic production. *Caulerpa racemosa* lotion has a pH is 5.72; density is 1,0255 g/cm³ and viscosity is 900 Cps.

CONCLUSION

Based on the results of research, it can be concluded that value antioxidant activity of *Caulerpa racemosa* lotion is very higher, value IC₅₀ reach 0,58 ppm using DPPH method (a compound is called a very strong antioxidant if the value of IC₅₀ is <50 ppm) and the results test of *Caulerpa racemosa* lotion i.e pH is 6,15; viscosity is 900; density is 1,0255; Total microbial contamination is 10 x 10³. It's appropriate with quality standard SNI 16-4399-1996 about the standards sunscreen preparations for the skin.

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Conflicts of Interest

The authors declare no conflict of interest.

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