FORMULATION AND EVALUATION OF SEA GRAPE *(Caulerpa racemose)* EXTRACT AS HAND CREAM AND ITS ANTIOXIDANT ACTIVITY TEST

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Abstract

Caulerpa racemosa (C. racemosa) is a seaweed containing folic acid, thiamine, ascorbic acid, and phenol as secondary metabolites that function as antioxidants. This study aims to formulate a hand moisturizing cream containing *C. racemosa* extract and evaluate the quality of the cream preparation. The manufacture of hand cream from *C. racemosa extract* was carried out in several stages, namely *C. racemosa* extraction, antioxidant testing, formulation of cream preparations, and evaluation. Evaluation of hand cream preparations of *C. racemosa* extract included organoleptic tests, homogeneity, pH measurement, viscosity, spreadability, and adhesion. The yield of *C. racemosa* from the extract was 33%. The IC_{50} of the *C. racemosa* extract was 159.8 ppm. The results of the organoleptic test of hand cream preparations showed physical characteristics in terms of pH, viscosity, homogeneity, dispersibility, and adhesion following the requirements of cosmetic preparations from the Directorate General of BPOM.

Keywords*: formulation, hand cream, sea grape, Caulerpa racemose, antioxidant*

Introduction

The cream is a semi-solid preparation in the form of a thick emulsion used for external use (Aulton, 1991). Creams usually consist of 2 phases in which one phase is dispersed in the other. There are two types of simple creams, namely the water-in-oil (W/W) type and the oil-in-water (W/A) type (Allen, 1999). One of the cream dosage forms on the market is hand cream. Hand cream is a cosmetic preparation used to protect the skin of the hands to keep it smooth and soft, not dry, scaly, and easily broken. Hand cream preparations must be easily spread on the skin surface (Wirantara, 2011). Therefore hand cream must have good physical properties and stability. The basis of skin moisturizing is the emollient effect that prevents dryness and skin damage caused by sun or aging skin while also making the skin look radiant. The water content in normal skin cells is more than 10%, which can be reduced due to excessive evaporation of water (Rowe et al., 2006).

The ingredients used in the manufacture of hand creams are increasingly varied, so researchers in the industry are competing to find formulas to produce hand creams that are economical, hygienic, do not endanger health, are easy to process, easy to obtain and have affordable selling values. In Indonesia itself, they are currently starting to develop a moisturizing cream containing natural plant extracts. The addition of natural plant extract that are safe for health have a positive influence or certain function on the hand cream produced. These functions include giving a soft impression, moisturizing the skin

and having antioxidant activity and giving a fragrant aroma when used.

Caulerpa racemosa (C. racemosa) is a type of seaweed that can be cultivated because it is known and favored by some people. *C. racemosa* has been widely used for consumption as food and as a mixture of antifungal drugs (Suhartini, 2003). In Indonesia *C. racemosa* is known as Latoh (Javanese), Bulung Boni (Bali), Lawi-Lawi (Sulawesi). The serving of *C.racemosa* sea grapes in Indonesia has been limited as a side dish at meals. Meanwhile, the analysis of the chemical composition of sea grapes shows that sea grapes have a high water content of 9.82%, so they are easily damaged (Santoso et al., 2006). Seagrape commodities require a fast handling process to save seagrapes from spoilage. They can be available for a more extended period so that their utilization can be optimal.

Various studies have proven that *C. racemosa* contains folic acid, thiamine, ascorbic acid, and phenols (Chew et al., 2008). These compounds make *C. racemosa* can be used as functional food and medicine (Anwar et al., 2016). In addition, sea grapes also contain natural fat that can increase fluidity to prevent large pores and overcome dry skin (Agrozine Editor, 2020). Recent studies have shown that sea grapes also have excellent antimicrobial and antioxidant properties (Marraskuranto et al., 2021). In connection with the above, we are interested in making hand cream from sea grape extract which has the potential to moisturize dry skin based on its nutritional content and natural fat. Then an evaluation of the antioxidant activity of sea grape extract and testing of the quality of sea grape hand cream was carried out.

Therefore, this study aimed to formulate a hand moisturizing cream containing *C. racemosa* extract and evaluate the quality of the cream preparation. Currently, no publication on the formulation and evaluation of cream preparations containing *C. racemosa* extract.

Research Methods

Materials and tools

The materials used in this study were sea grapes (*C. racemosa)* from Gowa Regency, South Sulawesi; methanol 99.8 % (Merck); aquadest; DPPH (Sigma Aldrich); stearic acid (Sigma Aldrich); cetyl alcohol (Sigma Aldrich); oleum cocos/coconut oil; Triethanolamine /TEA (Supelco); glycerol 85% (Supelco); methylparaben (Supelco); propylparaben (SAFC); and butylated hydroxytoluene/BHT.

The tools used include mortar and pestle; BUCHI rotary evaporators; Brookfield DV-E viscometer; IKA vortex - 2; pH meter, SHIMADZU UV-Vis spectrophotometer; EBA 20 HETTICH centrifuge; IKA hotplate magnetic stirrer; MEMMERT UN 110 oven; water bath EMMERT WN B14 RING; OHAUSS analytical balance; and other glassware

Sea grape processing preparation

Fresh sea grapes are cleaned of impurities, such as sand, stones, shells, and other types of seaweed. After cleaning, the green algae *C. racemosa* was washed with running water five times. Furthermore, fresh algae that have been washed weighed as much as 1572 grams and chopped (cut into small pieces) then dried in the sun to dry for about 2–3 days.

Sea grape extraction

C. racemosa was weighed as much as 50 g, then added 300 mL of methanol pa solvent. Extraction was carried out by maceration (soaking) for 3 x 24 hours in a closed vessel and protected from direct sunlight. During the extraction process, stirring is carried out periodically. After obtaining the liquid extract, the liquid extract was then concentrated using a rotary evaporator at 40^{o}C for 5 hours to obtain a concentrated extract. Next, the

concentrated extract was evaporated again in a water bath to obtain a thick extract.

Sea grape antioxidant activity test

At different concentrations, aliquot 0.5 mL of sea grape methanol extract was mixed with 0.5 mL of 1 mM DPPH (dissolved in methanol p.a to 5 mL). The concentration of *C. racemosa* extract used was 50, 100, 150, 200, and 250 ppm. The mixture was homogenized using a vortex and incubated for 1 hour in a dark room. The absorbance was read at a wavelength of 515 nm using a UV-Vis spectrophotometer. The inhibition of DPPH radical activity in percent (I%) was calculated based on the equation 1.

$$\%I = \frac{[(A_{blank} - A_{sample})]}{A_{blank}} \times 100\% \quad (1)$$

Where A_{blank} is the absorbance of the blank solution (containing all reagents except the test sample), A_{sample} is the absorbance of the sample. The IC_{50} value was determined from a plotted graph of the damping activity against C. racemosa samples. The same work was carried out on the BHT comparison with BHT concentrations of 2, 4, 8, 16, and 23 ppm.

Sea grape extract hand cream formulation

To make sea grape extract cream is as follows; as the oil phase, mix 1.5 g cetyl alcohol, 6 g stearic acid, and 2.2 g oleum cocos put into a porcelain dish, heat in a bath until melted, and stir until homogeneous. In the aqueous phase, mix 1.5 g TEA, 1.8 g glycerin, and 0.2 g methylparaben into a porcelain cup, heat in a bath until melted, and add 30 mL of distilled water, stirring until homogeneous. Added the oil phase to the water phase by stirring until the mixture thickens and cools. Furthermore, 4 g *C. racemosa* extract, which has been dissolved with 40 mL of aquadest, is added to the mixture, added to the remaining aquadest, and stirred until homogeneous (Allen, 2002).

Evaluation of sea grape extract cream

1) Organoleptic test

The organoleptic test was carried out visually; the components evaluated included the odor, color, shape, and texture of cream preparations (Azkiya et al., 2017). The organoleptic test is a descriptive test. This test can completely describe hand cream products, see differences in samples between 2 product formulas, perform unique identification such as aroma, and show certain intensity and quality (Tarwendah, 2017).

2) Homogeneity test

A tiny layer of cream is applied on a clean and dry slide, which is then covered with a glass slide. The cream is considered homogeneous if it has a flat texture and does not coagulate when examined under a microscope (Khopkar, 1990).

3) pH measurement

A pH meter is used to determine the pH of the preparation. To display the pH value, the pH meter was calibrated with a neutral standard buffer solution (pH 7.01) and an acidic pH buffer solution (pH 4.01). After that, the electrodes were cleaned and dried with distilled water. The sample was prepared at a concentration of 1%, and 1 g of the preparation was weighed and dissolved in 100 ml of distilled water. The electrode is then dipped into the solution. Allow the gadget to display the pH value until it is stable. The pH of the preparation is indicated by the number displayed by the pH meter (Lubis, 2012).

4) Viscosity

As much as 100 ml of the preparation was put in a container, the spindle was inserted to the immersion limit, and the rotor was run. Viscosity was measured using a Brookfield Viscometer model DV-E LV series with adjustable spindle and speed. This measurement is carried out at a temperature of 25 oC, and it will obtain the absolute viscosity of the preparation (Sundari, 2012).

5) Spreadability test

A total of 0.5 grams of the formulated cream was weighed and placed on a Petri coated with graph paper, given a petri dish on top of it, and left for 1 minute; the preparation area was calculated. Then given, a load of 50, 100, and 150 grams, respectively, is left for 60 seconds, and then the area of the preparation produced is calculated.

6) Adhesion test

As much as 0.3 grams of cream is smeared on a glass object with a known area. Place another glass object on the cream and then press it with 1 kg for 1 minute. The object-glass is installed on the test equipment, then a load weighing 80 grams is recorded, and the time is recorded until the two object glasses are separated (Engelin, 2013).

Results and Discussion

Sea grape extraction

Fresh sea grape samples were washed with running water, then soaked (maceration) for 3 x 24 hours using methanol, and protected from direct sunlight in a closed vessel. During the extraction process, stirring is carried out periodically. After obtaining the liquid extract, the liquid extract was then concentrated using a rotary evaporator at 40 ^{0}C for 5 hours to obtain a concentrated extract. Then the concentrated extract obtained was then dried using a freeze dryer; the aim was to maintain sample quality. According to Muchtadi and Sugiyono (1992), the dried material is first frozen and then followed by drying using low pressure for the freeze dryer process. The water content that has become ice will immediately turn into steam, known as sublimation.

Seagrape extract, after evaporation, obtained a thick extract of 50 mL, then after freeze-drying, the dry weight of the extract was obtained as much as 16.5 g. After calculating by equation 2, the yield obtained is 33 % of the total wet weight of 50 g sea grapes.

$$\%\text{Yield} = \frac{\text{Extract weight}}{\text{Sample weight}} \times 100\% \quad (2)$$

Sea grape extract antioxidant activity test

Antioxidants can stop, postpone, or slow down the oxidation process. The DPPH technique was used to measure antioxidant activity in this study. A nitrogen radical molecule is used in the DPPH method (1,1-diphenyl-2 picrylhydrazyl). DPPH will extract hydrogen atoms from a chemical, such as phenol compounds. The occurrence of DPPH is caused by electron transfer as a reaction mechanism. Because the DPPH approach is simple, quick, fast, and sensitive, and only requires a tiny sample, a quantitative antioxidant efficiency test was chosen. The presence of antioxidant effects in the sample caused the solution's hue to change.

Tabel 1. Sea grape antioxidant test results

Concentration (ppm)	Absorption	% Inhibition
50	0,9722 0,9537	37,53
100	0,8206 0,9051	40,38
150	0,8662 0,6362	48,65
200	0,8596 0,8459	57,89
250	0,8140 0,8115	59,49
Blank	1,2094	

Topical antioxidants are believed to catch free radicals that strike the skin as well as other free radicals. The cream is a non-sticky, easy-to-clean preparation with a moisturizing effect on the skin and excellent spreading capacity. The results of antioxidant measurements can be seen in Table 1. From the results of % inhibition

and concentration, a regression equation can be made to determine IC_{50}. The IC_{50} value is defined as the concentration of the test compound that can reduce free radicals by 50%. The smaller the IC_{50} value, the higher the free radical scavenging activity. From the results of testing the activity of antioxidant cream against DPPH for the formula that has been made, the IC_{50} value is 159.8 ppm. It is categorized as a weak antioxidant with range of 150-200 ppm (Molyneux, 2004). The results of the regression equation obtained can be seen in Figure 1.

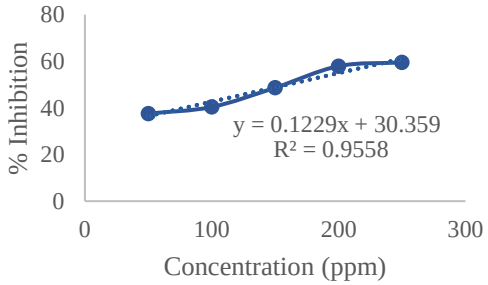

Figure 1. Sea grape antioxidant regression equation

Figure 2 shows the relationship between % inhibition and BHT concentration for comparison. The linear regression equation obtained for BHT is y = 2.6829 + 6.6404. From the linear regression equation, the IC50 value was 16.16 mg/L for BHT.

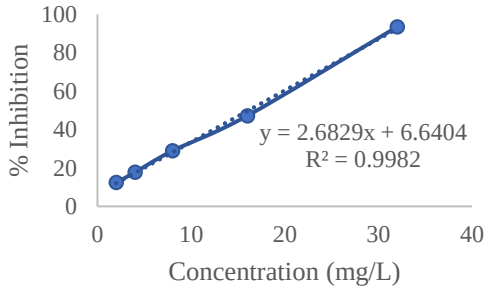

Figure 2. BHT antioxidant regression equation

Hand cream preparation

This hand cream is based on a standard hand cream formula taken from the Allen method (1999), but the ingredients used are modified. The ingredients used are stearic acid, cetyl alcohol, VCO coconut oil, triethanolamine, glycerine, methylparaben, and aquadest. The stearic acid in this formula serves as the oil phase.

Triethanolamine (TEA) functions as a strong base which, together with stearic acid, will react with saponification to form a monovalent soap emulsifier (Anief, 2000), often referred to as triethanolamine stearate soap with glycerol as a byproduct cetyl alcohol can function as an emulsifying agent, increasing the consistency and stability of the cream. VCO coconut oil functions as an oil phase that is also an emollient to prevent dehydration of the skin (Tumbelaka et al., 2019). Glycerine in this hand cream formula is used as a humectant, maintaining skin moisture. Methylparaben is used as a preservative in hand cream preparations. Grape odors are added because the extracted sea grapes still smell fishy (unpleasant).

In this formula (Table 2), there are 2 phases, namely the oil phase (phase A), which consists of stearic acid, cetyl alcohol, and VCO coconut oil. The aqueous phase (phase B) consisted of glycerin, TEA, methylparaben, and aquadest. In making hand cream, the oil and water phases are mixed. First, the oil and water phases were heated at the same temperature (60 ^{o}C). Heating aims to facilitate mixing because not all starting materials are in the form of liquids. Mixing liquid materials will be easier and more homogeneous than semi-solid materials so that the emulsification process can occur optimally. The resulting cream preparation can be seen in Figure 3.

Table 2. Sea grape hand cream formula

No.	Materials	Materials Weight	Function
1	Sea Grape Dried Extract	4 g	Active material
2	Stearic acid	6 g	Emulsifier
3	Cetyl alcohol	1.5 g	Stabilizer
4	Glycerin	1.8 g	Humectants
5	Methyl Parabens	0.2 g	Preservative material
6	TEA	1.5 g	Alkalizing agent
7	VCO Oil	2.2 g	Emollient
8	Aquadest	82.8 g	Solvent
9	Odors grapes	3 mL	Scent

Figure 3. Preparation of sea grape hand cream

Evaluation of hand cream preparation

1) Organoleptic test

The descriptive organoleptic observations showed that the cream had a light greenish-cream color, semi-solid dosage form (cream), soft texture and not too sticky, and had a characteristic wine smell mixed with a distinctive smell of coconut oil. The green color is obtained from the color of sea grapes, the soft texture, and the distinctive smell of coconut because it uses coconut oil as one of the ingredients to make hand cream. Then the hedonic acceptance test results were obtained from the rating scale/score on the intensity of the product, which has been divided into two formulations. Formulation 1 is a hand cream containing 1% sea grape extract added with BHT. While formulation 2 is a hand cream with 1% sea grape extract without additional synthetic ingredients. The assessment given by the panelists was based on the parameters of color, texture, aroma, moist impression, and sticky impression. This test is intended to determine the quality intensity and the presence or absence of significant differences between the hand cream formulas of pure sea grape extract and hand cream formulas that have been added with synthetic antioxidants (BHT) through a statistical t-test (Table 3).

Table 3. Hedonic acceptance test results

Panelist	Color		Texture		Scent		Moist effect		Sticky effect	
Formula	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**
1	7	7	7	6	7	7	8	7	8	7
2	5	6	4	7	5	7	5	7	6	6
3	6	6	7	3	7	4	7	3	7	5
4	5	5	7	7	5	7	4	5	3	3
5	5	5	7	7	7	7	7	7	3	3
6	7	7	6	6	4	4	8	7	4	3
7 8	5	5	8	7	4	4	7	5	7	5
9	5	5	7	6	5	5	7	6	5	5
10	5	5	5	4	5	4	5	4	5	5
11	5	5	4	4	4	4	4	4	5	4
12	8	8	8	8	6	7	8	7	8	8
13	7	7	4	6	5	7	8	8	4	4
14	7	7	6	8	5	6	8	6	7	7
15	7	7	7	8	5	5	7	8	6	8
16	7	7	8	8	6	7	7	8	8	8
17	4	4	4	8	3	3	7	5	4	4
18	6	7	8	9	3	4	7	7	6	5
19	5	5	6	6	4	4	3	7	4	6
20	6	6	5	5	6	7	7	6	7	6
21	6	7	7	6	6	5	6	7	7	6
22	5	7	6	8	3	3	6	7	9	9
23	4	4	7	5	3	3	7	6	7	7
24	6	6	4	7	3	3	7	8	4	6
25	6	6	4	6	3	2	7	7	7	6
26	4	7	4	7	4	7	5	7	4	7
Average	**5.77**	**6.08**	**6.00**	**6.46**	**4.69**	**5.00**	**6.54**	**6.38**	**5.73**	**5.72**
Standard Deviation	**1.107**	**1.093**	**1.470**	**1.449**	**1.320**	**1.673**	**1.392**	**1.329**	**1.733**	**1.671**

Note: F1 (1% Sea grape extract + BHT); F2 (1% Sea grape extract)

The T-test is one of the statistical tests used to test the truth or falsity of the hypothesis, which states that between two mean samples are taken randomly from the same population, there is no significant difference (Sudijono, 2010). T-statistics is a value used to see the level of significance in hypothesis testing by finding the value of T-statistics through the bootstrapping procedure. In hypothesis testing, it can be significant when the T-statistics value is greater than 1.96. In contrast, if the T-statistics value is less than 1.96, it is considered insignificant (Ghozali, 2016). Decision-making is done by looking at the significant value in the Coefficients table. Usually, the basis for testing the regression results is done with a confidence level of 95% or with a significance level of 5% ($\alpha = 0.05$). The criteria for the t statistical test (Ghozali, 2016):

a. If the significance value of the t-test > 0.05, then H_0 is accepted, and Ha is rejected. This means that there is no influence between the independent variables on the dependent variable.
b. If the significance value of the t-test < 0.05, then H_0 is rejected, and Ha is accepted. This means an influence between the independent variables and the dependent variable.

Table 4. Hedonic acceptance test results analysis

Test Type	Significance Value of Each Parameter				
	Color	**Texture**	**Scent**	**Moist effect**	**Sticky effect**
Variety	0.950	0.943	0.242	0.818	0.860
T- Independent	0.318	0.260	0.465	0.685	0.982

From table 4, it can be seen that the significance value of variance and independent t of each parameter is below the value of α (0.05). This shows that each parameter of color, texture, scent, moist effect and sticky effect of the two hand cream formulas tested organoleptically was not significantly different and did not show a significant difference. The results obtained are considered quite good, indicating the quality of the hand cream of sea grape extract organoleptically has no difference with hand cream, which contains synthetic antioxidants.

2) Homogeneity test

The homogeneity test results showed homogeneous results in the preparation of sea grape cream. Homogeneous results in the preparation are seen in undissolved granules when the preparation is squeezed between two object glasses. The results used indicated that the formulation used is quite good. The results of homogeneity can be seen in Figure 4.

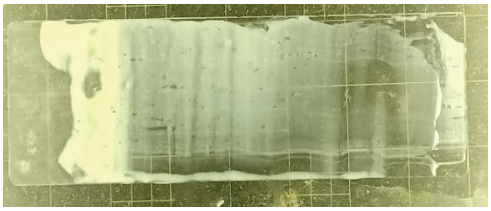

Figure 4. Homogeneity test results

3) pH measurement

The pH observations were carried out two times, namely 6.94 and 6.90. The pH requirement of a topical preparation is 5.5 - 10 (Soebagio et al., 2009). According to Soebagio et al. (2009), the results obtained show that the pH of the hand cream preparations has met the requirements. However, it is known that the physiological pH of the skin is 4.5 – 6.5, so the pH of the cream should melt the physiological pH requirements of the skin so that the skin does not become dry. The hand cream that is made already contains coconut oil which moisturizes the skin so that even if the pH is more than 6.5, it will not dry out the skin. This is also supported by the pH requirements of cosmetics that are safe for the skin, namely 4.5 - 8 (SNI 4399, 1996). The results of the pH test can be seen in Table 5.

Table 5. pH and viscosity test results

Repeat	pH	Viscosity (cps)	
		12 rpm	**20 rpm**
1	6,94	27800	25320
2	6,90	28800	24210
Average	6,92	28300	24765

4) Viscosity test

Measurement of the viscosity of the preparation was carried out using spindle R4 with RPM of 12 and 20. It has been carried out using another spindle, but the instrument cannot read. The viscometer used is the Brookfield viscometer. The results of the viscosity test can be seen in Table 5. The preparation's viscosity follows the requirements of 2000 – 50000 cps (SNI 4399, 1996).

5) Spreadibility test

The spreadability test was carried out to ensure an even distribution of the cream when applied to the skin. The ability to spread a good cream will ease application on the skin surface. In addition, the distribution of the active substances on the skin will be more evenly distributed so that the effects of the active substances become more optimal. The greater the spreadability of the cream, the better because the more comprehensive contact between the skin

and the cream so that the active substances contained can spread nicely and evenly. The dispersion obtained in table 6 is d = 5 cm, which meets the 5–7 cm (SNI 4399, 1996).

Table 6. pH and viscosity test

Spreadability Test Result	Adhesion Test Result
d = 5 cm	2 minute
Surface area = 19.63 cm^2	53 second

6) Adhesion test

Testing the stickiness of the cream was carried out to determine the stickiness of the cream on the skin by measuring the length of time the cream was attached. This will relate to the time the cream contacts the skin until the desired therapeutic effect is achieved. Good adhesion of the cream is 2-300 seconds from the test results obtained 2 minutes 53 seconds or 173 seconds. This is following the requirements for the stickiness of the cream (SNI 4399, 1996).

Conclusion

Based on the research that has been done, it can be concluded that sea grapes have a weak antioxidant power with a value of 159.8 ppm. The sea grape extract can be formulated into hand cream preparations. Based on the hedonic test, the hand cream formulation with the addition of sea grape extract was not significantly different from the hand cream containing BHT (synthetic antioxidant). This preparation has physical characteristics in terms of pH (6.92), viscosity (28300 and 24765 cps), homogeneity, spreadability (d = 5 cm), and adhesion (2 minutes 53 seconds) following the requirements for cosmetic preparation from the Directorate General of BPOM.

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