Formulation, Physical Characteristics and Antioxidant Activity of Chlorophyll-c Emugel Preparation from Brown Seaweed (*Sargassum polycystum*)

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Abstract. Brown seaweed (*Sargassum polycystum*) contains a distinc- tive pigment, chlorophyll-c. Chlorophyll is known to exhibit strong antibacterial activity against gram-positive bacteria. Emulgel is one of the topical preparations that offers several advantages, including being non-greasy, easy to spread, easy to clean, soft, easy to wash, having a longer shelf life, transparent and comforta- ble when used. The purpose of this study was to formulate chlorophyll-c isolate in the form of an emulgel preparation to be tested for physical characteristics, antioxidant activity, and irritation tests on rabbits. The research was conducted by isolating chlorophyll-c using column chromatography and preparative thin layer chromatography (PTLC) methods. Emulgel dosage formulations were cre- ated with different chlorophyll-c content as the active substance, consisting of FI 0.2%, FII 0.4%, and FIII 0.6%. The results showed that there was an influence of chlorophyll-c concentration on the characteristics of the emulgel preparation. The higher the concentration of chlorophyll-c, the greener the color of the emulgel, the pH decreases, the spreadability decreases, but the viscosity and stickiness increase. Testing of chlorophyll-c emulgel on antioxidant activity showed that the higher the concentration of chlorophyll-c, the greater the antioxidant activity. Giving chlorophyll-c emulgel to rabbits causes very mild irritation with the appearance of erythema or redness and edema that is not very clear.

Keywords: Emulgel, Chlorophyll-C; Brown Seaweed; Irritation Test; Physical Characteristics; Antioxidant.

1 Introduction

Indonesia is a maritime country that is rich in marine natural resources. One of them is seaweed, as many as 550 types of economically valuable seaweed variants exist in Indonesia. Brown seaweed such as Sargassum polycytum is the type that is most commonly found and used [1]. This type contains compounds that can be used as antioxidants. A typical compound that can be found and has antioxidant activity is chlorophyll c [2].

Chlorophyll possesses antioxidant activity, serving as a compound capable of counteracting free radicals. The chlorophyll molecule is composed of 4 pyrrole rings with

Z. B. Pambuko et al. (eds.), *Proceedings of 5th Borobudur International Symposium on Humanities and Social Science (BISHSS 2023)*, Advances in Social Science, Education and Humanities Research 856,
https://doi.org/10.2991/978-2-38476-273-6_48

Mg as the core. While pigments show great potential as antioxidants, they are less stable and easily degraded when exposed to light or oxygen, thus affecting their antioxidant activity in emulgel preparations with carbopol 940 as the gelling agent. Emulgels when used dermatologically, have several beneficial properties such as being thixotropic, non-greasy, easy to spread, easy to clean, soft, easy to wash, longer shelf life, transparent and comfortable when used [3].

Based on the description above, an emulgel preparation formulation will be conducted using active substance chlorophyll-c from *Sargassum polycystum* which meets the physical characteristics test including organoleptic, homogeneity, pH, spreadability, adhesiveness, and viscosity. In addition, it tests the antioxidant activity of chlorophyll-c extract emulgel preparations and the safety test of the preparation, namely the irritation test on rabbit test animals.

2 Methods

2.1 Extraction and Isolation of Chlorophyll-c from *Sargassum polycystum*

A total of 100 grams (20 x replicates) of fresh brown seaweed (*Sargassum polycystum*) was macerated with 1liter mixture of acetone: methanol (7:3) solvent, along with the addition of CaCO3 and antioxidant BHT [4]. Maceration was carried out in a place protected from light and at room temperature. The resulting macerate had its solvent evaporated using a rotary evaporator at 35 ºC to yield a thick extract. The thick extract undewent liquid-liquid separation with n-hexane as the solvent, to separate the compounds based on their polarity. The separation was assisted by the addition of saturated salt solution and tap water [5]. The green n-hexan fraction suspected of containing the target compound chlorophyll-c was tested for compound confirmation using the TLC method with a mobile phase of n-hexan: ether: acetone (6:3:2).

Chlorophyll-c isolation was carried out using the column chromatography method with a mobile phase of n-hexan: ether: acetone (6:3:2). Column fractions were collected in each vial and the separation of the band color was observed. Each column fraction underwent purity testing to identify pure compounds and those still mixed with others. Column fractions that are still not pure are further separated by TLCP (Preparative Thin Layer Chromatography) method with the same mobile phase as the column. This process produced distinct chromatogram bands, with chlorophyll-c exhibiting a specific yellowish-green color Chlorophyll-c isolate underwent purity testing with three different mobile phases and its spectra pattern was analyzed using a UV-Vis spectrophotometer at wavelengths ranging from 350 to 800 nm.

Emulsions are made by mixing the oil phase into the water phase (M/A). The emulsion was made by mixing liquid paraffin and span 80 (oil phase) with tween 80 and distilled water (water phase) on a 70°C water bath. The two phases were mixed in a porcelain cup and stirred until homogeneity was achieved [6]. Subsequently, chlorophyll-c (0.2%, 0.4%, and 0.6%) was added to the prepared emulsion base and stirred until a uniform and well-distributed dosage mass was formed. Carbopol 940 0.5% was

dispersed in 20 ml of hot water with constant stirring until homogeneity was achieved, and the pH was adjusted using triethanolamine. It was then dissolved using methyl paraben and propyl paraben with propylene glycol, and the mixture was added to the previously formed gel base [6]. The emulsion base, mixed with chlorophyll-c, was gradually added to the gel base until homogeneity was achieved. The remaining distilled water was added while stirring continuously until homogeneity was reached, then put in a container. Next, the emulgel was stored in a dark place at a temperature of 10 °C-15°C. Formulation of chlorophyll c show at Tabel 1.

Table 1. Formulation of chlorophyll c

Material	F I (%)	F II (%)	F III (%)	Utility
Isolate chlorophyll c	**0.2**	**0.4**	**0.6**	**Active substance**
Carbopol 940	0.5	0.5	0.5	Gelling agent
Trietanolamin	0.43	0.43	0.43	Alkalizing agent
Tween 80	2.5	2.5	2.5	Surfactant (water phase)
Span 80	2.5	2.5	2.5	Surfactant (oil phase)
Propylene Glycol	5	5	5	Cosolvent
Propylparaben	0.015	0.015	0.015	Preservative
Methylparaben	0.03	0.03	0.03	Preservative
Liquid paraffin	2.5	2.5	2.5	Emollient (oil phase)
Aquadest	Ad 50	Ad 50	Ad 50	Solvent

Information:
FI: Formula I emulgel with the active compound chlorophyll-c as much as 0.2%.
FII: Formula II emulgel with the active compound 0.4% chlorophyll-c.
FIII: Formula III emulgel with the active compound chlorophyll-c as much as 0.6%.

2.2 Physical Characteristics Test of Preparations

Organoleptical test were conducted by observing the physical appearance of the preparation including its shape, color, and odor. The homogeneity test involves applying the preparation to a glass object, where it should exhibit a homogenous composition without any coarse grains. The pH test of the preparation uses a pH meter, with the constant number shown indicating the pH of the preparation. The pH requirements for topical preparations typically range from 4.5-6.5 [3]. Viscosity test of the preparation was measured using a Brookfield Viscometer with spindle 64. The viscosity value for semi-solid preparations is 6000-50000 cP [6].

The spreadability test involves placing 1 gram of the preparation on glass, then covering it with another glass and applying a 50-gram load for 1 minute, recording the diameter. Weights are added periodically until the diameter remains constant [7]. The requirement for good emulgel spreadability is 5-7 cm [6]. In the adhesion test, several samples are positioned on a glass object and then pressed by a 1 kg load for 5 minutes. The glass object is then affixed to a testing device suspended by an 80-gram load, and the time it takes for the two glass objects to detach is recorded. This time is calculated as the adhesion.

2.3 Antioxidant Activity

The pigment isolate was dissolved in methanol and various concentration series were made. The blank was a methanol solution, while the sample solution consisted of 4 ml DPPH plus 1 ml extract. Both the blank and the sample were incubated for 30 minutes in a dark room, then their absorbances were measured at a wavelength of 517 nm using a single UV-visible spectrophotometer Shimadzu 1240 [8].

$$\% \text{ Inhibitory} = \frac{[DPPH]_0 - [DPPH]_s}{[DPPH]_0} \text{X}100\%$$

$[DPPH]_0$ = Initial DPPH concentration

$[DPPH]_s$ = Final DPPH concentration remaining

2.4 Irritation Test of Chlorophyll-c Emulgel Preparation

The irritation test was conducted on adult male albino rabbits. Rabbits that have been acclimatized for 24 hours, then shaved 24 hours before the experiment. The test was conducted using the patch test method. The rabbit's back was divided into 4 shaved areas measuring 2 x 3 cm with the codes FI, FII, FIII, and KN (emulgel system without chlorophyll-c). The test preparation as much as 0.5 gram was applied to the back skin (shaving area). After drying, the test area was covered using non-irritant gauze. The treatment was observed at the 3rd minute, 1st hour, 4th, 24th, 48th and 72nd hours after the application of the preparation [9].

3 Result and Discussion

The isolation of pigments from brown seaweed (*Sargassum polycystum*) using the column chromatography method resulted in six fractions: yellow, orange, yellow- green, blue-green, gray, and yellow. The dry fraction obtained from column chromatography was identified by thin layer chromatography method to determine the stain profile that appears, so that the purity of the compounds obtained can be known. The TLC analysis, employing the same mobile phase, revealed that the separation achieved by column chromatography was not entirely pure. Some compounds were still mixed, as indicated by the appearance of four stain profiles on the TLC plate.

The isolation of chlorophyll-c pigment compounds was furthered through the Preparative Thin Layer Chromatography (PTLC) method applied to the impure column fraction. A silica stationary phase was used for preparative chromatography, employing the same n-hexane: ether: acetone (6:3:2) mobile phase as in the column chromatography isolation. The outcomes of the *Sargassum polycystum* chlorophyll-c separation using preparative thin-layer chromatography.

Purity test of chlorophyll-c isolates resulting from separation by preparative thin layer chromatography (PTLC) was carried out using the identification method of separation by thin layer chromatography. There are 3 mobile phases used, namely n-hexane: ace- tone: ether (6:3:2), n-hexane: ethyl acetate (7:3), n-hexane: acetone (8:2). The stain that appears on the stationary phase (silica gel) shows a yellowish green color and only shows a single stain. Based on the test results obtained, it is concluded that chlorophyll-c is pure and not mixed with other compounds. The presence of a single stain in some of these TLC tests indicates that a compound with a high level of purity has been obtained [11].

Identification of chlorophyll-c isolate spectral pattern was carried out with the aim of ensuring that the isolate taken was true chlorophyll-c. Identification of spectral pattern using UV-Vis spectrophotometer instrument at wavelength (λ) 350-800 nm. The spec- tra pattern of the research results were compared with the spectra pattern in the literature [10] with the same solvent shown in Fig 1.

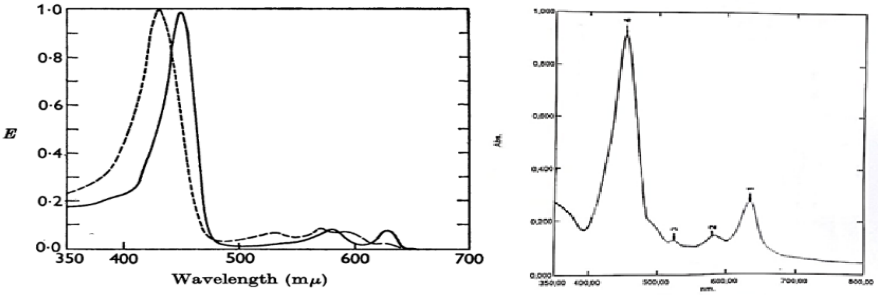
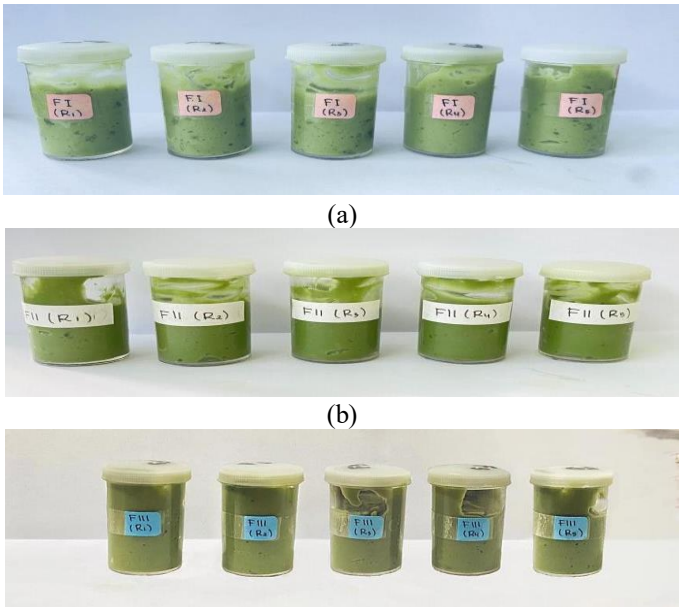


Fig. 1. Chlorophyll c spectrum pattern; literature (a) and chlorophyll c isolate (b)

The results of the spectra pattern of chlorophyll-c isolates have a pattern that is almost the same as the spectra pattern in the literature. There are 4 peaks in the spectra of chlorophyll-c with a wavelength of 451-632 nm. According to [10] the maximum ab- sorption of chlorophyll-c is at 446.1-629.1 nm where when compared with the spectra pattern of wavelength identification results still meet the range and in accordance with the literature.

Chlorophyll-c isolate was then formulated in an emulgel preparation. Emulgel was chosen because the active substance chlorophyll-c is a non-polar compound so that it is easier to mix on a preparation base that also has non-polar properties such as emulsion. Formulation of emulgel preparations with 3 different active substance formulas, namely chlorophyll-c concentrations of 0.2%; 0.4%; and 0.6%. The results of the emulgel physical characteristics test are presented in Fig 2 and Table 2.



(a)

(b)

(c)

Fig. 2. Emulgel formulation (a) formula I, (b) formula II and (c) formula III in 5 replication

Table 2. Physical Characteristics Test of Chlorophyll-c Emulgel

Physical Characteristics Test	Formula I	Formula II	Formula III	Standard	Meets Standards/Not
Organoleptic: Form Smell Color	Semi solid Typical base Light yellow green (+)	Semi solid Typical base Light yellow green (++)	Semi solid Typical base Light yellow green (+++)	Semi solid	Meets Standards
Homogeneity	Homogenous	Homogenous	Homogenous	No coarse particles	Meets Standards
pH	5.82 ± 0.015	5.57 ± 0.029	5.15 ± 0.028	4.5-6.5	Meets Standards
Viscosity (cP)	17968± 99.239	18497± 157.098	19080± 123.027	6000-50000 cP	Meets Standards
Spread power (cm)	5.7 ± 0.071	5.5 ± 0.114	5.2 ± 0.084	5-7 cm	Meets Standards
Sticking power (second)	4.15 ± 0.027	4.23 ± 0.022	4.37 ± 0.024	> 4 second	Meets Standards
Antioxidant activity (% inhibisi)	26	37	42	Have activity	Meets Standards

The safety parameters of the emulgel preparation when used are known based on the results of the irritation test. Irritation tests are carried out to determine the presence of irritating effects on the skin and to assess and evaluate the characteristics of a substance when exposed to the skin [9]. Observation of the acute dermal irritation test is carried out qualitatively by observing the presence or absence of edema and erythema that arise and is carried out quantitatively by determining based on the value of the edema and erythema score based on ISO guidelines.

According to [12], the primary irritation index of chlorophyll-c emulgel preparations can be grouped based on the category of irritation on rabbit test animals. The assessment of the irritation index that appears on the rabbit's back is calculated until the average result is obtained, which is clearly shown in Table 3.

Table 3. Primary irritation index score

Treatment	Mean primary irritation index	Irritation category [12]
Formula I	0.04	Very mild
Formula II	0.016	Very mild
Formula III	0.016	Very mild
Base	0.32	Very mild

The results of the irritation test indicate that the concentration of chlorophyll-c in the emulgel influences the occurrence of irritation, as evidenced by the onset of erythema following the application of the preparation on the rabbit's back. It is observed that higher concentrations of the active substance in the formula correspond to a lower onset of irritation on the rabbit's back. This irritation is believed to be associated with the resence of components that have the potential to cause moderate skin irritation, including methyl parabens and propylene glycol.

Irritation on the base application area is higher than the formula, this may be due to the pH factor. Chlorophyll-c emulgels and bases are stored at cold temperatures in the refrigerator, with the aim of maintaining the chlorophyll-c content in preparations that are very susceptible to temperature, heat, or light. Storage temperature can also affect changes in the pH of the preparation which becomes increasingly alkaline. The pH of the emulgel base is known to be around pH 5.96 and increases at low storage temperatures. Irritation can also arise during the shaving treatment of test animals where the friction between the shaver and the skin surface is too deep which triggers irritation on the rabbit's skin.

4 Conclusion

The difference in chlorophyll-c concentration in the emulgel formula affects the characteristics of the preparation and the antioxidant activity. As the concentration of the active substance in the formula increases, so do the viscosity, adhesion, and antioxidant activity, while the pH value of the preparation decreases, along with the spreadability

and skin irritation on the rabbit's back. The observed irritation, including erythema and edema, is classified as very mild for male albino rabbits.

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