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Production of Liquid Chlorophyll from The Leaves of Green Grass Jelly (*Premna oblongifolia Merr.*)

Novelina, Tuty Anggraini and Rudi Hermansyah

Faculty of Agricultural Technology, Andalas University, Padang, West Sumatera, Indonesia
E-mail: novelinasutanto@yahoo.com

***Abstract*— Chlorophyll is known to be used as a natural dye. The last few years it is known that chlorophyll has an important role as a source of antioxidants that are good for health. The availability of sources of chlorophyll in Indonesia is very large, one of which is the green grass jelly leaves (Premna obliongifolia Merr). The research objective is to get grass jelly leaf extract as a source of chlorophyll and know the characteristics of the resulting extract chlorophyll. The process of extraction is done by maceration with ethanol and addition of 7% NaHCO3. The yield of the concentrated extract obtained was 35% -36%. Concentrated extract of leaves of grass jelly contains the highest levels of total chlorophyll 1184.475 mg / L. The antioxidant activity of IC 50 (ppm) is 6533.9. Likewise, the results of toxicity tests is known that chlorophyll extract treatment response inactive with LC50> 1000 mg / mL is 1170.5 to 1504.8 mg / mL.**

***Keywords*— Leaves of grass jelly; chlorophyll; antioxidants**

I. INTRODUCTION

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Availability of chlorophyll in nature is very large. Gross [1] stated that the average chlorophyll content of leaves of 1% (dry basis). Similarly, its biological properties, has a great opportunity to make chlorophyll to be developed as a supplement or functional food. Supplement or functional food based chlorophyll circulating in Indonesia, almost all of which are imported products and has a selling price which is quite high, whereas the availability of sources of chlorophyll in Indonesia is very large. It is therefore necessary exploration of the sources of chlorophyll which is very potential to be developed, one of which is the green leaves of grass jelly (*Premna obliongifolia* Merr). Utilization of grass jelly leaves are still not optimal addition of this plant is a plant that is grown locally because of geographical conditions that support. The potential of chlorophyll from the leaves of green grass jelly nice to be developed in Indonesia can invite people to be able to cultivate the green grass jelly leaves, so that the community can also increase revenue by producing products that are good for health such as liquid chlorophyll products and applications to other food products, which are crucial for public health.

Chlorophyll susceptible to damage during processing so that it takes the application of science and technology in creating products that have antioxidants. The application of science and technology are performed in this study is a way to keep the loss of $Mg2^{+}$ ions at the center of the chlorophyll structure so that the color and antioxidant capacity of the extract obtained can be maintained. For that we need an alternative in maintaining the color and antioxidant capacity during processing, one of which is using $NaHCO_3$ as well as a good application processing. The addition of $NaHCO_3$ during the process of extraction of chlorophyll can increase the pH value in the solution so as to reduce damage to chlorophyll. Ferruzi [2] said that with the influx of Na + ions into the structure of chlorophyll is expected chlorophyll produced is hydrophilic so that the resulting increased antioxidant activity. The purpose of research is to produce grass jelly leaf extract as a source of chlorophyll and know the characteristics of the resulting extract chlorophyll.

II. MATERIALS AND METHOD

A. Materials and equipment:

The materials used are the leaves of fresh grass jelly, ethanol, $NaHCO_3$ and chemicals for analysis. The equipment used is the incubator Shaker, Rotary evaporator, spectrophotometer, pH meter, scales of analysis, rough

scales, stainless steel knives, stainless steel scissors, plastic containers, refrigerator and a set of tools glasses.

B. Extraction of Chlorophyll

Extraction of chlorophyll based on research [3] which has been modified in the following order: (1) The leaves of green grass jelly sawed ± 1 cm, then weighed as much as 50 grams. Followed by the addition of ethanol 1: 3 (w / v); (2) Then added $NaHCO_3$ 0.7% of the amount of ethanol used, then dissolved into 10 ml of water with the aid of heat. (3) After the maceration extraction process is done by using a shaker at room temperature for 24 hours. (4) The results of the extraction of filtered using filter paper with the aid of a vacuum filter. (5) The resulting filtrate is collected in erlenmeyer, while the residue extracted again with the same procedure to obtain a filtrate with a low absorbance indicating that all the chlorophyll has been extracted.(6) The extract obtained was then evaporated the solvent with the help of a rotary vacuum evaporator until the concentrated extract obtained. (7) Preparation of chlorophyll concentrations can be obtained in which the concentrated extract then frozen first and then the remaining solvent was evaporated with the aid drying freezer. (8) Preparation of liquid chlorophyll is done by obtaining chlorophyll concentrations dissolved in water to 100 ml.

C. Observation

Observations were conducted on the resulting yield of chlorophyll, total chlorophyll with spectrophotometer, phyto-chemicals contains with qualitative methods [4], the toxicity by Brine Shrimps methods [5], and antioxidant activity by DPPH method [6].

III. Results and Discussion

A. The Yield of Chlorophyll Extract

The yield of the extract was calculated based on the yield of the extract in water, where the calculation results as shown in Table 1.

TABLE I
YIELD OF CONCENTRATE AND LIQUID CHLOROPHYLL FROM THE GREEN GRASS JELLY LEAVES

Sample	Green grass jelly Leaf (g)	$NaHCO_3$ Maceration I (g)	$NaHCO_3$ Maceration II (g)	Concentrated (g)	The yield of extract (%)	Liquid (g)	The yield of the extract in water (g)
A1	50,0125	1,0513	1,0511	18,01	36,01	180	10,00
A2	50,0254	1,0511	1,0521	18,12	36,22	181	10,01
A3	50,0174	1,0512	1,0531	18,14	36,27	181	10,02
Average				18,09	36,17	180,67	10,01

This study begins with the manufacture of concentrated extract chlorophyll. The resulting concentrated extract chlorophyll has an average weight of 18.09 g with a yield of 36.17%. Concentrated extract derived chlorophyll concentrations were high that needs to be done in order to obtain Liquid Chlorophyll dilution. Liquid chlorophyll is a food supplement that is beneficial to health, where there is content to have high levels of chlorophyll. Liquid chlorophyll has produced an average weight of 180.67 g with a yield of 10.01.

According to Laborde and Von Elbe (1994) *cit* Alsuhendra [7] that the addition of some alkaline substance in vegetables can retain the green color of chlorophyll due to the increase in pH, then with high pH, and high stability chlorophyll.

B. Concentrated and Liquid of Chlorophyll Extract

The results of the analysis of total chlorophyll can be seen in Table 2 Total chlorophyll concentrated grass jelly leaves have the highest levels of 1184.475 mg / L, then the total chlorophyll liquid has amounted to 219.94 mg / L. Green grass jelly leaves that had been consumed by the public turned out to contain chlorophyll which is quite high, but the measurement results were lower than the results obtained by other research. According to the research results Kusharto *et al* [8], leaves of grass jelly contains the highest chlorophyll 1709 ppm. This is due to differences in the use of solvents.

TABLE II
CHLOROPHYLL TOTAL

Material	Sample Code	Abs λ : 645 nm	Abs λ : 663 nm	Preliminary Chlorophyll Levels (mg / L)	Dilution Factor	Final Chlorophyll Levels (mg / L)	Average Chlorophyll Total (mg / L)
Concentrate	A1	0,352	0,657	123,1618	10	1231,618	**1184,475**
	A2	0,31	0,579	108,4978	10	1084,978	
	A3	0,353	0,661	123,6828	10	1236,828	
Liquid	B1	0,698	1,001	220,0198	1	220,0198	**219,94**
	B2	0,699	0,995	219,7388	1	219,7388	
	B3	0,699	0,999	220,0596	1	220,0596	

C. Toxicity tests with the "Brine Shrimps" method

The results of toxicity test chlorophyll extracts of green grass jelly leaf by the method of "Brine Shrimps" can be seen in Table 3. Based on the calculation results obtained LC50 values for each treatment still.

The result of the calculation is known that, the three treatments were not actively responding to the value of LC50> 1000 mg / mL is 1170.5, 1386.4 and 1504.8.

TABLE III
RESULTS OF TOXICITY TESTS WITH THE METHOD OF "BRINE SHRIMPS"

Treatment	Concentration (ppm)	The number of dead larvae			The death average (%)	Probit Value (Y)	Log concentration (X)	LC_{50} (µg/mL)
		Vial I	Vial II	Vial III				
A	1000	3	2	2	23.33	1170.5	3	
	100	2	1	1	13.33		2	
	10	0	0	0	0		1	**1170.5**
Control	0	0	0	0	0			
B	1000	2	2	2	20	1386.4	3	
	100	1	1	1	10		2	
	10	0	0	0	0		1	**1386.4**
Control	0	0	0	0	0			
C	1000	2	2	2	20	1504.8	3	
	100	1	1	0	6.67		2	
	10	0	0	0	0		1	**1504.8**
Control	0	0	0	0	0			

Toxicity of the plant extracts against larvae of shrimp can be determined from its LC50 value. If the LC50 <1000 mcg / mL, then said to be active and when LC50 <100 mg / mL, then said to be very active. According to Meyer *et al*., [9] a compound is said to be active or cytotoxic if it has LC50 values (concentrations that kill 50% of larvae shrimp) below 1000 ppm.

D. Antioxidant Activity of the Chlorophyll Concentrated

Measurement of antioxidant activity obtained the highest antioxidant activity in concentrated of green chlorophyll leaves of grass jelly with 6533.9 ppm which may counteract free radicals by 50%. According to Endo *et al* (1985) *cit* Prangdimurti [10] chlorophyll has the ability to capture the DPPH radical. The antioxidant activity of the extract was measured by its ability to donate an electron to the free radical DPPH (colored purple) that reduces to DPPH-H (yellow or colorless).

E. Phytochemicals Content of Chlorophyll Extracts

The results of the analysis of liquid chlorophyll phyto-chemical compounds detected include phenolic compounds, flavonoids, triterpenoids, steroids, alkaloids, and coumarin characterized by a positive (+), but does not contain saponins. The more positive value indicates that the material contains a compound that is high, such as phenolics, steroids, and coumarin**.**

Identification of phenolic compounds give positive results. This is caused by the formation of a complex between the iron compounds with phenol gives a green color [4]. Phenolic compounds contained in plant dragon scales have antioxidant activity because these compounds can capture radicals and peroxides can bind ferrous metals which catalyze peroxide fat. Usually the compounds that have antioxidant activity are phenolic compounds that have a hydroxy group substituted in the ortho position and the -OH group and -OR [11]. Similarly, other compounds are flavonoids tested showed positive results. Flavonoids are one of the classes of phenolic compounds which can inhibit the activity of free radicals by donating an electron pair on the hydroxyl group.

TABLE IV
RESULTS OF PHYTOCHEMICAL ANALYSIS OF CHLOROPHYLL LIQUID

Phytochemical components	Results
phenolic	++++
flavonoids	++
triterpenoids	++
steroids	++++
alkaloids	++
coumarin	++++
saponins	-

Other secondary metabolites are compounds tested alkaloids, triterpenoids and steroids. The results showed that the positive alkaloid with the formation of white precipitate, triterpenoids positively by giving a red color with Lieberman-Burchard reagent. Similarly positive steroid test results of testing by giving a green color. The content of triterpenoids in plants is as anti insects [4].

IV. CONCLUSIONS

The results showed that the research that has been done, the manufacture of chlorophyll concentrate can be done by maceration method and the addition of $NaHCO_3$ to 7% and evaporate the residual solvent with a rotary evaporator and a

water bath. Concentrate obtained has chlorophyll content 1184.475 mg / L. Liquid product made by adding water to the concentrate at a ratio of 1:10 still showing solid green color. Products are safe to eat and have chlorophyll content of phenolic compounds, Flavonoids, Triterpenoid, steroids, alkaloids, and coumarins. The antioxidant activity of chlorophyll concentration is 6533.9 ppm.

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