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## Synthesis Biomaterial Chlorophyll E. dulcis modified Albumin for Photosensitiser Organic.

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### ABSTRACT

Chlorophyll is a green pigment present in the chloroplast together with carotene and xanthophyll in all living organisms that carry out photosynthesis. Chlorophyll *Eleocharis Dulcis* can be applied as Optical Biomaterials of Organic Exogenous Photosensitiser in Photodynamic Cancer Cell Therapy is very important because chlorophyll has several peak wavelengths. Chlorophyll will work optimally if its wavelength has maximum intensity. Although varied, all chlorophyll have almost the same chemical structure, which consists of closed porphyrin (cyclic), tetrapyrrole, with magnesium ions. The green colour of the leaves is influenced by the presence of Mg atoms on the ring at its centre and the tail is terpenes. Both of these groups are chromophores and are capable of exciting electrons when exposed to light at certain wavelengths. In general, chlorophyll has several wavelengths between 400 - 700 nm. Albumin is a protein that can remind stability and does not change the basic properties of chlorophyll, maximum absorption results at 658 nm, and particle size of 181 nm, toxicity test results of mammary cancer cells in a safe concentration of 0.01 mg / ml with mortality rate of 18 - 25%.

**Keywords:** Chlorophyll, Albumin, Photosensitiser, Photodynamic Therapy

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## INTRODUCTION

Photosensitizer is the most important part in photodynamic therapy, photosensitizer is a chemical compound that is useful to increase light stimulation to a network or cell, requirement High purity and chemical composition known, Easy to produce, High Quantum Yield, Strong absorption at 680 - 800 nm, Extinction coefficient 50.000 - 100.000 / M cm, Low Toxicity, Stable, Complementary Material (Ormond and Freeman, 2013).

Chlorophyll is a green pigment present in the chloroplast together with carotene and xanthophyll in all living organisms that carry out photosynthesis. In all green plants, most of the chlorophyll is in two forms: chlorophyll a and b. Chlorophyll a is less polar and is blue in green, while chlorophyll b is polar and yellow in green. Chlorophyll is green because it absorbs strongly in the red and blue regions of the visible light spectrum. The empirical formula of chlorophyll a is  $C_{55}H_{72}O_5N_4Mg$ , whereas chlorophyll b is  $C_{55}H_{70}O_6N_4Mg$ , at Figure 1. Chlorophyll structure a and b (Hung et al, 2014). The pigment represents a porphyrin containing a tetrapyrrole base ring. The four rings bind to the ions  $Mg^{2+}$ . The fifth isocyclic ring lies close to the third pyrrole ring. Chlorophyll has an absorption wavelength of between 400 - 700 nm. Maximum intensity is at 400 - 450 nm and 650 - 700 nm with color conversion from blue to red. The number of absorption peaks will cause a small intensity.

Figure 1: Extraction *Eleocharis dulcis*



Chlorophyll has been extensively explored including chlorophyll a stable from acidic processes (Budiyanto et al, 2008), Chlorophyll for acne therapy (Song, 2014) Chlorophyll for bioimaging (Mitra et al, 2014), chlorophyll used is chlorophyll which has a level of stability which is low because Mg atoms can be easily replaced by hydrogen atoms so that in the extraction process additional substances are needed such as  $ZnCl_2$  (Ozkan & Bilek, 2015). Chlorophyll can be extracted by the method of adding the chemical solution of diethyl ether and sodium sulphate or other polar chemical solutions such as ethanol with high purity chlorophyll and high molecular stability (Dentuto et al, 2007). Plants that can be extracted to take chlorophyll such as papaya leaves, spinach and other leaves but its use will interfere with other benefits, from the type of grass found that chlorophyll content such as reeds contain  $2326.3 \mu\text{g} / \text{g}$  and elephant grass contains  $2673.2 \mu\text{g} / \text{g}$  Chlorophyll while plants of grass and the benefits are still low as purun mice in the swamp of South Kalimantan, purun rats are usually in a dry state will cause a fire.

*Eleocharis dulcis* is a typical plant of swamp (Indrayati, 2014) which has erect stem, unbranched, gray to shiny green color with length 50-200 cm and thickness 2-8 mm, 32% Cellulose, Lignin 26 %, While chlorophyll has not been extracted (Wianto et al., 2012), while the highest yield of extraction for vegetables is owned by papaya leaves of  $29.5 \text{ mg} / \text{g}$  (Setiari & Nurchayati, 2010).

Albumin is the main protein present in human blood. Regulating osmotic pressure in the blood is a major function of proteins produced by this liver. The balance of albumin is needed to keep the fluid contained in the blood from leaking into the tissues of the body. Albumin in addition to the can also be used for coating in an effort to get a stable photosensitizer.

## MATERIALS AND METHODS

Eleocharis dulcis obtained from Banjarbaru swamp, ethanol, aquades, albumin. The chemicals in use are technical

### Ekstraktion

Extraction is a method of separation in which components of a solid or liquid are transferred to another liquid which acts as a solvent. The extraction may be carried out for mixtures having adjacent boiling points, so they can not be separated by distillation. The inter-phase shift occurs when there is a difference in concentration which moves from a higher concentration system to a lower-concentration system (Ozkan and Bilek, 2015). Chlorophyll extraction is carried out in a stirred batch reactor.

The pre-treated powdered mice and 500 ml of solvent in the form of 80% acetone were introduced into the batch extractor according to the ratio of feed and solvent mass (F: S) of 1: 17.1. Acetone will lead to the occurrence of protein denaturation that binds chlorophyll so that chlorophyll can escape from the bond with the protein and participate extracted in the solvent (Hung et al 2014).

With this method yield (percentage of chlorophyll obtained compared to chlorophyll content in raw material which indirectly shows the effectiveness of extraction) obtained is 92,9796%. Thus, using the above method can be obtained by optimal green dye. And furthermore this chlorophyll can be used as an extract for further processing.

The method for making coated is 10 µg chlorophyll and 10 µg albumin dissolved in 2 ml chloroform and evaporated on thin film. Thin film washed with nitrogen to remove chloroform. Phosphate buffered saline (PBS) (1 mL) is inserted into the flask and stirred for 30 minutes followed by sonification for 15-20 minutes. After the sonification of the solution is kept at 40C and in the dark (Maoquan et al, 2014)

### Analysis

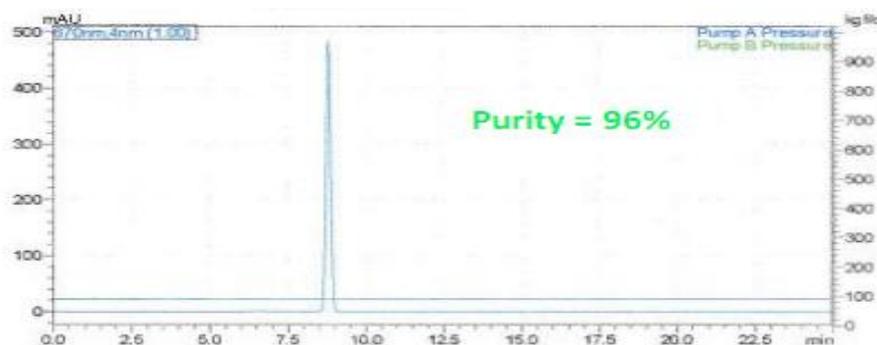
The analysis used UV-Vis to determine the absorption wavelength and Particle Size Analyzer (PSA) as an instrument for analysis, microscopy, FTIR and equipment for toxicity test, as well as mammary cancer cells

## RESULTS AND DISCUSSION

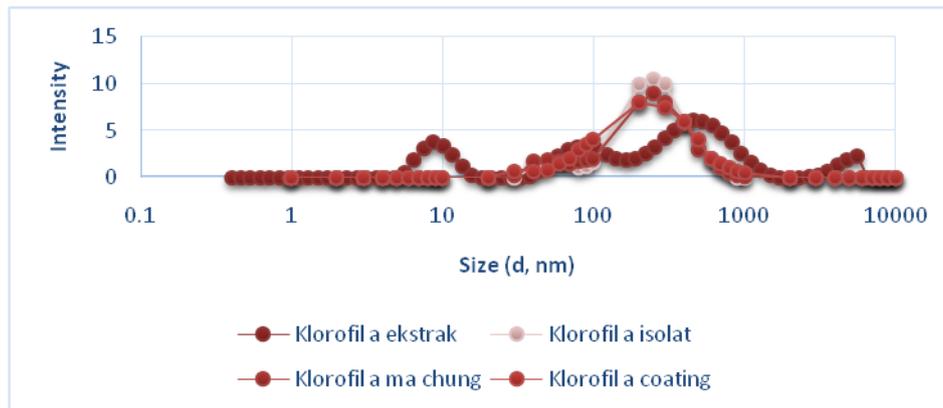
### Material

Eleocharis dulcis is a research material containing chlorophyll 2673,2 µg / g chlorophyll at Figure 2 Eleocharis dulcis and Figure 3 is Purity of chlorophyll a extract, as weeds then this content is equivalent to the content of chlorophyll present in alang-alang is 2326,3 µg / g while the highest is papaya leaf 29,5 mg / g (Setiari & Nurchayati, 2010).

Figure 2: Purity of chlorophyll a extract



**Figure 3: Particle Size Distribution**



**Particle Size Distribution**

The particle size will have an effect on the reaction on the sensitizer because the surface area reacting with this subject is indicated by composites with nanosilver (Falco et al 2015), whereas to be used as a sensitizer in addition to meeting various requirements, one is particle size. With time variation the particle size can be made, the result of the extraction of 182.5 nm chlorophyll particle size as shown Fig. 4

**Figure 4: Chlorophyll a. coated pluronic with HRTEM (Maoquan et al, 2014) b. Coated albumin**



From the measurement results with the size analyzer particle (PSA) it was found that with the change of time it was found that the average particle size decreased, partly increased the size when viewed in Figure 4 because it collected some particles into one so it was detected bigger. So that measured is the rearata. Size becomes important if it sees an influence in nanomedicine in its application as a photodynamic sensitizer (Alyssa et al, 2013).

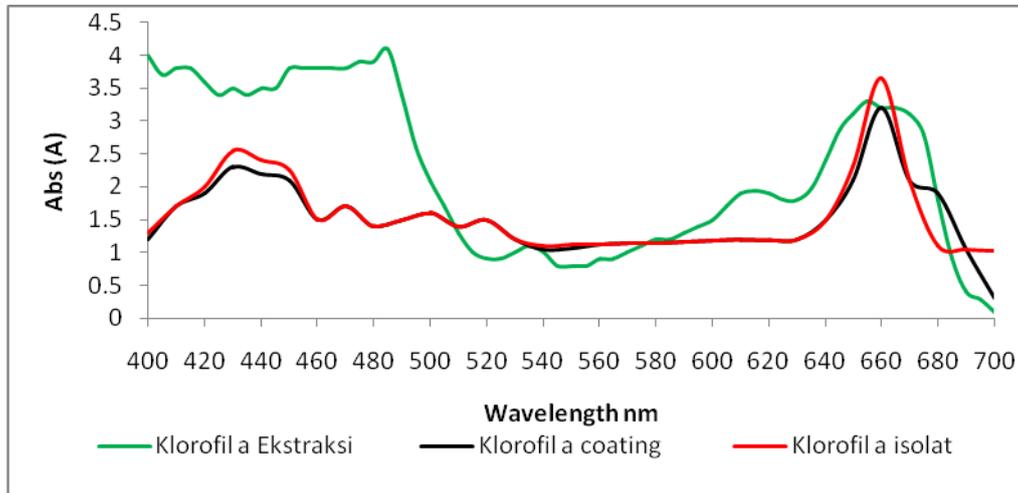
**Wavelength Absorbtion**

Chlorophyll is an organic compound that can undergo resizing but needs to be considered compound stability. By changing the size remains stable chlorophyll is evidenced by the absorption patterns found in normal intensity like chlorophyll standards.

The photosensitizer absorbance test was performed using a UV-Vis spectrophotometer. The principle of this spectrophotometer is the electronic transitions caused by UV Vis capable of excited electrons from orbitals in the ground state (ground state). Absorbance represents the amount of light absorbed by the thin layer of the total illuminated light. The absorbance of a compound at a certain wavelength increases with the number of transition molecules (Christian, 2003). In this study, the test absorbance at SIGMA Aldrich photosensitizer standard chlorophyll, chlorophyll a Machung, Chlorophyll Elecharis Dulcis and chlorophyll of any modifications, the photosensitizer ingredient diluted with DMSO and measuring the wavelength of absorption at 400-700 nm.

From the results of chlorophyll a absorption in Figure 5 shows that the maximum at the blue and red wavelengths. These results are consistent with the Millenkovic et al (2012) study which states that chlorophyll porphyrin derivatives have two maximum peaks in the visible regions of red (Q band) and blue (B band). In the extraction chlorophyll obtained in the same pattern with the results of the literature but still have many other peaks because of chromophores in other substances contained in the extract. While the isolation results show the maximum peak is at 658 nm while the other peak is very small.

**Figure 5: Absorbtion Eleocharis Dulcis**



The photosensitizer toxicity test was performed to find a safe dose of the addition of photosensitizer on the cell, so cell death after treatment was purely a result of treatment, not due to photosensitizer poisoning. Cells were incubated in photosensitizer for 24 hours then observed under a microscope. Based on the initial toxicity test results found that the chlorophyll has nontoxic properties so that this chlorophyll can be used as photosensitiser material.

Toxicity test results can be seen in Figure 6. And in detail can be seen in of the chlorophyll toxicity test results, the safe concentration with the number of deaths below 50% is 0.1 mg / ml.

**Figure 6a: Control cells, b. Cells incubated with photosensitiser, c. Cells Nekrosis**



**CONCLUSION**

- Chlorophyll obtained by size 181 nm result from rat purun extraction, maximum absorption result at 658 nm, toxicity test result on mamae cancer cell in get safe concentration that is 0,1 mg / ml with death rate 18 - 25%.
- Absorption of chlorophyll in isolate rats and coatings obtained at 658 nm is expected to be utilized as an organic sensitizer in photodynamic therapy
- Potential application as optical biomaterials is very potential

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