Chlorophyll Extraction Methods Review and Chlorophyll Stability of Katuk Leaves (*Sauropus androgynous*)

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Abstract. Chlorophyll is one of the most used coloring materials derived from green leaves and other parts of almost all green plants. It contains several components that are beneficial to health: vitamins, antioxidants, antibacterial, anti-inflammatory; hence, chlorophyll is a nutraceutical. Chlorophyll has been developed by extracting green plants, for instance, katuk leaves (*Sauropus androgynous*). A review of various chlorophyll extraction methods from natural ingredients was done to obtain the most suitable katuk leaves. Preliminary experiments had been carried out to develop the chlorophyll stability of katuk leaves on the influence of temperature, pH, and storage time. The chlorophyll content of katuk leaves decreased at high temperatures. Extraction using supercritical/subcritical CO2 can extract components selectively so that it is suitable for extraction applications for products that are not heat resistant. Meanwhile, carbon dioxide (CO2) as a supercritical/subcritical solvent has several advantages, including producing an inert gas that is non-toxic, non-flammable, and cheap. Under normal gas conditions, it is straightforward to separate CO2 and dissolved components without heating and producing extracts free of solvents. The most suitable chlorophyll extraction method of katuk leaves was supercritical/subcritical CO2 extraction because it could prevent chlorophyll degradation.

1. Introduction
Chlorophyll is one of the most used coloring materials derived from green leaves and other parts of almost all green plants. It contains several components that are beneficial to health: vitamins, antioxidants, antibacterial, anti-inflammatory; hence, chlorophyll is a nutraceutical. Chlorophyll has been developed by extracting green plants, for instance, katuk leaves (*Sauropus androgynous*). A review of various chlorophyll extraction methods from natural ingredients was done to obtain the most suitable katuk leaves. Preliminary experiments had been carried out to develop the chlorophyll stability of katuk leaves on the influence of temperature, pH, and storage time. The chlorophyll content of katuk leaves decreased at high temperatures. Extraction using supercritical/subcritical CO2 can extract components selectively so that it is suitable for extraction applications for products that are not heat resistant. Meanwhile, carbon dioxide (CO2) as a supercritical/subcritical solvent has several advantages, including producing an inert gas that is non-toxic, non-flammable, and cheap. Under normal gas conditions, it is straightforward to separate CO2 and dissolved components without heating and producing extracts free of solvents. The most suitable chlorophyll extraction method of katuk leaves was supercritical/subcritical CO2 extraction because it could prevent chlorophyll degradation.
consumed as vegetables to increase breast milk production. In Indonesia, katuk leaves are well known in every province as vegetables, medicine, food colorant, and even wedding tradition [4]. Katuk, also known as star gooseberry, belongs to the Euphorbiaceae family. The leaves are single because they are only strands and petioles, easy to obtain, and used by various food ingredients, including green coloring in sticky rice. Katuk leaves contain nearly 7% protein and 19% crude fiber, vitamin K, pro-vitamin A (beta carotene Vitamin B and C. The minerals contained are calcium (2.8%), iron, potassium, phosphorus, and magnesium. Katuk leaves have unique properties sweet, calm, and cleanse the blood, antipyretic, and anagogical properties [5].

Several methods were used to extract chlorophyll of natural ingredients from the plants, such as aqueous extraction, enzyme-assisted extraction, alcoholic/organic solvent extraction, and supercritical/subcritical extraction. This paper reviews the latest extraction methods’ scientific overview to know the best ways to extract chlorophyll of katuk leaves ( *Sauropus androgynous*). Also, the stability of chlorophyll content against temperature conditions, pH, and storage time had been carried out by preliminary experiments, especially from katuk leaves.

**2. Material and methods**

A scientific overview of the latest extraction methods was conducted to know the best ways to extract chlorophyll of katuk leaves ( *Sauropus androgynous*). This study reviewed many factors that affected chlorophyll extraction, such as operating conditions, raw material, and the chlorophyll yield. To find the most suitable method of extracting katuk leaves, a preliminary study was carried out by testing the stability of chlorophyll content in katuk leaves under the influence of temperature, pH, and storage time.

Katuk leaves were obtained from some garden plants in the Surakarta area. Katuk leaves extract was produced by simple mechanical extraction by blending the leaves using water as a solvent. The extract was separated through filtering. The mass ratio of leaves and solvent studied were 1:2 consisted of 100 grams of leaves and 200 ml of solvent. The katuk leaves extract was then tested for stability by examining the effects of temperature, pH, and storage time. Before being analyzed, the concentrated extract was diluted first (50 times dilution). Total chlorophyll content (chlorophyll a and b) (mg/l) was analyzed using UV-Vis Spectrophotometer with the following equation [6]. The concentrated extract was diluted first.

\[
\text{Total chlorophyll content} = 20.31A_{645} + 8.05A_{663}
\]

with \( A_{645} \) = the absorbance at 645 nm wavelength and \( A_{663} \) = the absorbance at 663 nm wavelength

**3. Result and discussion**

**3.1. Chlorophyll Extraction Methods**

Several experiments had been developed studies of factors affecting extraction. Operating conditions such as temperature, pH, and extraction time were reported affecting the yield of chlorophyll. Extraction takes place faster at high temperatures because it can increase the solubility and diffusion of the extracted compound and reduce the solvent’s viscosity. However, high temperatures can also degrade polyphenol compounds. A longer contact time between the solid and the solvent will increase an extract’s amount until the solution’s saturation point.

Besides that, the size of the material and the type and amount of solvent also played a role. Smaller particle size will increase the area of contact between the solid and the solvent, and it will be shortening the diffusion path, which makes the mass transfer rate higher. Polar solvents will dissolve polar compounds, and non-polar solvents will dissolve non-polar compounds; we call it the ‘like dissolving like’ principle. The more amount of the solvent used, the more results will be obtained because the particle’s distribution in the solvent spreads out, thus expanding the contact surface.
Table 1: Comparison of several extraction methods

| Extraction Methods                          | Raw Material                  | Result                  | Notes                                                                 | Reff. |
|---------------------------------------------|-------------------------------|-------------------------|----------------------------------------------------------------------|-------|
| Extraction by methanol (1:50) atmospheric   | Vegetables (cucumbers)        | Chl a: 626.15 mg/100 g  | Disadvantage: the methanol is toxic                                  | [7]   |
| Enzyme-assisted extraction (Pectinex Ultra SP-L) | Spinach                      | Chl b: 412 mg/100 g     | The highest chlorophyll release was at 8% enzyme, 45°C, and 30 min treatment. | [8]   |
| Ethanol-modified subcritical 1,1,1,2-tetrafluoroethane (R134a) | Laminaria japonica (seaweed)  | Chlorophyll derivatives yield. | The optimum extraction temperature 324.13 K, 17 MPa anda cosolvent amount of 4.73%. | [9]   |
| subcritical CO₂ extraction of a volatile oil-rich fraction | Black cumin/ Nigella sativa | Yields of chlorophyll a were predicted to be 2.326 g/kg at optimum condition. | Extraction using subcritical CO₂ (30°C and 70 bar) for 2 hours was able to obtain oleoresin with the highest thymoquinone content. | [10]  |
| supercritical carbon dioxide and ethanol as cosolvent | Microalgae N. gaditana and Synechococcus sp. | 0,5 and 2,4 μg/mg microalgae | The highest extraction yields of carotenoids and chlorophyll with CO₂ + 5% ethanol are obtained at 500 bar and 60°C. | [11]  |
| supercritical fluid CO₂ to extract chlorophyll a, b and c (Scenedesmus obliquus) | Microalgae | The highest yield chlorophyll a (0.033 mg/g) was obtained at a flow rate of 4.3 g CO₂/min | The higher chlorophyll yield was obtained at high operating pressure (250 bar), low temperature (40°C). | [12]  |
| Supercritical CO₂ extraction with a static modifier was applied to extract chlorophyll | Spirulina platensis | Chlorophyll extraction yield 6.84 mg/g | The optimal process: 21.2 ml of 40% water and ethanol as a modifier, 1 h static soaking time, 2 h dynamic extraction time, 48.7MPa and 326.4K, and 10 g min-1 CO₂ flow rate. | [13]  |

The solvent extraction method by methanol may produce organic solvent residuals in the products, which are harmful to consumers. The development of other experiments is needed since the traditional methods required a lot of water and time. The new efficient and environmentally friendly way for extracting pigments from natural materials was observed, such as supercritical/subcritical fluid extraction. The supercritical condition for CO₂ is achieved when the pressure and temperature are above the critical point of CO₂ (essential 31.1°C of temperature and necessary pressure 73.8 bar) [14]. Supercritical conditions have very high solubility due to their high density and diffusion coefficient, low viscosity, and surface tension. Thus they can penetrate small pores in the extracted material. If the temperature of supercritical CO₂ is lowered below 31.1°C, the CO₂ changes to a liquid phase called subcritical CO₂. Extraction using supercritical/subcritical CO₂ can extract components selectively and suitable...
for applications in products that are not stable to heat [15]. Carbon dioxide (CO₂) as a supercritical/subcritical solvent has many advantages: inert gas, non-toxic, non-flammable, and cheap. Under normal gas conditions, it is effortless to separate CO₂ and dissolved components without heating and producing extracts free of solvents. Nevertheless, the cost of supercritical/subcritical equipment is often outside the range of most of our finances. A high-pressure intensifier pump is used to achieve the required pressure, but the supercritical CO₂ condition was performed by other alternatives using dry ice. It is a solid phase of CO₂ when frozen. At standard temperature and pressure (STP), CO₂ usually behaves like a gas. If dry ice is put in an enclosed vessel, it will sublimate to become a gas, and the pressure will increase depending on the mass of dry ice until the desired pressure (supercritical pressure) is achieved. By supercritical CO₂ from dry ice, supercritical fluid extraction equipment will be simpler and cheaper [16]. The designed apparatus consisted of 2 main vessels. The first one was a high-pressure extractor for extracting process using supercritical CO₂ solvent. The second one was a separator vessel for separating the solvent and extract. [17].

### 3.2. Chlorophyll stability

Katuk leaves extract was obtained by simple mechanical extraction by blending the leaves with aqua dest solvent, with 1: 2 (100 grams of material and 200 ml of solvent). The total chlorophyll content in the extract was 15.164 mg/l. In other words, the chlorophyll extracted in the material was 1516.4 mg/kg of material. The stability of chlorophyll content in the leaves was tested under the influence of temperature, pH, and storage time.

### 3.3. The effects of temperature

The stability tests were carried out at three various temperatures; ambient temperature (30°C), middle - high temperature (40 - 100°C) by heating, and low temperature (10 - 20°C) by cooling. The effect of temperature on the chlorophyll content of katuk leaves extract analyzed using a UV-Vis spectrophotometer is presented in Figure 1.

![Figure 1: The effect of temperature on the chlorophyll content of katuk leaves extract](image)

The graph shows the chlorophyll content of katuk leaves extract under the influence of temperature from 10°C to 100°C. The chlorophyll content trend was stable at low until the ambient temperature decreased along with the increasing temperature. At 10°C, the chlorophyll content was about 15 mg/l and declined slightly until 60°C at about 13.5 mg/l. A sharp fall was observed at 70°C from the chlorophyll content was about 13 mg/l into about 6.2 mg/l at 100°C.
These significant decreases occurred because of the degradation of chlorophyll at high temperatures. Chlorophyll is degraded and turned brown due to the release of magnesium ions replaced by hydrogen. As a result, the formation of pheophytin compounds or a feotinization process happened.

3.4. The effects of pH
Katuk leaves are used as a natural coloring agent for food and beverages. The production and serving processes are carried out at various temperatures, pH values, and within a certain period of storage. Besides, food and drinks are often served also in acidic conditions. The stability test was carried out at low pH with the addition of citric acid. The effect of pH on the chlorophyll content of katuk leaves extract analyzed using a UV-Vis spectrophotometer is presented in Figure 2.

![Figure 2: The effect of pH on the chlorophyll content of katuk leaves extract](image)

The graph shows the chlorophyll content of katuk leaves extract under the influence of pH from 1 to 7. Chlorophyll content climbed as the pH increase from 1 at about 12 mg/l to 4 at about 15 mg/l. At pH 5 - 7, chlorophyll remains stable at about 15 - 15.5 mg/l.

3.5. The effects of storage time
The effect of storage time on the chlorophyll content of katuk leaves extract analyzed using a UV-Vis spectrophotometer is presented in Figure 3. The graph shows the chlorophyll content of katuk leaves extract under the influence of storage time and storage temperature. Chlorophyll content at low temperatures (10°C in the refrigerator) was more stable than at room temperature (30°C).

Of the three variables studied, the chlorophyll of katuk leaves was stable when stored at low temperatures, without heating treatment, and not at acidic pH. So far, extracting/taking the chlorophyll components from natural materials is carried out with a liquid solvent and then continued by evaporation/concentrating and drying processes. Those processes require high temperatures. Consequently, it will degrade and damage chlorophyll. The extraction method using supercritical/subcritical CO₂ can be done at low temperatures and prevent dissolved components’ degradation due to heating.

The chlorophyll content was reduced when treated at high temperatures through a preliminary experiment on the chlorophyll stability test of katuk leaves. The most suitable extraction method was using supercritical/subcritical CO₂ to prevent chlorophyll degradation.
Figure 3: The effect of storage time and temperature on the chlorophyll content of katuk leaves extract

4. Conclusion
The chlorophyll extraction from natural materials was practically carried out mostly using a liquid solvent, continued by evaporation/concentration, and drying required high temperatures. The extraction method using supercritical/subcritical CO\textsubscript{2} can be conducted at low temperatures, preventing dissolved components' degradation due to heat. Carbon dioxide (CO\textsubscript{2}) as a supercritical/subcritical solvent has advantages, such as an inert gas, non-toxic, non-flammable, and cheap. Under normal gas conditions, it is straightforward to separate CO\textsubscript{2} and dissolved components without heating and producing extracts free of solvents. From the study of the chlorophyll stability test of katuk leaves, the chlorophyll content decreased at high temperatures. Thus, the most suitable extraction method was supercritical/subcritical CO\textsubscript{2} extraction to prevent chlorophyll degradation.

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