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Isolation and Identification of Chlorophyll from Pandan Leaf Extract Using Column Chromatography

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ABSTRACT

Pandan is a plant whose leaves are often used by some people as an additional ingredient in food, especially as a natural dye and aromatizer. Pandan has green leaves due to the presence of chlorophyll in the leaves. Chlorophyll has benefits as an anti-cancer and is useful in cancer treatment therapy. Because pheophytin is able to activate radical compounds that are harmful to cells, this therapy is called photodynamic therapy. The purpose of this study was to isolate and identify chlorophyll in pandan leaf extract by column chromatography method using a combination of chloroform, ethyl acetate and ethanol eluents, with three variations of eluent, namely: Eluent I chloroform: ethyl acetate: ethanol (80:5:15), Eluent II chloroform: ethyl acetate: ethanol (85:10:5) and Eluent III chloroform: ethyl acetate: ethanol (90:5:5). The research stage was carried out by preparing test samples from pandan leaf ethanol extract, then isolation was carried out. Testing was carried out by column chromatography method to separate chlorophyll compounds, the isolate obtained was then identified for the presence of chlorophyll by uv-vis spectrophotometry method to determine the maximum peak at a wavelength of 665 nm. Based on the results of the study, the ethanol extract of pandan leaves was green in color and had a distinctive aroma of pandan leaves as much as 90 ml. The elution results obtained a green band suspected of being chlorophyll obtained as much as 30 ml with an elution time of 3 minutes 52 seconds. The results of the identification test with uv-vis spectrophotometry obtained a maximum wavelength at a wavelength of 665 nm and an absorbance value of 0.550. From the results of the study, it can be concluded that pandan leaf juice contains chlorophyll compounds and can be isolated by column chromatography method with a mixture of chloroform, ethyl acetate and ethanol eluents.

Keywords : Pandan leaves, isolation, chlorophyll, column chromatography

INTRODUCTION

Pandanus leaves (pandanus amaryllifolius) are one type of plant that is widely used to enhance aroma and flavor and as a food coloring by Indonesian people (Aryanti, 2016). In Malaysia, pandan leaves are widely used as a traditional medicine for diabetes sufferers (Dautzenberg, 2015). Dietary factors, including antioxidants, have a major effect in treating diabetes and its complications (Suryani, 2018). Pandan itself is a plant whose leaves are often used as an additive to food, especially as a natural dye and aroma. Pandan leaves contain chemicals including alkaloids, saponins, flavonoids, tannins, polyphenols, and dyes. Pandan is one of the plants that has the potential to produce essential oils (Tasia, 2014). Pandan leaves also have several pharmacological activities based on the solvents used, namely as antibacterials, antioxidants, anticancer, and antidiabetics (Raif et al., 2019).

Chlorophyll is a green pigment that can be found in chloroplasts along with carotene and xanthophyll in all living things that are capable of photosynthesis (Raif et al., 2019). Chlorophyll, commonly called green leaf substance, is a pigment possessed by various organisms and is one of the molecules that plays a major role in the process of photosynthesis. This chlorophyll can provide a green color to green plants (Maulid & Laily, 2015).

The function of chlorophyll in plants includes absorbing light and transferring it through plants during photosynthesis. Chlorophyll in plants can be found in thylakoids, chloroplasts. Chloroplasts in plants are exposed to sunlight which is then absorbed by chlorophyll which is finally combined with carbon dioxide with water, namely to make glucose or what is commonly called sugar. This process can also produce oxygen (Aryanti, 2016). Isolation of chlorophyll from pandan leaf juice can be done using column chromatography. Column chromatography is a method used to purify single chemicals from their mixtures. The advantages are low cost and ease of disposal of the stationary phase that has been used. The ease of disposal of this stationary phase prevents cross-contamination and phase degradation due to reuse or recycling (Iwo, 2018).

TOOLS AND MATERIALS

The tools used in this study were Measuring Cup, Erlenmeyer, Beaker Glass, Measuring Flask, Stirring Rod, Analytical Scale, Column, Stand and Clamp, Dropper Pipette, Uv-Vis Spectrophotometry, Rotary Evaporator, Funnel, Vial, Cuvette, Cotton Wool,

Aluminum Foil and Filter Paper, Oven, and Stopwatch. The materials used in this study were pandan leaf simplicia from Balokang Village, Banjar District, Banjar City, West Java, 96% Ethanol, Chloroform, Ethyl Acetate, Silica Gel 60 (size 0.063-0.200).

METHODS

Preparation of Pandan Leaf Raw Materials (Pandanus amaryllifolius)

The raw materials were obtained from the yard of a house in Balokang Village, Banjar District, Banjar City, West Java. The raw materials used were fresh pandan leaves with the aim of obtaining a higher compound content. The process carried out in the preparation of Pandan leaf raw materials (Pandanus amaryllifolius) was the collection of Pandan leaves (Pandanus amaryllifolius), wet sorting, washing, and chopping.

Making Pandan Leaf Juice (Pandanus amaryllifolius)

A total of 10 grams of selected leaf pieces were crushed by grinding using a mortar and dissolved in 100 ml of 96% ethanol, then left for 10 minutes. The crushed leaves were then filtered using filter paper until the filtrate was obtained. This filtrate was taken as a sample.

Chlorophyll isolation.

Preparing the eluent with three ratios, namely chloroform, ethyl acetate, ethanol. Three variations of eluent ratios were used, namely: Eluent I chloroform, acetic acid and chloroform (80:5:15). Eluent I was made as much as 100 ml. Add 80 ml of chloroform into an Erlenmeyer flask, then add 5 ml of ethyl acetate and add 15 ml of ethanol. Stir until homogeneous, then cover the Erlenmeyer flask with aluminum foil, so that the mixture does not evaporate. Eluent II chloroform, acetic acid and ethanol (85:10:5). Eluent II was made as much as 100 ml. Add 85 ml of chloroform into an Erlenmeyer flask, then add 10 ml of ethyl acetate and add 5 ml of ethanol. Stir until homogeneous, then cover the Erlenmeyer flask with aluminum foil, so that the mixture does not evaporate. Eluent III chloroform, acetic acid and ethanol (90:5:5)

Preparation of the chromatography column

Put a little cotton in the column and compress it. Then make silica slurry. Where the silica gel to be used is activated first, namely by oven at a temperature of 110 ° C for 2 hours. The silica gel to be used is silica gel (size 0.063-0.200 mm) which has a relatively small granule size and is widely used for the stationary phase in column chromatography.

A total of 20 grams of silica gel is dissolved using chloroform eluent: ethyl acetate: ethanol (90:5:5). Put the silica gel slurry that has been made into the column, then compress and tap slowly. Let stand for 24 hours to compact the stationary phase.

Chlorophyll Isolation by Column Chromatography

For component separation using column chromatography, 10 ml of pandan leaf extract is put into a column that already contains silica gel slurry that has been left for 24 hours slowly. Then flowed with a mixture of eluents from chloroform, ethyl acetate and ethanol while being vacuumed until all the mobile phases come out. The resulting fractions are then collected in an Erlenmeyer. Repeat the separation with a mixture of eluents based on different ratios. After that, all fractions are separated to be identified by uv-vis spectrophotometry.

Chlorophyll identification

After the pandan leaf juice is isolated by column chromatography, then the absorbance reading is carried out using the Uv-Vis Spectrophotometry tool. The chlorophyll isolates from each eluent are different, each is inserted into the sample cuvette, while the comparison cuvette is filled with 95% ethanol. Then measure the absorbance with uv-vis spectrophotometry at a wavelength of 665nm.

RESULTS

Pandan leaves were taken as many as 10 strands, then wet sorting and shredding were carried out and then weighed until 10 grams of pandan leaves were obtained. 10 grams of crushed samples were crushed by grinding with the addition of 96% ethanol solvent. The best type of solvent for making pandan leaf juice is ethanol because the chlorophyll contained in pandan leaves is very soluble in ethanol. After filtering, 90 ml of filtrate was obtained and the juice was dark green. Daun pandan yang diambil sebanyak 10 helai, kemudian dilakukan sortasi basah dan perajangan lalu dilakukan penimbangan hingga diperoleh daun pandan sebanyak 10 gr.

Table 1. Hasil isolasi klorofil dengan kromatografi kolom

Eluent	Volume	Isolation Time	Color
1	31	03.52	Green
2	30	02.57	Green
3	29	02.24	Green

Table 2. Results of chlorophyll identification using UV-Vis spectrophotometry

Eluent	Wavelength	Absorbance
1	665	0,550
2	665	0,821
3	665	0,772

DISCUSSION

Based on the table above, the results of the isolation of pandan leaf extract in the form of a clear green liquid are suspected to be chlorophyll compounds. The isolation method using the column chromatography method is based on the comparison of the combination of chloroform, ethyl acetate and ethanol eluents with three combination variations, namely eluent I, eluent II and eluent III. The largest volume was produced by eluent I as much as 31 ml, while the most effective eluent time was produced by eluent I, namely 2 minutes 24 seconds. Eluent III has the most effective time in the eluent process, this is because the chloroform composition is greater, so that the chlorophyll is more attracted because of the non-polar nature of chlorophyll. In addition, the thing that greatly influences this retention time is the polarity of the stationary phase of the column. Where the stationary phase used is silica gel which has a very high polarity, so that the eluent and the compounds entered are required not to bind to each other with silica.

After the chlorophyll separation, the next step is to identify the chlorophyll using UV-Vis spectrophotometry. This identification is done to ensure the chlorophyll content in the results of the isolation of pandan leaf extract. Chlorophyll shows a maximum wavelength at 665 nm. (Porra et al., 2017). From the results of the identification of the results of the isolation of leaf extract with three types of eluents, it was obtained that the three results showed a maximum absorption peak at a wavelength of 665 nm with an absorbance value of eluent I of 0.550, eluent II 0.821 and eluent III 0.772. Eluent II has the highest absorbance value, this indicates that eluent II is able to attract more chlorophyll. From the results of this study it can be concluded that in chlorophyll A, the highest peak is at a wavelength of 665 nm, while for chlorophyll B, the highest peak is at a maximum wavelength of 652 nm. For this reason, it can be said that pandan leaf juice using 96% ethanol contains chlorophyll compounds because the highest peak in the wavelength scanning is at 665 nm.

CONCLUSION

From the results of this study it can be concluded that chlorophyll can be isolated from pandan leaves by column chromatography method using three variations of eluents, namely eluent I chloroform: ethyl acetate: ethanol (80:5:15), Eluent II chloroform: ethyl acetate: ethanol (85:10:5) and Eluent III chloroform: ethyl acetate: ethanol (90:5:5). Eluent I can isolate the most chlorophyll, while the fastest isolation time is obtained by using eluent III. The highest chlorophyll content is obtained by using eluent II.

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