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# Determination of Anthocyanin Levels in Telang Flower (Clitoria Ternatae) Using the Differential pH Method Based on Three Types of Solvents

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**ABSTRACT**

Anthocyanins are pigments that are soluble in polar solvents naturally and belong to the flavonoid group found in various types of plants. As the name implies, this pigment gives color to the flowers, fruit, and leaves, and has been widely used as a natural colorant in various food products and other applications. Telang flower is an example of a plant that has high levels of anthocyanins. The research method to prepare the test sample used was extraction by maceration using 3 types of solvents ethanol 96%, n-butanol and ethyl acetate. The test was carried out using the differential pH method using UV-Vis spectrophotometry at 510 nm and 700 nm. The assay results obtained were the average anthocyanin content of telang flower extract in ethanol 96% 0,946%, n-butanol 1,684%, ethyl acetate 0,181%. The results of data analysis showed that all the average levels of telang flower extract from each solvent were different, in other words the average levels of telang flower extract from each solvent were significant differences. The highest anthocyanin levels in this study were produced in the telang flower extract with n-butanol as a solvent of 1,684% and the lowest inthe telang flower extract with ethyl acetate solvent of 0,181%.

## INTRODUCTION

Indonesia is known as one of the countries with the greatest biological wealth in the world. 90,000 plant species grow in Indonesia (Purgiyanti et al., 2023). This biodiversity is of course utilized by the people of Indonesia for various purposes, such as for making food, medicinal plants, customs, ornaments, and local technology. The plant is in wide use and is grown on farms or in the backyard. The use of a garden or terrace of the house not only serves to increase the aesthetic value of the house but also fulfills the needs of medicinal plants. One of the plants that can be grown as both an ornamental and a medicinal plant is the butterfly pea flower (Clitoria ternatea) (Purba, 2020).

The use of medicinal plants is increasingly in demand by the public. This is because people realize that drugs with natural ingredients have minimal side effects. Many plants have been used by the community in everyday life, one of which is a colored plant that has various pigments as a natural food coloring. These natural pigments are chlorophyll, beta-carotene, anthocyanin, betalain, and others (Hidayah, 2016). One of the plants that have this natural pigment is the butterfly pea flower. Butterfly pea belongs to the Fabaceae family. This plant thrives in full sun, but can also grow in the shade, such as in rubber and coconut plantations (Arnawa et al., 2017).

Butterfly pea flowers contain delphinidin, triglycerides, and phenols. The parts of Clitoria ternatea that are commonly used are the flowers and leaves. Clitoria ternatea flowers can treat red eyes, tired eyes, throat, skin diseases, and urinary disorders and are anti-toxic (Purba, 2020). The leaves increase blood circulation, prevent swelling, and regulate menstruation if pounded cauliflower leaves can treat festering wounds whereas if boiled and mixed with other herbs can treat leucorrhoea (Putri, 2019). Other pharmacological effects of the butterfly pea include toxic roots, laxatives, diuretics, stimulants of vomiting, and blood purifiers.

The chemical constituents of butterfly pea flower include tannins, plantains, saponins, triterpenoids, carbohydrates, phenolic flavonoids, flavanol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, stigma site 4-ene-3, 6 diones, volatile oils and steroids (Purba, 2020). Anthocyanins are a group of pigments that are blue/purple. Anthocyanins are secondary metabolites that dissolve in polar solvents, have many uses, and can be found in various types of plants. Anthocyanins can be found in flowers, fruits, and vegetables. One of the benefits of anthocyanins is as a natural pH indicator (Bondre et al., 2012).
In this study, the extraction method was used by maceration using three types of solvents namely n-butanol, 96% ethanol and ethyl acetate. The use of these three types of solvents aims to determine which concentration ratio will produce the highest anthocyanins based on the polar nature of anthocyanins which can dissolve in polar solvents. Maceration is intended so that the extraction process used is not heated so as not to reduce the anthocyanin content and the advantage of this extraction process by maceration is the method and equipment used are simple.

Total anthocyanin levels were measured using a differential pH spectrophotometry method. The spectrophotometric pH

difference method is a calculation based on the difference in the absorption of visible light at different pH values, namely at pH 1 and pH 4.5. This method is used because the most significant anthocyanin stability is affected by pH where acidic conditions will affect the extraction results. Conditions that are increasingly acidic, especially closer to pH 1, will cause an increasing number of anthocyanin pigments. Absorbance measurements will show an increasing amount of anthocyanins. Besides that, the increasingly acidic conditions cause more and more vacuole cell walls to break down so that more and more anthocyanin pigments are extracted (Pratiwi, Priyani, 2019). Based on the above background, a study was conducted with the title determination of anthocyanin levels in butterfly pea flower (Clitoria ternatae) using a differential pH method based on three types of solvents.

## METHOD

The type of research that will be carried out in this research is laboratory experimental research which aims to determine the levels of anthocyanins in butterfly pea flower (clitoria ternatea) based on three types of solutions. The sample to be used is the butterfly pea flower (clitoria ternatea) which is found in Mekarjaya Village, Sukaraja District, Tasikmalaya Regency. The research was carried out through several stages, namely sample preparation, extraction, preparation of solutions, and assays using UV-Vis spectrophotometry. This research is an observational study using a differential pH method based on three types of dilution to test the anthocyanin content in butterfly pea flowers.

**Tools and materials**

The materials used in this study were: saffron flower, aqua dest, KCL, HCL, NaOH, sodium acetate.

The tools used in this study include UV-Vis spectrophotometry, digital balance, metal glass, pipette, measuring flask, beaker glass, funnel, measuring cup, filter paper, pH meter.

**Research procedure**

1. Making Butterfly Pea Simplicia

Use fresh butterfly pea flowers. Furthermore, as much as 500 grams of butterfly pea flowers are cleaned with clean running water to remove impurities attached to the butterfly pea flowers. After cleaning, cut the small flowers of the butterfly + 3 cm so they dry quickly. Drying is done by drying it in the sun by covering it with a black cloth. After the butterfly pea flowers are dry, grind them using a blender.

2. Preparation of Butterfly Pea Powder Extract

The butterfly pea flower extract in this study was prepared by maceration based on the 2013 Indonesian Herbal Pharmacopoeia, 1st Edition, with a ratio (1:10). Weigh 10 grams of butterfly pea flower powder then put it in a beaker glass and pour 100 ml of 96% ethanol, do maceration extraction (Sinaga, 2019). The extraction process was carried out for 3 days. During the extraction process it was carried out at room temperature 25°C and carried out in the dark where aluminum foil was used to cover it. The maserate obtained is then evaporated using a Rotary Evaporator vaporizer at a temperature of not more than 50oC until a thick extract is obtained (Sinaga, 2019).

3. Anthocyanin Phytochemical Test

Proof of the existence of anthocyanins can be done in a simple way. The first method is the sample is heated with 2M HCl, then the sample color is observed. If the red color in the sample does not change (steady), it indicates the presence of anthocyanins. The second way is by adding

the sample with 2M NaOH drop by drop. According to Lestario et al (2011) if the red color changes to blue-green and fades slowly, it indicates the presence of anthocyanins (Anggraeni et al., 2018).

4. Total Yield Analysis

Yield analysis is calculated using the following equation:

Yield (%) =

$$\frac{massa\ ekstrak\ yang\ diperoleh\ (g)}{massa\ awal\ sampel\ (g)}\ x\ 100\%$$

5. Preparation of pH 1.0 and pH 4.5 solutions

Determination of anthocyanins was carried out using a differential pH method, namely pH 1.0 and pH 4.5. At pH 1.0 anthocyanins are in the form of oxonium compounds and at pH 4.5 they are colorless carbinol pH. This can be done by making an aliquot of anthocyanin in water with a pH of 1.0 and 4.5 and then measuring the absorbance. The wavelength is 510 nm and 700 nm (Suzery et al., 2010).

a. Solution pH 1.0. Approximately 1,490 grams of KCL was dissolved with distilled water in a 100 ml volumetric tube to the limit. Then mix 25 ml of KCL solution with 0.2 N HCL, add HCL again if necessary until the pH reaches 1.0 (Suzery et al., 2010).
b. Solution pH 4.5. Approximately 5,443 grams of sodium acetate (CH3COONa) was dissolved with distilled water in a 100 ml volumetric tube to the limit. Add 0.2 N HCL solution to pH 4.5 (Sundarni, 2017).

6. Measurement and Calculation of Total Anthocyanin Concentration with Differential pH Method.

a. Each viscous extract of 1 gram is dissolved with each solvent, then put into a 25 ml volumetric flask. Take 5 ml of condensed extract of butterfly pea flowers each
b. Prepare 2 solutions of each solvent, the first solution is used KCl buffer solution with pH 1.0 and for the second solution using sodium acetate buffer pH 4.5
c. Each solution is dissolved with a buffer solution based on a predetermined dilution factor
d. Samples dissolved using pH 1 buffer were left for 15 minutes before being measured.
e. Meanwhile, the samples dissolved with pH 4.5 buffer were ready to be measured after being allowed to mix for 5 minutes.
f. The absorbance of each solution at a wavelength of 510 and 700 nm was measured with pH 1 buffer and 4.5 buffer as blanks.
    1) The absorbance of the sample that has been dissolved (A) is determined by the following equation (Widyasanti et al., 2018): A= (A λ 510-A λ 700) pH 1.0 – (A λ 510-A λ 700) pH 4.5
    2) The content of anthocyanin pigment in the sample is calculated by the equation Total anthocyanin (ppm)

$$= \frac{A\ x\ BM\ x\ FP\ x\ 1000}{\varepsilon\ x\ b}$$

Information:

A = absorbance

Ɛ = molar absorptivity (26,900 L/(mol.cm))

b = cuvette thickness (1 cm)

BM = molecular weight (449.2 g/mol)

FP = dilution factor (Widyasanti et al., 2018)

**Data Analysis**

1. Normality Test

The goal of the normality test is to find out whether the data is normally distributed or not. This normality test uses a statistical test, namely the Shapiro-Wilk by taking a significance level of 5%. Guidelines for decision making with a significance level of 5% are as follows:

Significance value (sig) <0.05, the distribution is not normal.

Significance value (sig) ≥0.05, normal distribution (Subjects et al., n.d.).

2. Homogeneity Test

The objective of the homogeneity test is to find out whether the data obtained is

homogeneous or not. This homogeneity test uses the Levene statistical test by taking a significance level of 5%. The test criteria are as follows:
If the significance value (sig) <0.05, the data comes from a population that has an inhomogeneous variance. If the significance value (sig) is ≥0.05, the data comes from a population that has a homogeneous variance (Subjects et al., n.d.).
3. One Way ANOVA
The one-way ANOVA procedure or One-Way ANOVA is an analysis of variance with one dependent variable. This analysis of variance was used to test the hypothesis of similarity of means between two or more groups. This analysis technique is actually an extension of the two-sample t-test analysis technique (Dr. Abdul Muhid, 2019). In one-way ANOVA or One-Way ANOVA this produces: in each group the number of cases will be calculated, the average, the standard deviation, the average standard error, the minimum, the maximum, the average confidence interval, the Levene test for variance equality, and table of analysis of variance (Dr. Abdul Muhid, 2019). Data criteria that can be tested using one-way or One-Way ANOVA ANOVA, namely:

a. The value data of the factor variables must be integers (categorical data) and the dependent variable must be quantitative data (intervals and ratios).
b. Data must be independent of each other from random samples and normally distributed.
c. The variance of the samples is the same (homogeneous)
d. The samples are not related to one another.
e. In certain cases the ANOVA test can be used in research experiments that compare between groups.

If from the results of the ANOVA test it is known that there are different average data, these differences can be determined in further analysis (Post Hoc) (Dr. Abdul Muhid, 2019). If the One-Way ANOVA test cannot be carried out because it does not meet the parametric requirements, then the Kruskal Wallis test and the Mann-Whitney Post Hoc test are performed first by transforming the data.

## RESULTS AND DISCUSSION

1. Making Butterfly Pea Simplicia

The stages of making the butterfly pea flower simplicia (Clitoria ternatae) are wet sorting, drying, and the length of time to dry the butterfly pea flowers in indirect sunlight for 4 days.
After drying, the butterfly pea flower simplicia is dry sorted for the selection of simplicia in the extraction process. After that, the results obtained were weighed, namely 327 grams.

1. Extraction of Butterfly Pea Flower

The extract weight obtained was then calculated by extract yield, namely:

1) Rendemen ekstrak etanol= $\frac{17{,}23\ gram}{108\ gram}$ x 100% = 15,95%
2) Rendemen ekstrak n-butanol= $\frac{18{,}7\ gram}{108\ gram}$ x 100% = 17,31%
3) Rendemen ekstrak etanol= $\frac{1\ gram}{108\ gram}$ x 100% = 0,93%

**Tabel 1**

**Yield of Butterfly Pea Flower Extract**

| No | Sampel | SimpliciaWeight (g) | Obtained Extract (g) | Yield (%) |
|---|---|---|---|---|
| 1 | 96% Ethanol Extract | 108 | 17,23 | 15,95 |
| 2 | n-butanol extract | 108 | 18,7 | 17,31 |
| 3 | Ethyl Acetate Extract | 108 | 1 | 0,93 |

2. Identification of Anthocyanin Compounds in Butterfly Pea Flowers (Clitoria Ternatae)

**Table 2. Results of the Butterfly Pea Phytochemical Screening Test**

| Test | Extract | study | results |
|---|---|---|---|
| Heated with 2M HCl for 5 minutes with temperature 100°C | Etanol 96% | Red Excellent | Yellowish red (Positive Anthocyanin) |
| | n-Butanol | | Great Red (Positive Anthocyanin) |
| | Etil Asetat | | Orange (Low Anthocyanin) |
| Dropped with 2M NaOH drop by drop | Etanol 96% | Red Turns To Blue Green And Fades Slowly | Fading Green (Positive Anthocyanin) |
| | n-Butanol | | Faded Green (Anthocyanin Positive) |
| | Etil Asetat | | Fading Yellow (Low Anthocyanin) |

3. Determination of Anthocyanin Levels Using the Differential pH Method

**Table 3. Total Anthocyanin Levels of Butterfly Pea Flowers**

| Butterfly pea extract | Content (%) | Average content (%) |
|---|---|---|
| Etanol 96% | 0,752<br>1,586<br>0,501 | 0,946 |
| N-Butanol | 1,920<br>2,714<br>0,418 | 1,684 |
| Etil asetat | 0,125<br>0,167<br>0,251 | 0,181 |

1. Making Butterfly Pea Simplicia

The first simplicia of the butterfly pea flower (Clitoria ternatae) is wet sorting to select good flowers and to separate them from dirt/things that are not used. After washing with running water several times so that it is completely clean. Then the drying process is carried out which is carried out under direct sunlight, covered with a black cloth and must be turned back and forth so that the drying is evenly distributed. The aim of indirect drying under the sun is to avoid direct contact with ultra violet rays from sunlight which can cause damage to the levels of compounds contained in the simplicia. The length of time to dry the butterfly pea flowers in indirect sunlight is 4 days, with unstable weather conditions.

2. Extraction of Butterfly Pea Flowers

The maceration process is used because the anthocyanins are not resistant to heating, the maceration process is carried out by immersing the simplicia in an appropriate solvent so that it can penetrate the cell walls of the sample so that the withdrawal of the anthocyanin compounds can be maximized. The process of maceration of the butterfly pea flower is done by first chopping it into 3 parts with a size of about 1 mm. then weighed 108g each in a container after which it was added to the respective solvents, namely 96% ethanol, n-butanol and 1000 ml of ethyl acetate, then allowed to stand for 3 days with stirring every 6 hours. Different types of dissolution are used to see differences in dissolution results that can attract greater anthocyanins. After soaking, the filtrate obtained is filtered using filter paper, after obtaining the maserate, it is then concentrated by placing the maserate in a steam cup in a magicom with a temperature of 50-60°C until the macerate thickens thickly. Magicom was used due to the limited number of rotary evaporators in the laboratory. The extract results were then weighed against the results obtained, namely 17.23 grams of 96% ethanol extract, 18.7 grams of n-butanol extract and 1 gram of ethyl acetate extract. To find out the yield of the extract is done by weighing the weight of the sample obtained and the weight of the extract obtained, the yield obtained from each solvent is 15.95%, 17.31%, 0.93%. According to the Indonesian Herbal Pharmacopoeia, the yield of the extract can be said to be good if it is not less than 7.5%. So the results of the yield of butterfly pea extract that did not meet the standards were only butterfly pea extract with ethyl acetate solvent.

3. Identification of Anthocyanin Compounds in Butterfly Pea Flowers (Clitoria Ternatae)

The results of the observations showed that the 96% ethanol and n-butanol extract of butterfly pea flowers positively contained anthocyanins which were marked with a solid red color on the addition of HCl which indicated the presence of flavilium compounds and faded green after dropping NaOH indicated the presence of a quinonoide base compound. Whereas for extracts with ethyl acetate solvent, low anthocyanin levels are marked with a red to orange color fading on the addition of HCl and a slightly green yellow color fading on the addition of NaOH. .

4. Determination of Anthocyanin Levels Using the Differential pH Method

Measurement of the total anthocyanin concentration was carried out using the UV-Vis spectrovotometry pH differential spectrophotometry method. This method is a solution through the difference in soinar absorbance seen at different pHs, namely 1.0 and 4.5 where at pH 1 anthocyanins are in the form of flavinium cations which give a red color. At a pH of 2-4, anthocyanins are in the form of a

green mixture of flavinium and quinoidal cations.

The absorbance results obtained from 96% ethanol extract, n-butanol and ethyl acetate with the absorbance from the dissolved sample (A) were determined through the following agreement (Asri Widyasanti & Wulandari, 2018):
A= (A510-A700)pH 1.0 – (A510-A700)pH 4.5
The content of anthocyanin pigments in the sample is calculated by the equation:

$$\textbf{Amount of anthocyanin (ppm)} = \frac{A \times BM \times FP \times 1000}{s \times b}$$

From the results of the calculation above, it can be seen that the average levels of each solvent are different, namely 0.946% 96% ethanol, 1.684% n-butanol and 0.181% ethyl acetate. The highest anthocyanin content was produced by the extract with the solvent used was n-butanol at 1.684%. While the lowest anthocyanin content was produced by the extract with ethyl acetate solvent of 0.181%. This is because based on the polarity of anthocyanins which can dissolve in polar solvents. The total anthocyanin content of the extract with the three solvents above explains that the anthocyanin compounds in the butterfly pea extract are estimated to have the same polarity as n-butanol, where n-butanol can bind anthocyanin from the butterfly pea extract more than 96% ethanol and ethyl acetate solvents. In addition, the absorbance value which is smaller than the range that should be in the range 0.2 – 0.8 is caused by several factors including temperature, changes in pH, light and oxygen, then during the sample preparation process, sample conditions and sample storage period not directly used or tested after the extract is ready will also cause anthocyanins to degrade.

There are several conditions for fulfilling the One-Way ANOVA test, namely if the data is normally distributed then the test can be carried out or can be continued. The normality test results can be seen from the Shapiro-Wilk data because the data is less than 50. Based on the Shapiro-Wilk data the data is normally distributed because V-Palue > 0.05. Followed by the One-Way ANOVA test where the sig value of 0.124 means V-Palue > 0.05 and H0 is accepted or there is no significant difference between the % anthocyanin levels based on the type of solvent used. To see these differences can be seen from the descriptive column based on the average with a standard deviation. When viewed from the descriptive value it can be interpreted that the highest amount of anthocyanin levels or the highest was obtained by n-butanol which has a mean of 0.49100 with a standard deviation of 0.339796.

## CONCLUSIONS AND RECOMMENDATIONS

From the test for determining the levels of anthocyanin extract in the butterfly pea sample, it can be concluded that based on the results of anthocyanin assistance, the butterfly pea flower showed a positive result marked by a steady red color change after adding HCl indicating the presence of flavilium compounds and after dropping NaOH the color changed from green to fading green. indicating the presence of quinodal compounds. The average anthocyanin content in butterfly pea flower was 0.946% or 0.946g of anthocyanin from 96% ethanol extract from 100g extract, 1.684% or 1.684g anthocyanin from n-butanol extract from 100g extract, and 0.181 or 0.181g anthocyanin acetate extract from 100g extract. Based on the results of determining the anthocyanin content of the butterfly pea flower using the pH differential method, the highest concentration was found in the n-butanol extract at 1.684%.

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