

Determination of UV-Vis Spectrophotometry with Differential pH on Total Anthocyanin Levels of Ethanol Extract of *Cordyline fruticosa* (L.) A. Cheval Leaves

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ABSTRACT: *Cordyline fruticosa* (L.) A. Cheval is a traditional medicinal plant contains several chemical compounds including saponins, tannins, flavonoids, polyphenols, steroids, and anthocyanins. The purpose of this study was to determine the total anthocyanin levels of red andong leaf extract. In this study, red andong leaf was extracted using the maceration method with 70% ethanol solvent. Furthermore, qualitative testing was carried out by screening the phytochemicals of red andong leaf extract and continued with qualitative-specific testing for anthocyanins. Then the anthocyanin levels were measured using UV-Vis spectrophotometry with differential pH. The results showed that anthocyanins were of cyanidin type. Furthermore, the measurement of total anthocyanin levels was carried out using the differential pH method. Based on these results it can be concluded that the total anthocyanin levels of andong red leaf extract (*Cordyline fruticosa* (L.) A. Cheval) 17.76mg/L.

KEYWORDS: *Cordyline fruticosa* (L.) A. Cheval, Total Anthocyanin Levels, differential pH

1. INTRODUCTION

Red andong (*Cordyline fruticosa* (L.) A. Cheval) is a traditional medicinal plant that is proven to have various properties including as a (natural) medicinal ingredient, efficacious for treating gingivitis, diarrhea or dysentery, bleeding wounds, bleeding hemorrhoids, bleeding (hemostatic) (Dalimartha, S. 2006). Empirically, red andong leaf is used as a medicine for diarrhea and dysentery. One of the plants with anthocyanin substances is the red andong leaf which has a striking color. The previous study found that plant carriage has red leaves so it has the potential to be used as a dye or natural pigment (Susanto, 2014).

Almost all natural ingredients contain flavonoid compounds, one of which is anthocyanin. Anthocyanins are a class of flavonoid compounds broadly divided into plant polyphenols. Flavonols, flavan-3-ols, flavones, flavanones, and flavanols are classes of flavonoids that differ in anthocyanin oxidation. Flavonoid compounds are colorless or pale yellow (Sundari 2008). Utami's research confirms that this red andong leaf extract also contains flavonoids, triterpenoids, saponins, and tannins.

The stability of anthocyanins is influenced by several factors including pH, temperature, storage time, light, and oxygen (Harborne, 1996). In the research Tansiska (2007) and Rahayu., A (2010) stated the stability of anthocyanin with treatment at room temperature (25 °C) and cold temperatures (3 °C). The results stated that storage at cold temperatures better maintains the stability of anthocyanin. The longer the storage will further damage the color intensity due to the effect of free radicals which damage the anthocyanin color pigment.

Heat treatment can also cause the anthocyanin balance to tend towards a colorless form. Damage due to heating can occur during hydrolysis, where the anthocyanin glycosidic bonds produce unstable aglycones, then the aglycone rings form carbonyl and chalcone groups. This degradation can occur further if an oxidizing agent is present to form a brown compound (Ozela et al., 2007). stable or degraded at high temperatures and stable at low temperatures (Kwartiningsih, et al., 2016).

The content of compounds in the red andong plant greatly influences its pharmacological activity. Several studies support the extract. Utami, et al (2021) tested the potential of extract leaves as antioxidants to counteract DPPH radicals with IC values 50 64.5197 µg/mL in comparison of Vitamin C with IC value 50 2.12µg/mL. In addition, a cytotoxic test has been carried out on ethanol extract with an LC value₅₀ ie 60.36 µg/mL included in the toxic category (Utami., Y.P. et al, 2023). From the background above, it is necessary to research to determine the total anthocyanin content of the leaf extract.

2. EXPERIMENTAL SECTION

2.1. Sample collection

This research was conducted from August 2019 to January 2020 at the Pharmaceutical Biology Laboratory and research laboratory of the Makassar College of Pharmacy, Makassar. The research sample used Bone District, South Sulawesi. Then wet sorting is carried out and washed thoroughly with running water. Then chopped, then dried using an

oven at 50 °C for 2 days to reduce the water content. Then it is weighed and kneaded, then extraction is carried out. The type of research used is an experimental laboratory that utilizes specific reagents for qualitative analysis and uses the UV-Vis spectrophotometry method in quantitative analysis.

2.2. Sample Extraction

A sample of 500 g was irrigated in 3750 L of 70% ethanol for 3x24 hours and sometimes mixed. Once allowed to stand, the sample of red dandelion leaves is filtered, and the filter is collected. After that, continue using the same solvent until clear. Filters from maceration and re-maceration evaporate to obtain thick extracts.

2.3 Qualitative analysis

2.3.1. Identification of Alkaloids

The extract of 2 ml is inserted into the test tube, dropped with 2 N HCl, then divided into 3 test tubes. Each tube is added with each reagent. At the addition of the Mayer reagent, it is positive for the alkaloid if it forms a white precipitate. The addition of Wagner reagents is positive to the alkaloid if brown rain is formed. An orange leak is formed with the addition of the Dragendroff reagent, positive for the alkaloid. (Kusumawati, et al, 2003).

2.3.2. Identification of Flavonoids

As much as 0.5 g of extract was put into a test tube dissolved in 2 mL of 70% ethanol then stirred, added 0.1 g of magnesium powder and 3 drops of concentrated HCl. If an orange to-red color is formed, it indicates the presence of flavones, red to deep red indicates flavanols and solid red to-purplish red indicates flavanones (Kusumawati, et al, 2003).

2.3.3. Identification of Saponins

Put 2 mL of extract into a test tube, add 10 mL of hot water, cool then shake vigorously for 10 seconds. Positive for saponins if the foam is formed as high as 1-10 cm for not less than 10 minutes and on the addition of 1 drop of HCl 2 N, the foam does not disappear (Depkes RI, 1995).

2.3.4. Identification of Triterpenoids

As much as 0.5 g of extract was put into a test tube, added 2 mL of 70% ethanol, then shaken, 1 mL of chloroform, 1 mL of acetic anhydride, and then dried. After drying, H₂SO₄ was added. If a reddish color is formed, it is positive for triterpenoids (Mandal and Ghasal, 2012).

2.3.5. Identification of Tannins

As much as 2 mL of extract was put into a test tube and then shaken with hot water until homogeneous after FeCl₃ was added, if it produces a characteristic blue-black blue, it contains pyrogallol tannins. As for catechol tannins, it is considered positive if there is an addition of FeCl₃ solution, then it will be green or blue-green and precipitate (Kusumawati, et al, 2003).

2.3.6. Identification of Anthocyanin

Test the color with HCl: 50 mg of red andong leaf extract was added with 2 M HCl, then heated to 100 °C for 5 minutes (+) red. Color test with NaOH: 50 mg of red andong leaf extract was added drop by drop with 2 M NaOH while observing the color change that occurred (+) blue-green faded slowly.

2.4. Quantitative analysis

2.4.1. Determination of maximum wavelength

The sample was carried out using a visible spectrophotometer with 535 nm and 700 nm wavelengths.

2.4.2. Total anthocyanin test with differential pH method

Preparation of buffer solutions pH 1.0 and pH 5. Weighed as much as 0.86 g of potassium chloride (KCl) plus 100 mL of aquadest put in a glass beaker then added concentrated hydrochloric acid (HCl) little by little to a pH of 1 then the solution was transferred to a flask Measure 100 mL and add distilled water to a solution volume of 100 mL. To make a pH of 4.5, which is weighed 5.44 of sodium acetate (CH₃CO₂ .3H₂O) mixed with 100 mL of distilled water in a glass beaker. Then, with HCl 2 N, little by little to a pH of 4.5, the solution was transferred to a 100 mL volumetric flask, and distilled water was added to a volume of 100 mL to measure pH using a pH meter.

Determination of total anthocyanin test with different pH. 1 g of condensed extract was dissolved using 70% ethanol, then prepared two sample solutions, the first solution was a solution for pH 1 and the second solution for pH 4.5. Take 1 mL of red andong leaf extract each and dilute it using a pH solution each to a volume of 5 mL.

2.5. Data analysis

The absorbance of each dilution is measured. To determine the absorbance value, use 535 nm and 700 nm wavelengths. The absorbance obtained is calculated by calculating the anthocyanin content with the formula:

$$A = (A_{535}-A_{700})_{pH1.0}-(A_{535}-A_{700})_{pH4.5}$$

$$\text{Total Anthocyanin \%} = \frac{A \times MW \times DF \times 1000}{(E \times W)}$$

See also:

A : The absorption

MW : molecular weight of cyanidine 3-glucoside (449,2 g/mol)

E : Absorption coefficient of 26900 L/mol.cm⁻¹ is expressed as cyaniding-3-glucoside

W : The width of the kit (1 cm)

DF : The Reduction Factor

3. RESULTS AND DISCUSSION

The sample used in this study is red andong leaf. Traditionally, red andong leaves are used as a (natural) medicinal ingredient, efficacious for treating gingivitis, diarrhea or dysentery, bleeding wounds, bleeding hemorrhoids, and bleeding (hemostatic). Empirically, red andong leaves are used as a medicine for diarrhea and dysentery (Dalimartha, 2006).

Samples of red carriage leaves were obtained from Bone Regency, South Sulawesi Province. Then extracted using the maceration method. The maceration method was chosen because the damage factor for the active substance is smaller. This method does not use heat which can damage the withdrawn active substance. The main emphasis of this method is to provide sufficient contact time between the solvent and the extracted tissue. Maceration is carried out by immersing the sample powder in the solvent (solvent). The solvent will penetrate the cell wall and enter the cell cavity which contains the active substance (Hanum, 2000).

In this method, the sample is immersed in 70% ethanol which has been purified. Ethanol was chosen because it is easy to obtain and is a polar solvent that can dissolve the active compounds or secondary metabolites contained in the leaves of red andong. The maceration results obtained a viscous extract with a calculation of the percent yield of 3.41%.

Then a phytochemical screening was carried out to determine the active compounds' content in the sample of red andong leaf leaves. This study carried out phytochemical screening tests starting from the alkaloid, flavonoid, saponin, triterpenoid, tannin, and saponin tests. The results of identifying the chemical content of the ethanol extract of andong red leaves can be seen in **Table 1**:

Table 1. Phytochemical Screening of Red Andong Leaf Extract

Chemical Compounds	Observation Result	Reference	Information
Alkaloid			
Mayer	No separation occurs	White precipitate	(-)
Wagner	No separation occurs	Brown precipitate	(-)
Carrying liquor	No separation occurs	Orange precipitate	(-)
Flavanoids	Colored Red	Red, orange and yellow	(+)
Triterpenoids	Colored Red	Blue-green (steroids) Red-purple (triterpenoids)	(+)
Saponin	Foam formed	Formed foam	(+)
Tannin	Blackish green	Blackish green	(+)

The results of the phytochemical screening of red andong leaf extract with 70% ethanol solvent show that the alkaloid test, namely Mayer, Wagner, Dragendrof, showed negative results. On testing the flavonoids, triterpenoids, saponins, and tannins, showed positive results.

Furthermore, a preliminary anthocyanin test was carried out to identify anthocyanin compounds as a color test by weighing 50 mg of the condensed extract of red andong leaves and adding 2 M HCl. After adding 2 M HCl, then heating at 100 °C for 5 minutes and the results obtained by the red andong leaf extract did not change color, then carried out again by adding 2 M NaOH drop by drop until the color changed from green to fading slowly. This is in accordance with research conducted by Yulfriansyah and Novitriani (2016) which states that the addition of 2 M HCl produces a red color, whereas the addition of 2 M NaOH drop by drop produces a color change to yellowish green and fades slowly. Anthocyanin type of leaf extracts red carriage after the addition of 2 M HCl and 2 M NaOH, namely cyanidin. According to the journals Le Bellec (2006) and Rifka Hardiyanti (2018) states that the addition of 2 M HCl gives a red color and 2 M NaOH gives a blue green color that fades slowly. Chemically, anthocyanins are derivatives of a single aromatic structure, namely cyanidin, and all of them are formed from cyanidin pigments with the addition or reduction of hydroxyl groups, methylation and glycosylation (Harborne, 1996). can be seen in **Table 2**

Table 2. Preliminary Anthocyanin Test Red Andong Leaf Extract

Testing	Result	According to Harbone (1996)	Information
Sample + 2 M HCl and heated for 5 minutes at 100 °C	The color remains red	Fixed color red	(+) cyanidin
The sample was added with 2 M NaOH solution drop by drop	Color change turns green and fades slowly	The color changes to blue-green and fades slowly.	(+) cyanidin

The following quantitative analysis uses a UV-Vis spectrophotometer with a differential pH method. The pH difference method was used to compare anthocyanin compounds produced at different pH, namely pH 1 because anthocyanins were stable at pH below 4 and less stable at pH 4.5. Buffers were added at pH 1 and 4.5, and absorbance was measured. This test aligns with research on measuring anthocyanin levels conducted by a previous study (Utami et al., 2016).

Before determining the amount of anthocyanin, the wavelength is determined to see the maximum wavelength of anthocyanin enters. It is determined at a wavelength of 400-800. From the results anthocyanin testing from red andong leaf extract for cyanidin type maximum wavelength of 535 nm. According to Harbone, 1996 the maximum wavelength for anthocyanins ranges from 475 nm to 550 nm.

The 535 nm wavelength obtained is the maximum wavelength for cyanidin-3-glucoside, while the 700 nm wavelength is for correcting precipitates that are still present in the sample, if it is obvious then the 700 nm wavelength is 0 (Utami et al, 2016). The table above shows that the red andong leaf extract contains a total anthocyanin of 17.76 mg/L in **Table 3**.

Table 3. Quantitative Analysis of Measurement Results of Red Andong Leaf Extract

Sample	pH	Absorbance	Wavelength (nm)	A	Total Anthocyanin (mg/L)
Red Andong Leaf Extract	4.5	0.685	535	1.064	17.76 mg/L
		0.488	700		
Extract	1	1.752	535		
		0.491	700		

4. CONCLUSION

Based on the results of this study it can be concluded that the total anthocyanin levels of red andong leaf extract using the UV-Vis spectrophotometry method with a differential pH of 17.76 mg/L. The hope of the researchers from this study is as a reference for further research, which is to optimize the temperature and storage time of the total anthocyanin levels of the sample. Thus, the most optimal anthocyanin levels data will be obtained and samples will be used as active ingredients in the manufacture of pharmaceutical preparations.

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