Ad-Dawaa JOURNAL OF PHARMACY

ISSN 2987-4157

V0l. 2 No. 1 Page 27-34 DOI : 10.52221/dwj.v1i1.225

Determination of Anthocyanin Levels in Saffron Flower (*Crocus sativus* L) Infusion with Differential pH Method

Fitriyani[1 *], Davit Nugraha[1]

STIKes Muhammadiyah Ciamis, Indonesia.

Correspondance: Davit Nugraha

Email: *Fitriyani95@gmail.com*

Address : Jl. K.H. Ahmad Dahlan No.20, Ciamis, West Java, Indonesian

ABSTRACT

Saffron (Crocus sativus L), as an important herbal plant in medicine, cosmetics and ather health industries. Saffron (Crocus sativus L) is used in tradisional medicine to treat various types of diseases, including to trear chronic diseases such as asthma and artritis, also used to treat coughs and fevers. The saffron plant (Crocus sativus L) contains active compounds, namely, carotenoids, anthocyanins, vitamins (tiboflavin and thiamine), and minerals. The purpose of this study was to determine the levels of anthocyanins in saffron flowers (Crocus sativus L). anthocyanins are a group of pigments that cause a reddish color, found in water-soluble cell fluids. Anthocyanin compounds function as antioxidants and free radical scavengers, thus playing a role in preventing aging, cancer, and degenerative diseases. The research method used is aquadest solvent with temperatures of 25^{o}C, 50^{o}C, dan 80^{o}C with a steeping process. The test was carried out using a differential pH, then the absorption was measured using a UV-Vis spectrophotometer at a wavelength 510 and 700 nm. Determination of the levels that have been obtained the average anthocyanin content of steeping saffron flowers with a temperature of 25^{o}C 0,047%, 50^{o}C 0,289%, 80^{o}C 0,116%. The results of data analysis show that all the average levels of steeping saffron flowers from each temperature are different, in other words the average levels of steeping levels of saffron flowers from each temperature there are significant differences. The highest anthocyanin levels in this study were produced by steeping saffron flowers at a temperature of 50^{o}.

Keywords : Saffron Flower, Anthocyanin, Differential pH.

INTRODUCTION

Saffron is used in traditional medicine to treat various types of diseases. Saffron is used to treat chronic diseases such as asthma and arthritis. Saffron is also used to treat coughs and fever (Afifah & Hasanah, 2020). Saffron is considered one of the important herbal plants in Indonesia medicine, cosmetics and other health industries. Chemical analysis shows that more than 150 chemical components are contained in saffron stigmas, where These three components are mainly crocins (crocetin), picrocrocin (intermediate safranal) and safranal. These components create exclusive color, taste and aroma from saffron. Apart from these three components, saffron also contains other components, such as carotenoids, carbohydrates, raw fiber, protein, fat, anthocyanins, flavonoids, vitamins (riboflavin and thiamine), minerals and other nutritional elements which is considered a nutritional element and is beneficial for health (Sitepu & Simanungkalit, 2019).

Saffron has a taste Typical bitterness due to the presence of the monoterpene glycoside picrocrosin. The antioxidant content in saffron includes phenolic compounds and carotenoids (Pharmacy & Science, 2021). Chemically, anthocyanins come from a single aromatic structure, namely cyanidin, where all types of anthocyanins have differences based on the bonds between them R3' and R5' groups with aromatic anthocyanin rings. Here's the basic structure anthocyanins (Priska & Et al, 2018). Anthocyanins are more stable in acidic conditions compared to alkaline or neutral conditions (Hetty Nur Handayani et al., 2021). Anthocyanins can specifically absorb light in the absorption range ultraviolet (UV) to violet, but stronger in the visible region of the spectrum. Anthocyanins are absorbed at a wavelength of 250-700 nm, with two peaks as a sugar group (glycone) at a wavelength of approximately 278 nm, and The main peak is anthocyanin (aglycone) at a wavelength of 490-535 nm (Siti Rahmah K.R, et al., 2023).

In this research, research will be carried out to determine anthocyanin levels In saffron flowers, anthocyanins are a group of pigments that cause color reddish, found in water-soluble cell fluid. Anthocyanin compounds functions as an antioxidant and scavenger of free radicals, so it plays a role in preventing aging, cancer and degenerative diseases (Pham, et al. 2022). Apart from being functional As antioxidants, anthocyanins have other uses, including as natural indicators, and as a dye in the textile and food industries (Pratiwi & Priyani, 2019).

TOOLS AND MATERIALS

The tools used are analytical scales, beaker glass, watch glass, measuring flask, beaker glass, pH meter, UV-Vis spectrophotometry. The ingredients used are saffron flowers, potassium chloride, hydrochloric acid, sodium hydroxide, sodium acetate and distilled water.

METHODS

1. Making Saffron Flower Infusion

   The saffron flowers in this study were brewed based on use in society. 40 mg of saffron flowers are taken and then added into a beaker glass and pour 10 ml of distilled water at a temperature of 25°C, 50°C, and 80°C. The stirring process was carried out for 15 minutes.

2. Anthocyanin Phytochemical Test

   Proving the presence of anthocyanins can be done by: simple. The first way is to add 2M HCl to the sample, then observe the color of the sample. If the red color of the sample does not change (steady), then indicates the presence of anthocyanins. The second way is by adding samples with 2M NaOH drop by drop. According to (Lestario et al., 2012) If color red turns to green blue and fades slowly then shows the presence of anthocyanins (Anggriani et al., 2017).

3. Making pH 1.0 and 4.5 Solutions

   Anthocyanin determination was carried out using the pH difference 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 in the form of colorless carbinol. This can be done by creating an aliquot of an anthocyanin solution in water whose pH is 1.0 and 4.5 for then the absorbance was measured (Suzery et al., 2015).

4. Measurement and Calculation of Total Anthocyanin Concentration by Differential pH Method

   The results of steeping saffron flowers using distilled water at temperatures of 25°C, 50°C and 80°C are 40 mg dissolved in 10 ml of solvent water. Then put it into a measuring flask. Take 5 ml from the measuring flask. Prepare two solutions. In the first sample, add KCL buffer with a pH of 1.0 and for the second sample, use a sodium acetate buffer with a pH of 4.5. Each sample is dissolved in a buffer solution based on a predetermined dilution factor.

Samples dissolved in a pH 1.0 buffer solution were left for 15 minutes before being measured. For samples dissolved in buffer pH 4.5, it was measured after leaving it mix for 15 minutes. g. The absorbance of each solution with wavelengths of 510 and 700 nm was measured with a pH 1.0 buffer and a pH 4.5 buffer as a blank. The absorbance of the dissolved sample (A) is determined using the following equation (Widyasanti et al., 2018):

$$A = (A510-A700)pH\ 1.0 – (A510-A700)pH\ 4.5$$

RESULTS

Table 1. Phytochemical Test Results of Saffron Flowers

Added with 2 M HCL	Color changes to yellow	Positive anthocyanin (content low anthocyanins)
Added NaOH 2M drops	color changes to green	Positive anthocyanin (content low anthocyanins)

Table 2. Absorbance of Saffron Flower Infusion at 25°C

pH	**λmax (nm)**	**Absorbance**
1,0	510	1 = 0,024
		2 = 0,023
		3 = 0,023
	700	1 =0,002
		2 = 0,001
		3 = 0,001
4,5	510	1= 0,001
		2 = 0,003
		3 = 0,002
	700	1 = 0,001
		2 = 0,000
		3 = 0,001

Table 3. Absorbance of Saffron Flower Infusion at 50°C

pH	λmax (Nm)	Absorbance
1,0	510	1 = 0,133
		2 = 0,137
		3 = 0,135
	700	1 = 0,001
		2 = 0,003
		3 = 0,005
4,5	510	1 = 0,023
		2 = 0,020
		3 = 0.032
	700	1 = 0,002
		2 = 0,004
		3 = 0.023

Table 4. The result of anthocyanin levels in Saffron Flower Infusion

Saffron infusion	Levels (%)	Average (%)
	0,019	
25°C	0,009	0,047
	0,019	
	0,093	
50°C	0,099	0,289
	0,097	
	0,036	
Suhu 80°C	0,042	0,116
	0,038	

DISCUSSION

Based on the results in table 1, it shows that the observations obtained using the first method using 2 M HCl resulted in a low anthocyanin content indicated by a fading yellow color. Observations obtained using the second method show that the anthocyanin content is low, characterized by a greenish yellow color that fades slowly after being dripped with NaOH. This is because the anthocyanin contained in the saffron solution has been degraded. To see more clearly the anthocyanin content, a concentration test was carried out using UV-Vis spectrophotometry on the next step.

This section describes, explains and interprets in detail the meaning of the data and variables from the research results. relate our results to previous research. Discussion 600-1000 words in English. Explain the strengths and limitations of the study. This method is carried out in the first way, namely making a pH 1.0 buffer solution by mixing 1.49 grams of KCL with distilled water to a limit of 100 ml in a measuring flask, then adding HCL until the pH becomes 1.0, while for the pH 4 buffer, 5, namely by mixing 5.44 grams of sodium acetate with distilled water until the 100 ml mark in the measuring flask, then adding HCL until the pH becomes 4.5. After making a pH buffer, the second step is to brew 20 mg of saffron flowers each with 10 ml of distilled water at temperatures of 25^{o}C, 50^{o}C and 80^{o}C and take 5 ml of each, then prepare 2 solutions from each. temperature, the first solution is added with KCL buffer with a pH of 1.0 and the second solution is added with a sodium acetate buffer with a pH of 4.5, then the pH of each solution is first checked, if the pH of each solution has not reached pH 1.0 and 4.5 then added again with the pH buffer that has been made until the pH of each solution is appropriate. After the pH was appropriate, it was allowed to stand and then the absorbance was measured using UV-Vis spectrophotometry with wavelengths of 510 and 700 nm.

The absorbance of the dissolved sample (A) is determined using the following equation (Asri Widyasanti & Wulandari, 2018):

$$A= (A_{510} - A_{700})\text{pH } 1.0 – (A_{510} - A_{700})\text{pH } 4.5$$

Based on the data in the table above, it shows that the anthocyanin levels of saffron flowers from each temperature have different levels. The lowest anthocyanin content is produced at a temperature of 25^{o}C with an average of 0.047%, this is because at a temperature of 25^{o}C the anthocyanin is less bound, thus causing a low absorbance value, and the highest anthocyanin content is produced at a temperature of 50^{o}C with an average of 0.289%, this is because the anthocyanin is stable at this temperature. 50^{o}C-60^{o}C, this result is obtained from the calculation of multiplying the absorbance result times the anthocyanin molecular weight times the dilution factor times 1000 divided by the molar absorptivity times the thickness of the cuvette to get the concentration results. where the temperature of 50^{o}C can bind more anthocyanins from infusion of saffron flowers than at temperatures of 25^{o}C and 80^{o}C, at a

temperature of 80ºC it produces an average of 0.116% less than a temperature of 50ºC. This causes degradation, because anthocyanins are sensitive to changes in temperature, light, oxygen , so that there is a change in pH during the spectrophotometric test preparation process which causes the anthocyanin to be less stable, thus affecting the concentration test. Based on statistical tests, the effect of temperature shows significant differences in results.

CONCLUSION

Based on the research that has been carried out, it can be concluded that the infusion of saffron flowers contains anthocyanin, the average anthocyanin content of infusion of saffron flowers at a temperature of 25ºC is 0.047%, 50ºC 0.289%, 80ºC 0.116%.. The highest anthocyanin content is produced in infusion of saffron flowers at temperatures 50ºC.

ACKNOWLEDGEMENTS

We would like to express our gratitude to our colleagues for the publication of this research article as well as to STIKes Muhammadiyah Ciamis for their assistance with laboratory facilities so that this research could be completed well.

REFERENCES

Afifah, M. N., & Hasanah, A. N. (2020). Saffron (Crocus sativus L): Kandungan dan Aktivitas Farmakologinya. Majalah Farmasetika, 5(3), 116–123. https://doi.org/10.24198/mfarmasetika.v5i3.26291.

Anggriani, R., Ain, N., & Adnan, S. (2017). Identifikasi Fitokimia Dan Karakterisasi Antosianin Dari Sabut Kelapa Hijau (Cocos Nucufera L Var Varidis). Jurnal Teknologi Pertanian, 18(3), 162–172. https://jtp.ub.ac.id/index.php/jtp/article/view/599

Hetty Nur Handayani, Kaliawan, & Nanang Setio Pambudi. (2021). Perbandingan Penentuan Antosianin Pada Bunga Rosella Dengan Menggunakan Uv-Vis Spektrometri Dan Pencitraan Warna. Jurnal Teknik Ilmu Dan Aplikasi, 9(1), 15–17. https://doi.org/10.33795/jtia.v9i1.5

Lestario, L. N., Rahayuni, E., & Timotius, K. H. (2012). Kandungan Antosianin dan Identifikasi Antosianidin dari Kulit Buah Jenitri (Elaeocarpus angustifolius Blume). AgriTECH, 31(2). https://doi.org/10.22146/AGRITECH.9731

Pham, T. N., Le, H. T., Pham, V. T., & Le, T. H. N. (2022). Optimization Of The Microwave Assisted Extraction Of Anthocyanin From Violet Glutinous Rice (Oryza Sativa) And Its Antioxidant Activities. Materials Science Forum, 1077, 175–18

Pharmacy, J., & Science, H. (2021). Efektivitas Senyawa Antioksidan dalam Saffron (Crocus Sativus L). 02, 36–42

Pratiwi, S. W., & Priyani, A. A. (2019). Pengaruh Pelarut dalam Berbagai pH pada Penentuan Kadar Total Antosianin dari Ubi Jalar Ungu dengan Metode pH Diferensial Spektrofotometri. EduChemia (Jurnal Kimia Dan Pendidikan), 4(1), 89. https://doi.org/10.30870/educhemia.v4i1.4080.

Priska, M., & Et al. (2018). Antosianin dan Pemanfaatannya. Cakra Kimia (Indonesian E-Journal of …, 6(2), 79–97. https://ojs.unud.ac.id/index.php/cakra/article/view/46629.

Sitepu, Y. R. B. T. P. D. melitus T. 1. 2019. 89-94, & Simanungkalit, J. N. (2019). Potensi Saffron Sebagai Antidiabetes. Y Sitepu, 1(November), 89–94. http://jurnal.globalhealthsciencegroup.com/index.php/JPPP/article/download/ 83/65.

Siti Rahmah Kurnia Ramdan, Resi Lestari. 2023. Determination of Anthocyanin Content of Purple Sweet Potato (Ipomea batatas L) Extract Using the Differential pH Method. Jurnal Kesehatan Mucis. Vol.10 No.2. https://doi.org/10.52221/jurkes.v10i2.371.

Trikas ED, Papi RM, Kyriakidis DA, Zachariadis GA. A sensitive LC-MS method for anthocyanins and comparison of byproducts and equivalent wine content. *Separations*. 2016;3(18):1-12.

Widyasanti, A., Nurlaily, N., & Wulandari, E. (2018). Karakteristik Fisikokimia Antosianin Ekstrak Kulit Buah Naga Merah Menggunakan Metode Uae. Jurnal Ilmiah Rekayasa Pertanian Dan Biosistem, 6(1), 27–38.