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Influence pH to Rate Anthocyanins Extract Senggani Fruit (Melastoma malabathricum Auct, Non Linn)

Ratih Indrawati[1*], Gervacia Jenny.R[1], Maulidiyah Salim[1], Bagus Muhammad Ihsan[1], Ari Widiyantoro[2]

[1] Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak, Indonesia
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak, Indonesia

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Corresponding Author:
Bagus Muhammad Ihsan
ihsanfillah24@gmail.com

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Abstract: One of the plants that can be used as a natural dye is senggani fruit. This study aimed to determine the effect of acid pH on the anthocyanin levels of the senggani fruit extract. The method used in this study uses UV-Vis Spectrophotometry. The sample was senggani fruit extract, added with an acidic pH solution (1-6) with 3 repetitions for 18 samples. Based on the results of research that has been carried out, the average anthocyanin levels at pH 1 are 2.00% with a bright red color; pH 2 is 1.63% with a red-orange color, pH 3 is 1.45% with an orange color, pH 4 is 2.05 % with brownish red color, pH 5 is 1.38% with brownish orange color, and pH 6 is 1.11% with light brown color. Based on statistical tests using Simple Linear Regression, Ha was accepted so that it could be concluded that there was an effect of acid pH on the anthocyanin levels of the senggani fruit. Based on a study that has been done, it is known that the sourer the pH solution is, the taller the anthocyanin obtained. Matter happens Because absorbance is comparable with the rate of anthocyanin something sample.

Keywords: Acid pH; Anthocyanin; Extract; Melastoma malabathricum Auct non Linn

Introduction

Natural dyes have a higher economic value than artificial dyes. One of the natural coloring ingredients comes from fruit (Islam et al., 2024; Emekdar Karaman & Şahin, 2024; Aftab et al., 2024). Senggani fruit (Melastoma malabathricum Auct, non-Linn) is one of the fruits that can be used as a natural dye (Mustaqim, 2020; Peluru & Abram, 2021; Safrida et al., 2023). Senggani fruit (Melastoma malabathricum Auct, non-Linn) is a fruit that contains anthocyanins (Dolnicar et al., 2015).

Anthocyanins are water-soluble pigments that are naturally found in various types of plants (Khoo et al., 2017; Nassour et al., 2020). As the name suggests, this pigment provides color to green plants' flowers, fruit, and leaves and has been widely used as a natural coloring in various food products and other applications (Khoo et al., 2017; Qaisar et al., 2019; Shrikant Swami et al., 2020). The nature of anthocyanins at low pH (acid) is that the anthocyanin pigment is red, and at high pH, it changes to violet and then becomes purple-blue. The results of previous research entitled Senggani Fruit Anthocyanin (Melastoma Malabathricum Auct, Non-Linn) as a Bacterial Colorant in Differential Painting Techniques concluded that senggani fruit anthocyanin pigment can be used as a coloring agent Bacteria (Indrawati et al., 2022).

Anthocyanin pigments are classified as amphoteric compounds that can react with acids and bases. In acidic media, anthocyanins are red, like in vacuole cells, and turn purple and blue as the medium becomes more alkaline. Factors that affect the stability of anthocyanins are structural transformation and pH, temperature, light, oxygen, And pigmentation (Armanzah & Hendrawati, 2016; Khoo et al., 2017). This compound is polar and can be extracted with solvents. It is polar, too.

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Some polar solvents include ethanol, water, citric acid, and ethyl acetate (Noviyanty & Anggriani Salingkat, 2019; Wulaningrum et al., 2013; Lidya Simanjuntak et al., 2014; Pratiwi & Priyani, 2019).

The nature of anthocyanins at low pH (acid) is that the anthocyanin pigment is red; at high pH, it changes to violet and then becomes purple-blue (Ayun et al., 2022; Anggriani et al., 2017). According to Khasanah et al. (2014), Pradnya et al. (2023), pH and temperature influence the stability of teak leaf extract. The higher the pH and temperature values, the more the extract stability decreases (in terms of total anthocyanin content, antioxidant activity, and color quality). Stability Test of Red Anthocyanin Pigment from Young Teak (T. grandis) leaves against pH as a Natural Colorant. Several factors influence the stability of anthocyanin pigment, including changes in structure and pH, where hydroxy will reduce stability, while methyl will increase stability. Anthocyanins are more stable in acidic solutions than in solvent bases (Suena et al., 2023; Hidayah & Fikroh, 2023 ;Khairi et al., 2023). Based on previous research and several things that can affect the stability of the anthocyanin pigment, researchers are interested in testing the stability of the red anthocyanin pigment from senggani fruit and getting the right pH to maintain the stability of the anthocyanin of senggani fruit (Surianti et al., 2019; Sari, 2020; Purwaningsih et al., 2023).

This research is novel and valuable to the field of dye chemistry and natural products because of the following factors: the impact of acid pH levels, the anthocyanin content, and the specific focus on senggani fruit extract—all of which have not been previously studied in relation to natural dyes and their properties.

Method

The research design used is a quasi-experimental study in Laboratory Polytechnic Country Pontianak and Laboratory Chemistry Health Polytechnic Ministry of Health Pontianak. The research was carried out from March until August 2022. Population in the study: This is extracted fruit (Melastoma malabathricum Auct. non-Linn). This study treated sengggani fruit extract so that pH was obtained at 1, pH 2, pH 3, pH 4, pH 5, and pH 6.

Measurement and inspection rate of anthocyanin extract fruit will be done using spectrophotometry. Based on previous research and several things that can affect the stability of the anthocyanin pigment, researchers are interested in testing the stability of the red anthocyanin pigment from senggani fruit and getting the right pH to maintain the stability of the anthocyanin of senggani fruit.

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Making Reagents
Solution HCl 0.1 m

A total of 4.145 ml of 37% HCl solution was pipetted using a measuring pipette and inserted into a 500 ml measuring flask that contains Aquadest in a way slowly. After that, it was diluted with distilled water until the limit mark on the pumpkin measuring (Ramadhani & Octarya, 2017).

Make solution pH 1 (solution HCl 0.1 M)

Taken 75 ml solution HCl concentrated 0.1 m with pipette measuring, Solution the entered to in beakers glass, Added Aquadest to in beakers glass A little sake A little until it reaches pH Which desired., pH solution tested use pH meters, Solution poured into the bottle reagent, then labeled pH 1, and Solution pH 1 Ready used.

Make pH solution 2

Solution pH 2 made from dilution solution HCl pH 1, Prepared solution pH 1 Which has made previously., Taken 1 ml solution pH 1 with measuring pipette, Solution the put in in beakers glass 100 ml, Added Aquadest A little sake A little until reachpH Which desired, Solution homogenized until mixed flat, pH solution tested use pH meters, Solution poured to in bottle reagent, Then label it pH2, Solution pH 2 Ready used.

Make pH solution 3

Solution pH 3 made from dilution solution HCl pH 2, Prepared pH solution 2 Which has made previously, Taken 1 ml solution pH 2 with measuring pipette, Solution the put in in beakers glass 100 ml, Added Aquadest to in beakers glass A little sakeA little until it reaches pH Which desired, Solution homogenized until mixed flat, pH solution tested use pH meters, Solution poured into the bottle reagent, Then give label pH 3, Solution pH 3 Ready used.

Make pH solution 4

Solution pH 4 made from dilution solution HCl pH 3, Prepared solution pH 3 Which has made previously, Taken 1 ml solution pH 3 with measuring pipette, Solution the put in in beakers glass 100 ml, Added Aquadest to in beakers glass A little sakeA little until it reaches pH Which desired., Solution homogenized until solution mixed flat, pH solution tested use pH meters, Solution poured into the bottle reagent, Then give label pH 4, and Solution pH 4 Ready used.

Make pH solution 5

Solution pH 5 made from dilution solution HCl pH 4, Prepared solution pH 4 Which has made previously, Taken 1 ml solution pH 4 with measuring pipette, Solution the put in in beakers glass 100 ml, Added Aquadest to in beakers glass A little sakeA little until it reaches pH Which desired, Solution homogenized until solution mixed flat, pH solution tested use pH meters, Solution poured to in bottle reagent, Then give label pH5, and Solution pH 5 Ready used.

Make pH solution 6

Solution pH 6 made from dilution solution HCl pH 5, Prepared solution pH 5 Which has made previously, Taken 1 ml solution pH 5 with measuring pipette, Solution the put in in beakers glass 100 ml, Added Aquadest to in beakers glass A little sakeA little until reach pH Which desired, Solution homogenized until solution mixed flat, pH solution tested use pH meters, Solution poured to in bottle reagent, Then give label pH 6 and Solution pH 6 Ready used.

Procedure inspection

Making extract fruit senggani

Prepare 6 beakers labeled pH 1 on glass 1, pH 2 in glass 2, pH 3 in glass 3, pH 4 in glass 4, pH 5 on glass 5, and pH 6 on glass 6, Entered extract thick as much as 0.1 grams in each beaker's glass. Then, dissolved in 10 ml ethanol 96%, Add pH solution to each beaker glass until it reaches the desired pH. pH 1 solution at glass 1, pH 2 on glass 2, pH 3 on glass 3, pH 4 on glass 4, pH 5 on glass 5 and pH 6 on glass 6, Solution homogenized until mixed flat, pH of the solution was tested using pH meters, and The solution was poured into the bottle reagent and then labeled (Asni et al., 2020; Widowati et al., 2021; Risnayanti, 2020).

Addition solution pH

Prepare 6 beakers labeled pH 1 on glass 1, pH 2 in glass 2, pH 3 in glass 3, pH 4 in glass 4, pH 5 on glass 5, and pH 6 on glass 6, Entered extract thick as much as 0.1 grams in each beaker's glass. Then, dissolved in 10 ml ethanol 96%, Add pH solution to each beaker glass until it reaches the desired pH. pH 1 solution at glass 1, pH 2 on glass 2, pH 3 on glass 3, pH 4 on glass 4, pH 5 on glass 5 and pH 6 on glass 6, Solution homogenized until mixed flat, pH of the solution was tested using pH meters, and The solution was poured into the bottle reagent and then labelled (Fatonah et al., 2016; Mufidah et al., 2021).

Determination Total Rate Anthocyanin

Factor dilution, which is the appropriate sample, must be determined. Moreover, formerly with the method, dissolve the sample with buffers KCL pH 1 until an absorbance of less than 1.2 is obtained long wave 510 nm, Furthermore, measured absorbance Aquadest on the long waves (510 and 700 nm) to look for point zero. Long wave 510 nm is the extended wave maximum for cyanidin-3-glucoside, whereas a wavelength of 700 nm is needed to correct deposits still in the sample. If the sample is apparent, absorbance on 700 nm is 0, Two solution samples were prepared. The first used KCl buffer with pH 1, and the second sample used buffers of Na acetate with pH 4.5. Each sample was dissolved with a buffer solution based on DF (dilution factor/dilution factor) that had been determined previously. Samples dissolved using a pH 1 buffer were left for 15 minutes before being measured, whereas For samples dissolved with buffers, pH 4.5 was Ready to be measured after being mixed for 5 minutes, The absorbance of each solution at wavelengths of 510 and 700 nm was measured with pH 1 buffer and pH 4.5 buffer as the blank (Le et al., 2019; Inácio et al., 2013; Rigolon et al., 2020). Absorbance from the sample Which has dissolved (A) is determined with the formula:

Information :

A = anthocyanin absorbance

€ = molar absorbance of cyanidin-3-glucoside 26900 L/(mol.cm)

L = cuvette width = 1 cm

MW = molecular weight of cyanidin-3-glucoside = 449.2 g/mol DF = dilution factor

V = final volume or volume of pigment extract (L)

Wt = sample weight

Figure 1. Formula

Result and Discussion

Based on results study Which has done, obtained data following:

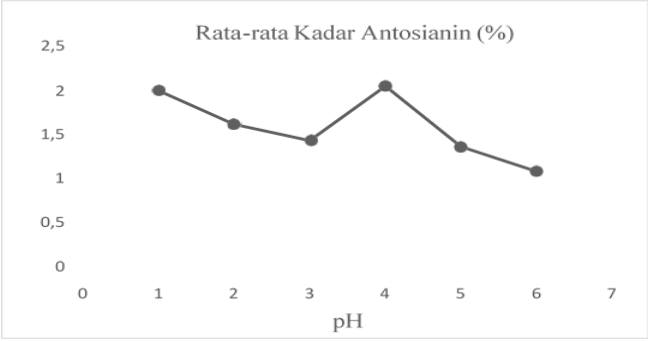

Figure 2. Chart Average Rate Anthocyanin Extract Senggani Fruit

From table in on, can seen that average rate anthocyanin higheston pH 4 namely 2.05%. This study found the highest anthocyanin levels in sample solution with pH 4, namely 2.05%. This happens because the pH 4 is pure senggani fruit extract without adding a pH solution. On inspection method, spectrophotometry UV-Vis uses light looks. The amount of light emitted will be absorbed by the checked solution. So, that amount of light absorbed will compare straight with the absorbance obtained. On the pH 4 solution sample obtained, the color is more intense than pH 1, so the amount of light absorbed is more significant. This causes the absorbance value at pH 4 to be greater and higher anthocyanin levels. At pH one, the anthocyanin level is high, caused by the increasingly acidic pH of the solution. Meanwhile, at pH 4, levels the anthocyanin is high not because the acid solution is added, but because at pH 4, the solution extract is pure without the addition of solution pH so that the color generated is the color of the original extract with respect the more concentrated and level the anthocyanin is higher.

Test Descriptive

The data obtained from the research results are then processed using descriptive tests to describe the results, which has been done. Descriptive tests can be seen in the table 1. Table 1 shows that the anthocyanin levels in the extracted fruit are highest at pH 4, 2.0533%.

Table 1. Descriptive Test of the Effect of Acid pH on Levels Anthocyanin Senggani Fruit Extract (*Melastoma malabatchricum Auct. non Linn)*

			Descriptive Statistics	
	N	*Minimum*	*Maximum*	*Mean*
pH 1	3	1.90	2.10	2.0000
pH 2	3	1.54	1.72	1.6300
pH 3	3	1.34	1.54	1.4533
pH 4	3	2.00	2.14	2.0533
pH 5	3	1.27	1.48	1.3767
pH 6	3	1.08	1.13	1.1100
Valid N (listwise)	3			

Test Normality

Test normality aims To know whether distribution data in the variables that will be used are normally distributed or not, so it can be known whether the data use statistics parametric (data normally distributed) or non-parametric (non-normal distribution data) (Adhelacahya et al., 2023; Alti et al., 2023). Condition data distributed normally is marked sig. or significance > 0.05 (Khoirunnisa & Amaliyah, 2023). Results test normality can seen in the table 2.

Table 2. Test Normality Influence pH Sour to Rate Anthocyanin Extract Fruit Senggani (Melastoma malabathricum Auct. non Linn)

			Shapiro-Wilk
	Statistic	df	Sig.
pH 1	1.000	3	1.000
pH 2	1.000	3	1.000
pH 3	.949	3	.567
pH 4	.855	3	.253
pH 5	.999	3	.948
pH 6	.893	3	.363

From table 2 it can be seen that all have a sig value > 0.05 so that it can interpreted that data on distribute normal.

Test Homogeneity

After testing normality data with the test, Shapiro-Wilk further tested homogeneity data. Test homogeneity aims To know whether the data is homogeneous or not so that you can determine which test is in accordance. Condition data homogeneous is marked sig. or significance, which generated > 0.05 (Kristi & Andriani, 2023; Ghasemi & Zahediasl, 2012).

Table 3. Homogeneity Test Effect of Acid pH on Concentration Anthocyanin Senggani Fruit Extract (Melastoma malabathricum Auct. non Linn)

Levene Statistic	df1	df2	Sig.
.587	5	12	.710

Table 3 shows the results of the data homogeneity test. Based on the test, this homogeneity obtained a sig value of 0.710, so it can be interpreted that the research data variance is homogeneous because the sig value is > 0.05. Then, the next test parametric is with test regression.

Analysis Bivariate

If Univariate analysis has been carried out, the results will be known characteristics or distribution of each variable, and analysis can be continued bivariate (Notoatmodjo, 2012). Analysis bivariate in study This was carried out after the results of the anthocyanin level examination were tested for normality and homogeneity data. It is known that the data that was obtained was distributed normally and homogeneously. So analysis bivariate in study This uses a simple linear regression test. Simple linear regression is an analysis for measuring the linear connection between two variables, where one variable is considered to influence the other variable. Variables that influence are called independent variables, and variables that influence influence are called variable dependent (Zakariah et al., 2020).

Table 4. Correlations Simple Linear Regression Test Effect of pH Sour to Rate Anthocyanin Extract Fruit Senggani (Melastoma malabathricum Auct. non Linn)

		Antosianin	pH
Pearson Correlation	Rate Anthocyanin	1	-.655
	pH	-.655	1
Sig. (2-tailed)	Rate Anthocyanin		.003
	Ph	.003	
N	Rate Anthocyanin	18	18
	pH	18	18

From the regression test, a significance value of 0.003 < 0.05 means a natural (significant) influence exists between the independent variables and the dependent variable. Table 5.5 shows that the value R = - 0.655 is in the range of 0.60 – 0.799, so the relationship (correlation) between acidic pH and anthocyanin levels is strongly negative. Negative meaning is the relationship between acidic pH and anthocyanin levels of fruit extracts (Melastoma malabathricum Auct. non-Linn) No one way, Meaning No one way here is the more low pH so rate anthocyanins from senggani fruit extract (Melastoma malabathricum Auct. non-Linn) will continue to increase. Vice versa, the higher the rate anthocyanin extract fruit (Melastoma malabathricum Auct. non-Linn) will drop.

From the test results in Table 5 and 6, the R-value is obtained, namely 0.655, which means that the correlation between the independent variable and the level of anthocyanin is 0.655. The R Square value obtained from the test results on that is 0.428, Which means the influence variable free to variable bound is 42.8% while the rest is influence caused by variables that have not been researched.

Table 5. Model Summary of Simple Linear Regression Test Influence pH Sour to Rate Anthocyanin Extract Fruit Senggani (Melastoma malabathricum Auct. non Linn)

Model	R	*R Square*	*Adjusted R Square*
1	.655	.428	.393

Table 6. Coefficients Test Regression Linear Simple Influence pH Sour to Rate Anthocyanin Extract Fruit Senggani (Melastoma malabathricum Auct. non Linn)

Model	B	Std. Error	Beta	Q	Sig
(Constant)	8.718	1.541		5.658	.000
Rate Anthocyanin	-3.253	.939	-.655	-3.464	.003

Based on table 6 show that model equality regression linear simple Which used for pH sour Which affect levels anthocyanin extract fruit please is : y = 8.178 – 3.253x

Where: Y: Anthocyanin content of Senggani fruit extract (Melastoma malabathricum Auct. non Linn) X: pH sour

The regression equation obtained is y = 8.178 – 3.253x. Table 5.7 shows the results of statistical test regression with a mark significance of 0.003 < 0.005. Then Ha is accepted, so it can be concluded that acidic pH influences anthocyanin levels in extract fruit (Melastoma malabathricum Auct. non-Linn.

Based on a study that has been done, it is known that the sourer the pH solution is, the taller the anthocyanin obtained. Matter happens Because absorbance is comparable with the rate of anthocyanin something sample. The condition solvent, the more sour, mainly approaching pH one, will produce significant levels of total anthocyanins because anthocyanins are more stable at acidic pH. Besides that is the situation, which is the sourest. It also causes more wall cell vacuoles, Which are broken to extract more anthocyanin pigments (Pratiwi & Priyani, 2019). pH conditions significantly affect the stability/equilibrium of the solution anthocyanin extract. Under very acidic pH conditions, anthocyanins form cations flavilium, where anthocyanin is most stable and colorful. Meanwhile, at a more alkaline pH, anthocyanin will be yellow (chalcone form), blue (quinoid form), or No colored (carbinol base) (Misbachudin et al., 2014; Saputra et al., 2014; Khoo et al., 2017; Hermanto et al., 2023)

Anthocyanin damage can be influenced by temperature, pH, light, oxygen, and enzymes. Heating dramatically affects the color stability of anthocyanin pigment and can cause the color to become pale. The decline in color stability is allegedly because of temperature. Because of the decomposition of anthocyanin from form, aglycone becomes calcone (Not colored). Temperature And long warmup cause decomposition And change structure, so that happens to bleach (Nasrullah et al., 2021; Eko Wiyono et al., 2022). Nalawati (2022), reported that temperature affected the color stability of anthocyanin extracts in Isabel grape skins, and controlling storage temperature is a factor important in guard stability of anthocyanin. Suzery et al. (2020), reports that treatment temperature influences the stability of anthocyanin. The more tall the temperature warms up, the more speed the decline rate of the anthocyanin. Enaru et al. (2021), states that anthocyanins are unstable at light intensity and high temperatures and in aqueous solutions. Letting go of group sugar causes aglycone anthocyanin, which forms, to fade quickly if exposed to light or enhancement temperature (Nurhidajah et al., 2023). In the study, the researcher controlled temperature and light. The temperature is controlled by storing the sample solution and checking the anthocyanin rate in place with a temperature room of 27.3°C, measured using a thermometer. The controlled method keeps the sample in place, Which is dark, that is, a bottle of glass chocolate, And puts it in a black plastic bag. This is done to maintain the stability of anthocyanins in the sample solution due to the activity of anthocyanins, polyphenol, oxidase, and peroxidase enzymes, resulting in a change in the color of anthocyanin through an oxidation reaction so that decreasing levels of anthocyanin (Turker et al., 2004; Simanjuntak et al., 2016; Suzery et al., 2020).

Conclusion

Average rate anthocyanin extracts fruit please on one as big as 2.00%, pH two as big as 1.63%, pH three as big as 1.45%, pH four as big as 2.05%, pH five as big as 1.38 and pH 6 of 1.11%. The color produced by senggani fruit extract at pH 1 is bright red; on pH two, it colored red-orange; on pH three, it colored orange; on pH four, it is brownish red; at pH five, it is brownish orange; and on pH six, colored chocolate young. Acidic pH affects the anthocyanin content of senggani fruit extract (Melastoma malabathricum Auct non-Linn) with p = 0.003.

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Author Contributions

Conceptualization, RATIH INDRAWATI.; methodology.; validation, GERVACIA JENNY.R. and RATIH INDRAWATI.; formal analysis, R MAULIDIYAH SALIM.; investigation, BAGUS MUHAMMAD IHSAN and ARI WIDIYANTORO.; resources, BAGUS MUHAMMAD IHSAN.; data curation, GERVACIA JENNY.R.: writing—original draft preparation, ARI WIDIANTORO and BAGUS MUHAMMAD IHSAN.; writing—review and editing, BAGUS MUHAMMAD IHSAN.: visualization, and MAULIDIYAH SALIM and R. GERVACIA JENNY.R . All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no confict of interest.

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Kolaka.