5th International Conference Eco-Innovation in Science, Engineering, and Technology
Volume 2024
http://dx.doi.org/10.11594/nstp.2024.4504

Conference Paper

Effect of Type and Concentration of Acid on Chemical Characteristics of Mexican Petunia (*Ruellia simplex* C. Wright) Flower Extract

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ABSTRACT

Mexican petunia (*Ruellia simplex* C. Wright) is a flowering plant which has blue to purple flowers which are thought to be anthocyanin components. The solvent used in the extraction of Mexican petunia flowers is distilled water which is a polar solvent with the addition of functional acids in order to denature plant cells so that maximum anthocyanin is extracted. The aim of this research was to determine the effect of acid treatment type and concentration on chemical characteristics of Mexican petunia flower extract and knowing its stability to pH, temperature and heating time for the best treatment. This study used a completely randomized factorial design with two factors, namely the first factor was the type of acid (acetic acid and citric acid) and the second factor was the acid concentration (0.05%; 0.1%; 0.15% ; 0.2%). The data obtained were analyzed using ANOVA next treatments that had a significant effect were tested further using the DMRT test at the 5% level. The best results were obtained in citric acid treatment with a concentration of 0.2% with initial pH 2.04, final pH 2.95, color intensity 0.412, and total anthocyanin 0.0728%, stable at acidic pH in the range of pH 3, heating temperature up to 60^{O}C and heating time up to 60 minutes.

Keywords: Mexican petuia, citric acid, acetic acid, stability

Introduction

The Mexican petunia flower is a species of flowering plant that includes in the genus Reullia, family Acanthaceae. In Indonesia, this plant has blue to purple flowers, blooms in all seasons and is easy to obtain. Flower colors are attributable primarily to flavonoid and carotenoid pigments. Flavonoids are the most common flower color pigment, and the predominant flavonoid pigments are the anthocyanins. Anthocyanins are composed of an anthocyanidin and sugar moieties. They are the basis for most orange, pink, red, magenta, purple, blue, and blue–black floral colors. Freyre at al. (2015) stated that *Ruellia simplex* C. Wright flowers are blue to purple in color, due to the presence of anthocyanin components in it and it has potential as a natural dye. This is also supported by Mattioli et al. (2020), anthocyanin is a pigment that contributes to red color light, purple and blue, can be obtained from red flower crowns, pink, purple and blue. Anthocyanin compounds are found in water extracts of plants, stable at low pH, sensitive to high temperature and soluble in polar solvents. One way to obtain the anthocyanin component in Mexican petunia flowers is by the extraction method. The extraction process suitable for taking color components from flowers is a maceration extraction process.

Maceration is one of the extraction methods, used to minimize damage to internal anthocyanin components in Mexican petunia flowers, apart from that the maceration method is simple and cheap (Kurniawati, 2019). Maceration is carried out by immersing the sample in solvent. The solvent will penetrate the walls cells and enter (diffuse) into the cell cavity containing the active substance. The active substance will dissolve due to the difference in concentration

How to cite:
Sarofa, U., Puspitasari, S., & Rosida. (2024). Effect of type and concentration of acid on chemical characteristics of Mexican Petunia (*Ruellia simplex* C. Wright) Flower Extract. *5th International Conference Eco-Innovation in Science, Engineering, and Technology*. NST Proceedings. pages 20-25. doi: 10.11594/ nstp.2023.4504

between the active substance solutions inside the cell and outside the cell, the concentrated solution is forced out. These events repeat themselves so that there is a balance between concentration solution outside the cell and inside the cell (Mukhriani, 2014).

The solvent used in the extraction of Mexican petunia flowers is distilled water which is a polar solvent with the addition of functional acids denatures plant cells so that anthocyanins are extracted maximally. Based on Hidayah et al (2014), solvents are often used to extract anthocyanin is a polar solvent in combination with acid, such as hydrochloric acid, acetic acid, formic acid or ascorbic acid. The acid functions to denature the membrane plant cells, then dissolve the anthocyanin pigment so that it can come out of the plant cells, as well as preventing the oxidation of flavonoids. Factors that influence extraction efficiency include methods extraction, type and concentration of solvent, particle size of the material to be extracted, extraction time, extraction temperature, solvent to material ratio, and pH extraction (Zhang et al., 2018). Differences in total anthocyanins produced for each type of organic acid closely related to the differences in degree of dissociation of each acid type. Degree of dissociation for hydrochloric acid, acetic acid, and citric acid respectively are 1, 1.8x10-5, and 8.4x10-4 (Day & Underwood, 1998). The greater it is the degree of dissociation is the stronger the acid because the greater the number of ions hydrogen released into solution. Besides that, the acid causes more and more vacuole cell walls to break down so anthosaine pigments are increasingly being extracted (Mattioli et al, 2020). Previous research extracted natural dyes from dragon fruit using distilled water as a solvent with the addition of citric acid and acetic acid at a concentration of 10 % and the material and solvent ratio of 1:10 has a total anthocyanin yield 8.355 mg/100gr and 0.751 mg/100gr respectively. (Hidayah et al, 2014). Based on this, it is necessary to research the influence of the type and concentration of acid on the color extraction of Mexican petunia flowers (*Ruellia simplex* C. Wright) and it's stability.

Material and Methods

The main ingredient used in the research was Mexican petunia flowers obtained in Sidoarjo Regency. The materials used for analysis are distilled water, acetic acid, citric acid, potassium acetate, KCl, HCl, FeCl3 (Sigma Aldrich), Mayer's reagent (HgCl2 and KI), Wagner's reagent (Iodine and KI), Liebermann Burchard's reagent (chloroform, anhydrous acetic and concentrated sulfuric acid). The equipment used are spectrophotometer, analytical balance, pH meter, hotplate, magnetic stirrer, water bath and glassware.

The method used is maceration, that was, Mexican petunia flowers were cleaning, weighing, cutting, solving in acid solutions according to the type and concentration (0.05%;0.1%;0.15%;0.2%). Then were extracting using magnetic stirrer on 27^{o}C temperature for 20 minutes and then filtering. The extract was then measured for total anthocyanin, pH measurement, and color intensity. The extract with the highest total anthocyanins was tested for stability by measuring absorbance at a wavelength of 517 nm at pH (3-6) heating temperature 60oC – 90oC) and heating time (0 – 90 minutes).

This research used a Completely Randomized Design (CRD), arranged in a factorial pattern, consisting of two factors, namely the first factor was the type of acid (acetic acid and citric acid) and the second factor was the acid concentration (0.05% ; 0.1% ; 0.15% ; 0.2%). The data obtained were analyzed using ANOVA next treatments that had a significant effect were tested further using the DMRT test at the 5% level.

Results and Discussion

Chemical analysis of Mexican petunia flowers extract

Analysis of Mexican petunia flower extract includes pH, color intensity and total anthocyanins. The results of this analysis can be seen in Table 1.

Table 1. Average pH, color intensity and total anthocyanin of Mexican petunia flowers extract

Treatment				
Acid type	Concentration (%)	pH	Color Intencity	Total Anthocyanin (mg/100g)
Acetic acid	0,05	3.86 ± 0,014[g]	0.275 ± 0,021[a]	0,013 ± 0,001[a]
	0,10	3.62 ± 0,007[e]	0.351 ± 0,011[b]	0,032 ± 0,001[d]
	0,15	3.44 ± 0,007[d]	0.438 ± 0,007[cd]	0,046 ± 0,000[c]
	0,20	3.37 ± 0,035[c]	0.468 ± 0,025[d]	0,048 ± 0,002[d]
Citrc acid	0,05	3.68 ± 0,042[f]	0.430 ± 0,026[cd]	0,027 ± 0,000[b]
	0,10	3.42 ± 0,000[cd]	0.412 ± 0,003[c]	0,054 ± 0,003[e]
	0,15	3.18 ± 0,035[b]	0.409 ± 0,014[c]	0,064 ± 0,000[f]
	0,20	2.95 ± 0,014[a]	0.391 ± 0,042b[c]	0,073 ± 0,004[g]

Note: Average values followed by different letters mean significantly different ($p \leqslant 0.05$)

Table 1 shows a decrease in pH as the acid concentration given increases. Acetic acid has a pH ranging from 3.86-3.37, but citric acid has a lower pH than acetic acid, namely around 3.68-2.95. This is because the higher the acid concentration, the more H+ ions are released, thereby lowering the pH of the solution. The decrease in pH of citric acid is lower than that of acetic acid, this is because the acidity level of citric acid is lower than that of acetic acid. The acidity level in citric acid is 3.15 and in acetic acid 4.76 (Ali et al., 2013). Besides that, the degree of dissociation in citric acid is higher than in acetic acid, where the degree of dissociation is greater the stronger an acid is, because the greater the number of hydrogen ions released into solution (Mukhriani, 2014).

Table 1 shows that the color intensity results for acetic acid increased along with the increase in concentration, whereas for citric acid there was a decrease along with the increase in concentration, although it was not significant. This is because the higher the acid concentration, the lower the pH produced, as a result of which other compounds such as tannins are also extracted. According to Kurniawati (2019), extraction in acidic conditions can affect the color intensity, this is because by extracting samples in acidic conditions, not only anthocyanins are dissolved, but also other compounds, such as tannins.

Table 1 shows that the higher the acid concentration, the total anthocyanin produced in citric acid and acetic acid increases, but the total anthocyanin in citric acid is higher than in acetic acid. This is because the higher the acid concentration, the lower the pH of the solvent, so that it can help the process of removing anthocyanin pigments from cells by breaking down the cell vacuole walls. The pH of the citric acid solvent is lower than the pH of the acetic acid solvent, so the total anthocyanin produced in the solvent with acetic acid is higher than in citric acid. According to Hidayah et al. (2014), increasingly acidic conditions cause more vacuole cell walls to break so that more anthosaine pigment is extracted.

Stability test on the best treatment of mexican petunia flower extract

The best treatment was obtained from citric acid treatment with a concentration of 0.2%. Furthermore, during this treatment, color stability tests were carried out regarding pH, heating temperature and heating time.

Stability test against pH

The color stability test was carried out at a pH range of 3, 4, 5, and 6 to determine the changes that occur at varying pH conditions. The changes that occur can be seen from the color and absorbance numbers produced at each pH. The color results can be seen in Figure 1.

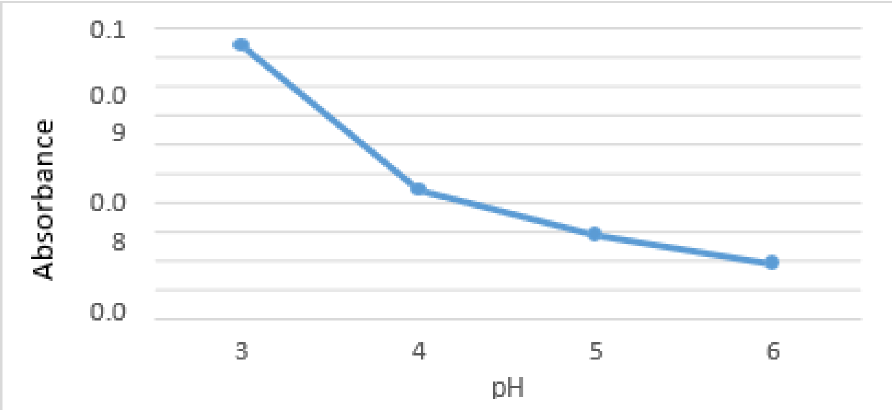

Figure 1. The relationship between pH and color stability

Figure 1 shows that the higher the pH, the lower the absorbance produced. This is due to structural changes in anthocyanins. The higher the pH, the more OH groups are bound to the anthocyanin structure and this causes a shift in the colored anthocyanin groups to chalcone or colorless groups. The absorbance figure is more stable at pH 3, whereas at pH above 4 the absorbance decreases significantly because anthocyanins tend to be easily damaged or degraded and change color to colorless or chalcone due to the presence of more OH groups. According to Freyre et al (2015), pH 4 and above causes damage to the anthocyanin pigment in arben fruit which causes the absorbance number to decrease because the color changes to colorless (bleaching occurs).

Stability test against heating temperature

Stability tests on heating temperatures were carried out at temperatures of 60°C, 70°C, 80°C, and 90°C to determine the changes that occur at varying heating temperatures. The results of the color change can be seen in Figure 2.

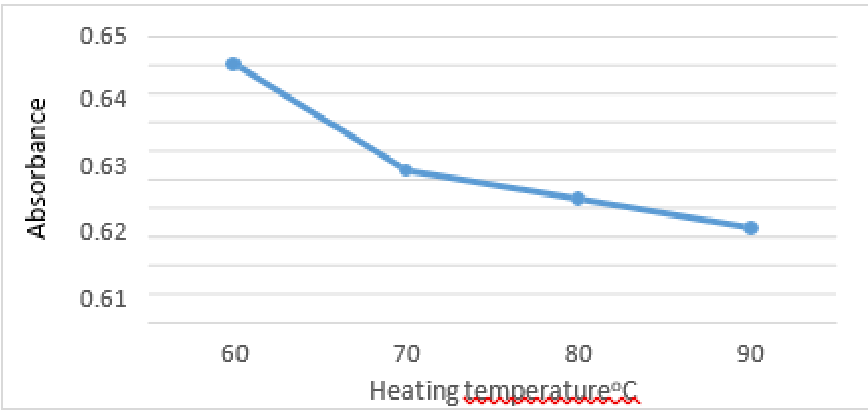

Figure 2. The relationship between heating temperature and color stability

In Figure 2, shows that the higher the heating temperature given, the lower the resulting absorbance number. This is because the higher the heating temperature used, the more damage there is to the anthocyanin component. Heating at temperatures above 60OC can cause damage to anthocyanin, where the anthocyanin component is degraded into a colorless substance or chalcone. A significant decrease in absorbance numbers occurred at temperatures of 60OC - 90OC, but when compared with the absorbance of the control without heating (1.318) at a temperature of 60OC there was a large decrease in absorbance. The decrease in the absorbance value of the dye extract at high temperatures is due to the decomposition of anthocyanins from aglycone form to chalcone (colorless) (Mattioli et al., 2020).

Stability test over heating time

Stability tests on heating duration were carried out after boiling for 0, 30, 60 and 90 minutes to determine changes that occurred under varying heating durations. The results of the color change can be seen in Figure 3.

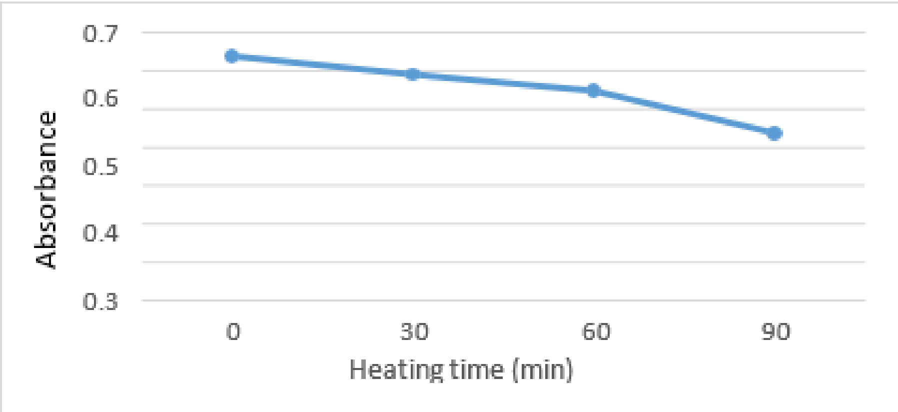

Figure 3. The relationship between heating time and color stability

Figure 3. Shows that the longer the heating time, the lower the absorbance results. This is because anthocyanins will be damaged or degraded when heated at high temperatures, the longer the heating time, the more anthocyanins will be damaged due to the heating process. The decrease in absorbance value was classified as stable at a heating time of 0 minutes to 60 minutes and experienced a very significant decrease at a heating time of 90 minutes. According to Hidayat et al. (2014), the higher the temperature and the longer the heating, the lower the anthocyanin absorbance value, so the color stability decreases.

Conclusion

The type and concentration of acid treatment had a significant influence and interaction on pH parameters, color intensity, and total anthocyanins. The best treatment is citric acid with a concentration of 0.2% produces Mexican petunia flower extract with a pH of 2.95, intensity color 0.412, and the highest total anthocyanin 0.0728%. Stable at pH 3, stable up to heating at a temperature of 60OC with a heating time of up to 60 minutes.

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