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Research Article

Determination of Total Flavonoid Content of Red Fruit Juice (*Pandanus conoideus* Lam) by a UV–Vis Spectrophotometer

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ABSTRACT: Determination of Total and Flavonoid Content of Red Fruit (Pandanus conoideus Lam) Using A UV – Vis Spectrophotometer. (Supervised by Mamat Pratama and Andi Trihadi Kusuma Kisra)
Red fruit (Pandanus conoideus Lam) belongs to the pandanacea family. This plant contains compounds such as tannins, flavonoids, steroids, triterpenoids and alkaloids and has properties in curing various diseases such as diabetes, coronary heart disease, hypertension, cancer and HIV/AIDS. This study aims to determine the total flavonoid content of red fruit juice (Pandanus conoideus Lam) using a UV - Vis spectrophotometer. The stages carried out in this research began with sample preparation, qualitative testing of flavonoid compounds and determining the total flavonoid content of red fruit (Pandanus conoideus Lam) samples. The results obtained in this study based on a qualitative test using NaOH reagent stated that red fruit juice (Pandanus conoideus Lam) was positive for containing flavonoids and in a quantitative test using a UV - Vis spectrophotometer the percent concentration obtained in the red fruit juice sample was 8.7258%.

KEYWORDS: Red fruit (*Pandanus conoideus* Lam.); flavonoids; UV-Vis Spectrophotometer.

1. INTRODUCTION

Indonesia is one of the world's mega-diverse countries for medicinal plants. The area of tropic forest in Indonesia is the second largest in the world after Brazil. Of the 40,000 plant species in the world, 30,000 species are found in Indonesia and 940 species are known to have medicinal properties that have been used in traditional medicine by various ethnic groups in Indonesia for generations. The number of medicinal plants covers about 90% of the number of medicinal plants in the Asian region (Fahrudin, 2014)

Red fruit (*Pandanus conoideus* Lam) is widely used by local Papuans as a source of food, natural coloring and traditional medicinal ingredients. Red fruit is known as a fruit with many benefits because red fruit is rich in various nutrients including beta-carotene, tocopherol, oleic acid, linoleic acid, decanoate, protein, calcium, vitamins, energy, fat and fiber (Sadolona and Agustin, 2021). In research conducted states that based on the results of screening red fruit (*Pandanus conoideus* Lam) contains alkaloids, flavonoids, glycosides and triterpenoids. The benefits of red fruit (*Pandanus conoideus* Lam) are empirically proven to cure various diseases such as cancer, heart disease, tuberculosis and respiratory tract disorders (Mellyana and Prabawati, 2022).

Fruit juice is the result of squeezed fruit that is filtered. The main purpose of making fruit juice is to extend the shelf life and usefulness of the fruit. There are slight differences in the preparation of fruit juice for each type of fruit but the principle remains the same. Juice contains crushed fruit and appears cloudy or clear (Hermawan, 2016)

Flavonoids are compounds included in the phenolic group which is the largest green plant. Flavonoids are found in almost all parts of the plant including the fruit, roots, leaves and outer skin of the stem. The flavonoid compound itself plays a role in giving color to fruits and flowers. Flavonoids also have functions as anti-inflammation, anti-allergic, antiviral, antioxidant and anticarcinogenic. Based on the above background, this study will determine the total flavonoid content in red fruit juice (Pandanus conoideus Lam) using UV-Vis Spectrophotometer.

2. EXPERIMENTAL SECTION

2.1. Sample collection

The sample population was the red fruit (Pandanus conoideus Lam) sourced from Mimika District, Papua Province and the sample used was the red fruit juice (Pandanus conoideus Lam).

2.2. Qualitative analysis

A total of 1 ml of sample juice was put into a test tube. Then added 2 – 4 drops NaoH 10 % . Positive for flavonoids if it produces yellow (Widyasari and Handayani, 2020).

2.3. Material and Tools

The materials used in this study arealuminum foil, aluminum chloride ($AlCl_3$), quettetin, 1 M potassium acetate (1 M CH_3COOK), juice of red fruit (Pandanus conoideus Lam) and aquadest. While the tools used are blender (Miyako), dropping pipette (Pyrex ®), brown bottle, porcelain cup, beaker glass (Pyrex ®), measuring cup, test tube (Pyrex ®),

1000 µl micro pipette (Dragonlab ®), volume pipette (Pyrex ®), cuvettes, vials, volumetric flask 10 mL, Ultraviolet Visible Spectrofotometer (*thermoScientific Tipe Fenesys 10s*) and analytical scales (*Kern ABT 220 -5DM*).

2.4. Quantitative analysis

2.4.1. Preparation of Quercetin Standard Curves

Weighed as much as 10 mg of quercetin standard and dissolved in 10 mL of ethanol p.a stock solution was pipetted as much as 1 mL then enough volume up to 10 mL with ethanol p.a so that a concentration of 100 ppm was obtained. From the 100 ppm quercetin standard solution, several concentration series were made, namely 14 ppm, 16 ppm, 18 ppm, 20 ppm and 22 ppm. From each concentration series, 0.7 mL (14 ppm), 0.8 mL (16 ppm), 0.9 mL (18 ppm), 1 mL (20 ppm) and 1.1 mL (22 ppm) were pipetted. Then each concentration series was added 1.5 mL of ethanol p.a, 0.1 mL of 10% $AICI_3$ and 0.1 mL of potassium acetate and then the volume was sufficient to the limit mark using distilled water using a 5 mL volumetric flask. After that, it was incubated for 30 minutes at room temperature. The absorbance was determined using uv-vis spectrophotometry method at a maximum wavelength of 446 nm (Fawwaz et al., 2023).

2.4.2. Maximum wavelength determination

Determination of the maximum wavelength of quercetin was done by running quercetin solution at a wavelength range of 400 - 800 nm. The maximum wavelength will be used to measure the absorbance of the sample (Bachtiar *et al.*, 2023).

2.4.3. ***Sample preparation and measurement***

A total of 0.5 mL (500 µl) of red fruit juice (Pandanus conoideus Lam) was put into a 5 mL volumetric flask. Then added 1.5 mL ethanol p.a.; 0.1 mL $AICI_3$ 10%; 0.1 mL potassium acetate and sufficed to the limit mark using distilled water. After that, it was incubated for 30 minutes at room temperature. The absorbance was determined using UV-Vis spectrophotometric method at the maximum wavelength. Where the sample is made in three replicates for each analysis so that the average absorbance value is obtained.

2.5. Data analysis

A calibration standard curve was obtained by uv-vis spectrophotometry. The peak area was plotted against concentration. The equation of the line can be used to determine the best garus fit for the curve. The correlation coefficient is used to measure linearity. Furthermore, the correlation coefficient, intercept and slope of the calibration curve were calculated. Where the best fit of the data can be determined by linear regression using the following equation:

$$y = bx + a$$

Where : y = Absorbance
x = Concentration (C) mg/L
b = Slop (slope)
a = Intercept
r = Coefficient of correlation

3. RESULTS AND DISCUSSION

The red fruit plant (Pandanus conoideus Lam) is one type of plant that is found endemically in the province of Papua and belongs to the pandanaceae family. This red fruit plant is evenly distributed throughout Papua from the lowlands to the highlands, where the Papuan people use red fruit as a daily food ingredient which is processed into food spices and in the form of sauce. Part of the red fruit (Pandanus conoideus Lam) used is the seeds of red fruit which contains many natural antioxidant components such as a-carotene, B-carotene, B-cryptosanthin, a-tocopherol and unsaturated fatty acids such as oleic, linoleate and palmitoleic acids as well as phenol components. Red fruit can be used as a source of raw materials for degenerative drugs such as coronary heart disease, liver, cholesterol, diabetes, gout, osteoporosis and HIV (Maran, Siburian and Hendri, 2022). Samples of red fruit (Pandanus conoideus Lam) used in this study were taken in Timika Papua, Mimika Baru sub-district, Mimika district, Papua province and the sample part used was the seeds of the red fruit plant.

This study was conducted with the aim of determining the % value of total flavonoid content of red fruit juice (Pandanus conoideus Lam) which was carried out with qualitative tests in the form of color reaction methods and quantitative tests using UV-Vis spectrophotometric methods. (Maran, Siburian and Hendri, 2022)

The first stage to be carried out is sample preparation. The stage of sample preparation is fruit washing, the fruit that has been taken is ripe and not attacked by pests. Sample washing is done with the aim of removing impurities contained in the sample used. Red fruit samples that have been washed and separated from the stump are taken as much as 400 grams and then put into a juicer. The first stage to be carried out is sample preparation. 400 grams are then inserted into the juicher tool so that the pulp and juice are separated, after which the juice is filtered again so that 160 mL of red fruit juice is obtained, the juice obtained is stored in a brown bottle container and stored in the refrigerator, then qualitative testing is

carried out to determine the presence of flavonoid content in red fruit which is carried out using a test tube as shown in **Table 1**.

Table 1. Qualitative testing results of flavonoid compounds in red fruit juice

Sample	Reagents	İnitial color	Color change	Description
Red fruit (Pandanus conoideus Lam)	NaOH	Orange	Yellow	+

For qualitative testing, it is carried out by adding NaOH reagent, where when adding NaOH reagent, it will experience a yellow color change that occurs due to flavon and flavonol compound derivatives which will be decomposed by bases into molecules such as acetophenone which is yellow due to the breaking of bonds in the isoprene structure (Lindawati and Ni'ma, 2022).

In this study, the determination of total flavonoid content was carried out using the UV-Vis colorimetric/spectrophotometric method. The treatment carried out to determine the total flavonoid content in the sample is the addition of $AlCl_3$ to the sample solution which functions to form a stable acid with C-4 ketone groups, then with C – 3 and C – 5 hydroxyl groups from flavones and flavonols, where the addition of $AlCl_3$ will also form a stable acid complex with orthohydroxyl groups on rings A and B of flavonoids and the addition of potassium acetate which serves to maintain wavelengths in the visible region where before measurement is carried out incubation for 30 minutes which aims to make the reaction run perfectly so that it can provide maximum color intensity (Jubaidah, 2018).

UV-Vis spectrophotometer has a working principle based on the absorption of light through a solution, some of the light will be absorbed, some reflected and some emitted (Yanlinastuti and Fatimah, 2016). In this study, the determination of total flavonoid content of red fruit juice (Pandanus conoideus Lam) Using quercetin as a comparator because quercetin is included in one type of flavonoid that is often used as a comparator and is one of the compounds that are widely distributed in plants (Maulana K *et al.*, 2019). Then the quercetin comparator was made in several concentration series, namely 14 ppm, 16 ppm, 18 ppm, 20 ppm and 22 ppm where the purpose of using a concentration series is to determine flavonoid levels using the standard curve method to obtain a linear line equation that will be used for flavonoid concentration calculations (Risma *et al*., 2023). Furthermore, the maximum wavelength of quercetin was determined.

Determination of the maximum wavelength of the quercetin solution was carried out at a wavelength range of 400 - 800 nm which aims to know the absorption area that can be produced in the form of absorbance values on the quercetin solution that will be measured using a UV-Vis spectrophotometer at a wavelength range of 400 – 800 nm. The absorbance value of the quercetin solution will be measured using a UV-Vis spectrophotometer in the wavelength range of 400 - 800 nm(Sukmawati, Sudewi and Pontoh, 2018).The results obtained at the maximum wavelength for quercetin solution were at a wavelength of 446 nm. The color obtained from the quersetin solution is yellow, where the higher the concentration level used, the more intense the color obtained. The range of total flavonoid content based on the absorbance value is between 0.2 - 0.8 (Bachtiar *et al.*, 2023).The following are the results of absorbance measurements of quercetin standard solution (**Table 2**):

Table 2. Absorbance measurement results of quercetin standard

Quercetin concentration (ppm)	Absorbance
14	0.242
16	0.417
18	0.555
20	0.697
22	0.884

The next step is to measure the absorbance of the sample solution of red fruit juice (Pandanus conoideus Lam) and the measurement results obtained in **Table 3** below.

Table 3. Measurement results of total flavonoid content of red fruit (Pandanus conoideus Lam)

Sample	Replication	Absorbance
Juice red fruit (Pandanus conoideus Lam)	1	0.830
	2	0.868
	3	0.874

The absorbance measurement results were then graphed to obtain the value of the linear regression equation. The calculation used is based on the principle of Lambert - Beer law which shows a unidirectional relationship between absorbance and analyte concentration. Where the measurement of absorbance of total flavonoid content to determine the calibration curve of quercetin at a wavelength of 446 nm resulted in a regression equation y = 0.0782 - 0.8486. For the standard solution of flavonoid compounds, there is a linear relationship between absorbance and concentration in absorbance measurements with a correlation coefficient of r = 0.9984. The value (r) which is close to 1 indicates that the

regression equation obtained is linear (Pratama, 2023).The following is a graph of the absorbance results of the quercetin standard (**Figure 1**).

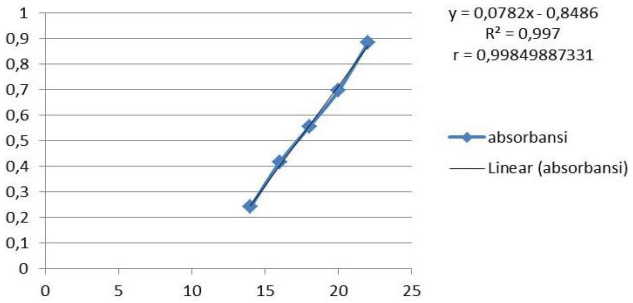

Figure 1. Linier regression curve of quercetin standard

The calibration curve equation above can be used to determine the levels of total flavonoid compounds in fruit juice. Determination of total flavonoid content of red fruit juice (Pandanus conoideus Lam) carried out against quercetin standard using UV-Vis spectrophotometer results obtained in flavonoid levels in replicates 1, 2 and 3 amounted to 21.465: 21.951 and 22.028 pl/mL. The results obtained were entered into the calculation formula of total flavonoid content and the results obtained for replication 1 amounted to 171.72 mg QE/L juice; replication 2 amounted to 175.608 mg QE/L juice and replication 3 amounted to 176.224 mg QE/L juice (**Table 4**). Then to get the average % value of total flavonoid content of red fruit juice (Pandanus conoideus Lam), the three replicates were summed up and then divided by 3 so that the average value obtained was 17.4517%. In measuring the total flavonoid content of red fruit juice samples (Pandanus conoideus Lam), 3 replications or repetitions were carried out. replication or repetition it is done because it aims to make the results obtained more accurate (Wahdaningsih et al., 2017).

Table 4. Percentage calculation (%) of total flavonoid content of red fruit (Pandanus conoideus Lam)

Sample	Replication	İnitial total flavonoid content (µl/mL)	Total flavonoids (mg QE/L juice)	Average total flavonoid content (mg QE/L juice)
Red fruit	1	21.465	171.72	
	2	21.951	175.608	174.517
	3	22.028	176.224	

4. CONCLUSION

The results of the calculation of the percent of total flavonoid content of red fruit prove that the flavonoid content contained in the red fruit juice sample used in this study is from the calculation of the average value obtained of 17.4517 mg QE/L juice which means that the flavonoid content in each liter of red fruit juice is equivalent to 17.4517 mL of quercetin.

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