

Total Flavonoid Content of the Bark Extract of *Salacca edulis* Reinw

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ABSTRACT: Salak fruit (*Salacca edulis* Reinw) is one of the fruits whose bark is not used but contains chemical components in the form of secondary metabolites that are beneficial to health. A previous study reported that the bark of salak fruit has potential as an anti-diabetic, antioxidant. The bark of Salak fruit is traditionally beneficial for the treatment of diabetes mellitus by drying then boiling and drinking the boiled water. Therefore, this study aims to determine the total flavonoid content of the bark extract of the pondoh and honey varieties of salak fruit. A sample of 30 g was dissolved with ethanol in a 10 mL volumetric flask, then 1 mL was pipette and added with 0.2 ml AlCl₃ and 0.2 mL potassium acetate then allowed to stand for 30 min at 25°C. After being allowed to stand, it was measured on a UV-Vis spectrophotometer with a wavelength of 442 nm. In the measurement with UV-Vis spectrophotometer, the total flavonoid content of the salak bark extract of pondoh and honey varieties were 5.18 mg QE/g and 5.77, respectively.

KEYWORDS: Flavonoid; salak; spectrophotometry; secondary metabolite.

1. INTRODUCTION

Salak (*Salacca edulis* Reinw) is a native fruit originating from Indo-Malaya which is now known as Southeast Asia (Sudjijo, 2009). Many varieties of salak can be grown in Indonesia. Superior varieties that have been released by the government for development include Pondoh, Ngulumut, Enrekang, Honey, and others (Tim Karya Mandiri, 2010). So far, salak is considered a fruit that can only be enjoyed by the fruit, but people do not know that besides fruit, the bark of the salak fruit can be used in the form of extracts because it contains flavonoid compounds (Fitrianingsih, 2014).

According to Fitrianingsih *et al* (2014), the results of phytochemical tests showed that the bark of the salak fruit contains alkaloids, polyphenols, flavonoids, and tannins. Flavonoids are secondary metabolites that have a C₆-C₃-C₆ core structure, namely two aromatic rings linked by 3 carbon atoms (Hanani, 2015).

Flavonoids have potential as antioxidants and have activity as antibacterial, anti-inflammatory, anti-allergic, and antithrombotic (Lipinski, 2011). Antioxidants are compounds that can counteract or reduce the negative effects of oxidants in the body, namely protecting the body from free radical attacks. Free radicals are molecules that do not have electron pairs so they will be very reactive and have irregular movements, if present in the body they will cause damage to various parts of the cell that can cause various degenerative diseases such as cataracts, cancer, atherosclerosis, and the aging process (Lipinski, 2011).

A previous study reported that the bark of salak fruit has potential as an anti-diabetic, antioxidant (Fitrianingsih *et al*, 2014; Anjani *et al*, 2015). The bark of Salak fruit is traditionally beneficial for the treatment of diabetes mellitus by drying then boiling and drinking the boiled water (Anjani *et al*, 2015; Sahputra, 2008). Based on the potential use of bark of salak, the total flavonoid content will be determined in the bark extract of salak fruit pondoh and honey varieties using the UV-VIS spectrophotometry method.

2. EXPERIMENTAL SECTION

2.1. General

The tools used are desiccator, micropipette (memmert), rotavapor (Ika® RV 10 basic), a set of glassware, sonicator, ultraviolet-visible spectrophotometer (Thermo scientific), analytical balance (Ohaus), vortex. The materials used in this study were aluminum chloride, bark extract of salak fruit (*Salacca edulis* Reinw.), ethanol, potassium acetate, quercetin. The reagent AlCl₃ 10% was prepared by weighed as much as 1 g and dissolved with distilled water up to 10 mL. Potassium acetate was prepared by weighed as much as 1.58 g and then dissolved with distilled water up to 10 mL.

2.2. Sample collection

Sampling of bark of salak from salak of pondoh and fresh honey variety. The bark is then cleaned of adhering dirt using running water and then dried by drying in indirect sunlight. After drying in a blender, it is ready to be extracted by the maceration method (Fawwaz, 2017).

2.3. Extraction

The bark of salak simplicia powder (100 g) was put into a maceration container (jar), added 1000 mL ethanol slowly until the simplicia was submerged, left for 3 days in a closed vessel, and protected from light while stirring occasionally. After 3 days the simplicia was filtered and the dregs (residue) were soaked again with a new liquid, this was done 3 times with 70% ethanol each time as much as 1000 mL. The results obtained are then collected and evaporated with a rotary evaporator until a thick ethanol extract is obtained and is ready to be used in research (Fawwaz, 2017).

2.4. Qualitative analysis

A total of 100 mg of extract was added with ethanol p.a. Then heated. The top layer was pipetted and added with a few drops of 2 N concentrated HCl and magnesium flavonoid powder. The appearance of red color indicates the presence of flavonoid compounds (Ditjen POM, 1989)

2.5. Quantitative analysis

2.5.1. Determination of maximum wavelength

A total of 100 μ L of quercetin solution with a concentration of 50 ppm was added 0.2 mL of 10% AlCl_3 , and 0.2 mL of 1 M potassium acetate. Then it was allowed to stand for 30 minutes at room temperature and the absorbance was measured on a UV-Vis spectrophotometer at 400 – 500 nm.

2.5.2. Concentration series of standard gallic acid

Quercetin stock solution (1000 ppm) by weighing 10 mg of quercetin standard and dissolved in 10 mL of ethanol p.a. From the standard solution of quercetin (1000 ppm) 5 mL was pipetted and dissolved in 10 mL of ethanol for 500 ppm. This solution (500 ppm) was diluted to prepare the concentrations 15, 20, 25, 30, 35, and 40 ppm. From each concentration of a standard solution of quercetin added 0.2 mL of 10% AlCl_3 , and 0.2 mL of 1 M potassium acetate. Then it was allowed to stand for 30 minutes at room temperature and the absorbance was measured on a UV-Vis spectrophotometer at the maximum wavelength.

2.5.3. Determination of total flavonoid

Determination of total flavonoid content in the ethanolic extract of salak fruit bark refers to the procedure of Cheng *et al* (2002). 1 mL of the test solution was taken, added with 0.2 mL of AlCl_3 and 0.2 of 1 M potassium acetate, then incubated for 30 minutes in a dark place at room temperature. Then the absorbance was measured on a UV-Vis spectrophotometer with a maximum wavelength. The sample solution was made in 3 replications so that the flavonoid content obtained was equivalent to quercetin (Cheng *et al*, 2002).

2.6. Data analysis

A calibration standard curve was obtained by running on UV-Vis spectrophotometry and then plotting peak areas against concentrations. For the curve, the best fit of the line was calculated by the equation of a line. Linearity was evaluated through the correlation coefficient (R^2). The correlation coefficient, intercept, and slope of the calibration curve was calculated. The best fit of data was determined by linear regression using the following equation: $Y = bx + a$, where:

Y = Peak area

b = Slope

x = Concentration

a = Intercept.

3. RESULTS AND DISCUSSION

The results of measuring total flavonoid levels in the bark extract of salak pondoh and honey varieties were by measuring the ethanol extract sample using a UV-Vis spectrophotometer and using the quercetin standard. The use of quercetin as a standard because quercetin is a type of flavonoid that is commonly used as a standard in determining flavonoid levels, and also because quercetin is the largest flavonoid flavonol group which has a keto group at C-4 and has a hydroxy group at C-3 or C-5 which are neighbors of flavones and flavonols.

The extraction results obtained from dissolving the sample with 70% ethanol were 5.47 grams for the Pondoh bark extract and 6.04 for the Honey bark extract. With the results of the % yield, respectively, were 5.47% and 6.04% (**Table 1**). The purpose of this extract is to determine the secondary metabolites carried by the solvent, but not to determine the type of compounds carried.

Table 1. The yield of bark salak ethanolic extract

Sample	Weight of sample (g)	Weight of extract (g)	Yield (%)
Pondoh	100	5.47	5.47
Honey	100	6.04	6.04

The qualitative test aims to determine the chemical components contained in samples of the bark extract of the Pondoh and Honey varieties of salak fruit bark with a color reaction using certain reagents, namely 2 N concentrated HCl

reagent and magnesium flavonoid powder. The identification results show that the bark extract of the salak fruit shows a positive red color containing flavonoid compounds.

The next research was carried out by quantitative testing of total flavonoid compounds using a UV-Vis Spectrophotometer to determine how much total flavonoid levels were in the bark extract of the Pondoh and Honey varieties of salak fruit using quercetin standards. This test was started by measuring the maximum wavelength by running a standard solution of 50 ppm quercetin in the range of 400-500 nm. The reason for using the maximum wavelength in spectrophotometer measurements is because at the maximum wavelength it has maximum sensitivity where the change in absorbance for each concentration unit is the largest. In this study, the wavelength obtained was 442 nm, the maximum wavelength was used to measure the absorption of samples of the bark extract of the Pondoh and Honey varieties of salak fruit. Furthermore, the standard solution of quercetin was made with 6 concentration variations, namely 15, 20, 25, 30, 35 and 40 ppm and filled in a 10 mL volumetric flask (**Table 2**). Then 1 mL of each concentration was pipetted then added 0.2 mL of 10% aluminum chloride (AlCl_3) which serves to give a bathochromic effect so that there is a shift in the wavelength towards the visible (visible) which is marked by the solution producing a more yellow color and the addition of 0.2 mL of 1 M potassium acetate which functions as a stabilizer so that the reaction that occurs can be maintained. After that, the standard solution was allowed to stand for 30 minutes at 25°C. The reason why it is allowed to stand for 30 minutes is so that the reaction that occurs can take place completely. The absorbance was determined using the UV-Vis spectrophotometric method at the maximum wavelength. Then measured at a maximum wavelength of 442 nm.

Measurement of the standard solution was carried out in order to obtain a calibration curve to obtain a linear regression equation. After obtaining the absorbance of each concentration of the standard solution and obtaining a linear regression equation, it is possible to determine the total flavonoid content in the bark extract of the Pondoh and Honey varieties of salak fruit.

Total flavonoids based on absorbance data of standard solutions from each concentration obtained a regression equation $y = 0.0231x - 0.1162$ with a correlation coefficient value (r) = 0.9965, where the correlation coefficient value is declared to be eligible if the value (r) = 0.995 - 0.999 and the value of V_{x0} obtained is 2.427%, which is eligible, where the V_{x0} condition is if 5% (Gandjar & Rohman, 2009).

Table 2. Concentration series of quercetin

Concentration (ppm)	Absorbance (442 nm)
15	0.220
20	0.349
25	0.457
30	0.599
35	0.691
40	0.794

Based on the absorbance data of the standard solution from each concentration, the regression equation $y = 0.0231x - 0.1162$ was obtained with the correlation coefficient (r) = 0.997 which can be seen in the **Figure 1**.

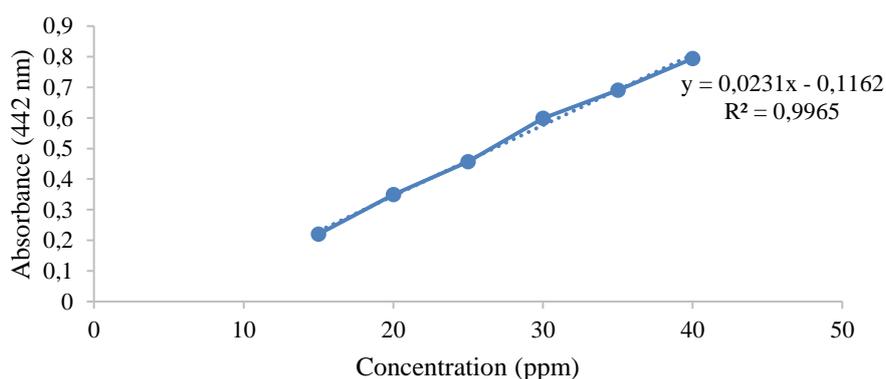


Figure 1. Quercetin calibration curve at a maximum wavelength of 442 nm

Furthermore, the determination of the total flavonoid content of the bark extract of the Pondoh and Honey varieties of Salak fruit bark, i.e. each extract was weighed as much as 0.03 grams for 3000 ppm for 3 times for both varieties as replication, then dissolved with ethanol and made enough in a 10 ml volumetric flask then pipette 1 ml and add 0.2 mL of 10% aluminum chloride (AlCl_3) and 0.2 mL of 1 M potassium acetate then let stand for 30 minutes at 25°C. For the blank, 1 mL of ethanol was added with 0.2 mL of 10% aluminum chloride (AlCl_3) and 0.2 mL of 1 M potassium acetate. Then the absorbance was measured at a wavelength of 442 nm. The results of the flavonoid content can be seen in the **Table 3** below.

Table 3. Total flavonoid content of the bark salak ethanolic extract.

Variety	Absorbance (Y)	Initial flavonoid content (mg/mL)	Flavonoid content (mgQE/g extract)	Average (mgQE/g extract)
Pondoh	0.255	16.07	5.30	5.18
	0.248	15.77	5.24	
	0.233	15.12	4.99	
Honey	0.274	16.89	5.57	5.77
	0.289	17.54	5.81	
	0.295	17.80	5.91	

4. CONCLUSION

From the results of the research that has been done, it can be concluded that the bark of the salak fruit of Pondoh and Honey varieties contains flavonoid compounds. The flavonoid content in the bark extract of the salak fruit of the pondoh variety was 5.18 mg QE/g and the total flavonoid content for the extract of the bark of the salak fruit of the honey variety was 5.77 mg QE/g.

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