

COMPARISON OF TOTAL FLAVONOID LEVELS OF N-HEXANE FRACTION, ETHYLACETATE, AND WATER ZALACCA PEEL ETHANOL EXTRACT USING VISIBLE SPECTROPHOTOMETRY

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Abstract. The Zalacca plant is one of the typical fruits from Indonesia which can be found in almost every region. The peel and flesh of malacca, according to phytochemical results, contain alkaloid, flavonoid, hydroquinone, steroid, and tannin compounds, as well as certain types of zalacca containing saponin compounds. Everyone needs a compound that is an antioxidant to protect body cells from the negative effects of free radicals. Flavonoids including aromatic compounds that are antioxidants, and active flavonoid compounds in counteracting free radicals are determined by the presence of a hydroxy functional group. This study aims to determine the differences in total flavonoid levels of the fraction of N-hexane, ethyl acetate, and water from the ethanol extract of the zalacca rind. The results of determining the total flavonoids from the zalacca rind extract, and fractions of n-hexane, ethyl acetate, and water were 0.95%, 4.55%, and 10.25%, respectively. The results of the statistical analysis of one-way variance (ANOVA one-way) showed that all antioxidant groups gave a significant value of $p < 0.05$, it can be concluded that there was a significant difference between the levels of flavonoids from the N-hexane fraction, ethyl acetate and water from the peel extract of the zalacca.

Key words: Zalacca peel ; Quercetin; Flavonoid ; Fraction

INTRODUCTION

Degenerative diseases, cancer, and premature aging are new problems in the world of health. One of the causes is due to the process of lipid peroxidation of body cells due to free radical compounds (Falahudin, 2010). Flavonoids were secondary metabolites contained in plants that belong to the polyphenol group (Ekawati, Suirta, & Santi, 2017). Flavonoid compounds were generally spread throughout all parts of plants including leaves, roots, wood, bark, flowers, fruit, and seeds (Zuraida *et al.*, 2017)

Flavonoids for plants function to protect themselves from disease and the surrounding environment, while the function of flavonoids for the human body is to prevent cardiovascular disease because flavonoids are phenolic compounds that have antioxidative properties that play a role in preventing cell damage by reactive free radical. One of the plants that contain flavonoid compounds is the zalacca plant

Zalacca (*Salacca zalacca*) was a plant native to Indonesia which belongs to the Arecaceae family, allied with coconut, oil palm, sugar palm (Renau), palms, and ferns with low branches. The thorny stems on this plant cause the growth of new shoots which can become flower buds in large quantities (Rahmah, 2016). Zalacca fruit has a high carbohydrate content, vitamin C, calcium, phosphorus, iron, and antioxidants (Dhayana Putri, Karta, & Krisna, 2016). In addition, zalacca fruit skin is also efficacious as medicine. Results of the phytochemical test showed that the skin of salak fruit contains flavonoids and tannins, as well as a small amount of alkaloids. The content of flavonoids contained in the skin of salak fruit affects reducing blood sugar levels in white Wistar rats induced by sucrose (Kanon, Fatimawali, & Bodhi, 2012). The purpose of this study was to determine differences in total flavonoid levels of the N-hexane, ethyl acetate, and water fractions of the ethanol extract of salak fruit peels.

METHODS

Making Simplicia Powder

The skin of the zalacca fruit is cleaned under running water and then cut and dried by aerating in the room. The sample was powdered and sieved with a no.40 mesh sieve.

Manufacture of Ethanolic Extract of zalacca Fruit Peel

Zallacca fruit peel powder is macerated with 96% ethanol for 2x24 hours while stirring occasionally then filtered and re-macerated until clear. The macerate obtained was concentrated using a rotary evaporator to obtain a thick extract.

Preparation of N-Hexane, Ethyl Acetate, and Salak Fruit Peel Water Fractions

The condensed extract of zalacca peel was put into a separatory funnel, added with 25 mL of distilled water and 25 mL of n-hexane solvent. Shaken until dissolved and allowed to stand until it separates. The insoluble n-hexane fraction was fractionated using 25 mL ethyl acetate then shaken until dissolved, allowed to stand, and separated between the ethyl acetate fraction and the water fraction. Replicated 3 times. The fraction results were upped using a rotary evaporator.

Determination of Total Flavonoid Levels

Determination of λ Maximum

The 1000 ppm quercetin mother liquor was diluted to a concentration of 200 ppm. Take 0.5 mL of the solution and add 1,5 mL ethanol p.a, 0.1 mL $AlCl_3$ 10%, 0.1 mL 1 M sodium acetate, and 2.8 mL distilled water. Absorption was measured in a wavelength range of 400-550 nm on the Visible spectrophotometer

Determination of Operating Time

The solution used is the same as the solution when measuring the maximum λ , measured at a wavelength of 442 nm with an interval of 1 minute.

Quercetin Standard Curve

A 1000 ppm quercetin mains solution was made in concentration series of 100, 150, 200, 250, and 300 ppm. Take 0.5 mL of each concentration and add 1.5 mL of ethanol p.a, 0.1 mL $AlCl_3$ 10%, 0.1 mL 1 M sodium acetate, and 2.8 mL distilled water. The solution was allowed to stand for 16 minutes and its absorbance was measured at wavelength 442 nm.

RESULTS AND DISCUSSION

Results Determination Total Flavonoid Levels

Results total flavonoid levels are presented in Table 1

Table 1. Total flavonoid levels.

Fraction	Extract Weight (g)	Absorbance	Equivalent Equality (ppm)	Flavonoid Levels Total (%)	$\bar{x} \pm SD$
N-hexane	0,025	0,204	4,5	0,45	0,95 $\pm$ 0,50
		0,213	9	0,95	
		0,224	14,5	1,45	
Ethyl Acetate	0,025	0,276	40,5	4,05	4,55 $\pm$ 0,52
		0,297	51	5,1	
		0,285	45	4,5	

Determination of total flavonoid content using quercetin solution as a standard solution. absorbance value from the measurement of quercetin solution from each concentration is used to find the linear regression equation which will later be used to determine total flavonoid content. The linear regression equation obtained from measuring the quercetin solution is $y = 0.002x + 0.195$ with $R = 0.999$ with an R-value close to 1 indicating that the regression equation is linear.

The determination of the flavonoid content of the zalacca peel fraction was carried out 3 times with replications measured at a wavelength of 442nm. Each fraction was reacted with $AlCl_3$. The results of the determination of total flavonoids from zalacca peel extract n-hexane fraction were 0.95%. The ethyl acetate fraction of zalacca peels obtained a total flavonoid content of 4.55% while in the water fraction of zalacca peels the total flavonoid content was 10.25%.

The total flavonoid yield of a plant can be affected by where the plant grows and other environmental factors such as soil composition, temperature, rainfall, and ultraviolet radiation. In addition, the solvent is an important factor in extracting the flavonoid component. Flavonoid compounds are widely distributed in plant tissues in the form of glycosides which causes flavonoids to dissolve easily in polar compounds, thus in this study the total flavonoid content of the water fraction was higher compared to ethyl acetate and n-hexane fractions because water is more polar than both. Meanwhile, the total flavonoid fraction of ethyl acetate of zalacca peel was higher than n-hexane because ethyl acetate is semi-polar and n-hexane is non-polar.

The results of the one-way statistical analysis of variance (one-way ANOVA) showed that all groups of total flavonoid content gave a significant value of $p < 0.05$. It can be concluded that there was a significant difference between the total flavonoid content of the water fraction, ethyl acetate, and n-hexane of zalacca peel.

CONCLUSION

Based on the research that has been done, it can be concluded that the n-hexane fraction, the ethyl acetate fraction, and the water fraction of the ethanol extract of salak fruit rind have flavonoid compounds. Tests for total flavonoid levels showed a significant difference based on statistical tests of the n-hexane fraction, ethyl acetate fraction, and aqueous fraction of ethanol extract zalacca peel with successive values of 0.95%, 4.55%, and 10.25%. The more polar the solvent, the more flavonoid compounds are attracted so that it shows a high total flavonoid content.

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Future researchers need to conduct further research on testing total flavonoid levels using other methods or further research to determine the types of flavonoids contained.

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