

EXTRACTION OF FLAVONOID CONTENT FROM BANANA PEEL (MUSA PARADISIACA L.) BY ULTRASOUND – ASSISTED EXTRACTION METHOD AND ITS SPF VALUE

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Abstract

In this study, banana peel was used as a natural sunscreen compound because of its natural antioxidants, such as flavonoids, tannins, triterpenoids, and phenols. Extraction is carried out using Ultrasound-Assisted Extraction method with variations in ethanol solvent concentration and material to solvent ratio at 45°C for 45 minutes. The ethanol concentrations used are 96%, 70%, and 50% and the material to solvent ratio 1/10, 1/20, and 1/30. From the results of the study, the highest yield and SPF value were obtained at ethanol concentration of 50% and material to solvent ratio 1/30. The yield in this variation is 15.2646%, and the SPF value is 8.9874 with the maximum protection category. The highest Total Flavonoid Content (TFC) results was obtained at 70% ethanol concentration and the ratio of material to solvent 1/30, namely 1.6559 mg QE/g. From the variation with the best TFC value, the mass transfer coefficient is 0.0151 mL/μg QE.min.

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Keywords:

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1. Introduction

Ultraviolet radiation, one of which is, will be very dangerous to human skin. Exposure to ultraviolet radiation will damage cells in the skin and can be at risk of becoming a cancer. One example of a disease caused by ultraviolet radiation is burning skin. One way to protect the skin from ultraviolet radiation is by using sunscreen. Sunscreen has an active ingredient that can protect the skin from UV radiation. The active ingredient consists of ultraviolet absorbent and ultraviolet reflector. Because sunscreen is only a protector, there will be a small amount of ultraviolet light absorbed into the skin. Therefore, the sunscreen used must contain a certain amount of SPF or Sun Protecting Factor [1].

Banana peel contains quite a lot of flavonoid compounds because of the high antioxidant content in bananas. Flavonoid compounds can be used as constituent compounds sunscreen. Flavonoid compounds are obtained by extraction, one of the extraction methods that can be used is Ultrasound-Assisted Extraction (UAE). In Indonesia, it is very rare to find the extraction of flavonoids using UAE. Whereas when compared to other extraction methods, such as maceration or soxhlet, UAE has advantages that can reduce extraction time and the use of solvents [2-5].

2. Experimental Section

A. Banana Peel Preparation

Banana peels are washed thoroughly using running water, then dried in an open room overnight. Then, the dry skin is blended until it goes into pieces.

B. Extraction Of Flavonoid Content in Banana Peel

Banana peels are weighed and put into an Erlenmeyer flask containing solvent. The solvent used, namely ethanol, was varied in concentration 96%, 70%, and 50% with each amount based on the raw material/solvent ratio that had been determined, i.e. 1/10 (g/mL), 1/20 (g/mL), 1/30 (g/mL).

Extraction was carried out using an ultrasonic water bath at atmospheric pressure with a temperature of 45°C for 45 minutes. The extract obtained was then filtered using a vacuum filter and then the filtrate was separated from the solvent by a vacuum oven at a temperature of 40-50 °C and a pressure of 0.2 bar. The yield of the extract for each variation was calculated as % (wt/wt) using the equation:

$$\%yield = \frac{\text{dried banana peel extract}^{\text{r}} \text{ mass}}{\text{raw banana peel mass}} \times 100\% \quad (1)$$

C. Phytochemical Screening

Flavonoid Test. 1 mL of banana peel extract was mixed with 1 mL of 96% ethanol, 0.1 gram of Mg powder, and 10 drops of concentrated HCl. If the extract contains flavonoids, it will form red, yellow or orange colour.

Triterpenoid Test. 2 mL of banana peel extract was mixed with 10 drops of CH₃COOH and 2 drops of concentrated H₂SO₄. Then shake gently and leave for a while. If the extract contains triterpenoids, it will form red colour.

Tannin Test. 2 mL of banana peel extract was mixed with 2 mL of aquadest and few drops of 1% FeCl₃. If the extract contains tannins, it will form blackish green colour.

Phenolic Compound Test. 1 mL of banana peel extract was mixed with 3 drops of 1% FeCl₃. If the extract contains polyphenol, it will form red, green, blue, purple or black colour.

Alkaloid Test. 10 mL of banana peel extract was mixed with 1.5 mL of 2 M HCl then heated for 5 minutes and added 5 drops of Dragendorff's reagents. If the extract contains alkaloid, it will form orange color and precipitate.

Saponin Test. 2 mL of banana peel extract was mixed with 10 mL hot aquadest, shake for 15 minutes then added a few drops of 2 M HCl. If the extract contains saponins, it will form a stable foam for a while.

D. Determination Of Total Flavonoid Content (TFC) Using AlCl₃ Method

Determination TFC of banana peel extract was carried out using the colorimetric method with quercetin was used to make a calibration curve. The results were expressed as µg quercetin (µg.QE)/gr banana peel.

25 mg of quercetin was weighed and put into a 25 mL volumetric flask, then added ethanol of all concentrations up to 25 mL as the main solution of 1000 µg/mL. Then diluted to standard solutions of 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL, and 120 µg/mL. Then, 0.5 mL of the standard solution and 0.5 mL of each extract solution were separated into several vials and added with 1.5 mL of 96% ethanol, 0.1 mL of 10% AlCl₃, 0.1 mL of 1 M potassium acetate and 2.8 mL of aquadest. These solutions were incubated for 30 minutes at 25°C then the absorbance of each solution was measured at a wavelength of 438 nm using a UV-Vis spectrophotometer. The absorbance data of each standard solution then made into a calibration curve by connecting absorbance as coordinate (Y) and concentration of standard solution as abscissa (X), while the absorbance data of each extract solution was used to calculate the TFC using the equation:

$$TFC = \frac{c \times V \times f}{m} \quad (2)$$

where TFC is the total flavonoid content (µg.QE/mg dry weight), c is quercetin equivalent (µg/ml), V is total product volume (mL), f is dilution factor (1), and m is raw banana peel mass (mg).

E. Determination Of Mass Transfer Coefficient

The mass transfer coefficient was calculated for the extract which had the highest TFC. Extraction was carried out with time variations of 5, 15, 30, 45 and 60 minutes. The calculation of the mass transfer coefficient is carried out by the following equation:

$$\frac{t}{C_t} = \frac{1}{k(C_e)^2} + \frac{t}{C_e} \quad (3)$$

where k is second-order mass transfer coefficient (mL/µg.min), t is time (minutes), C_e is concentration of solute in solution at equilibrium (ppm QE), C_t is concentration of solute in solution at a certain time t (ppm QE).

The mass transfer coefficient value is obtained by plotting the graph between t/C_t as the Y axis and t (time) as the X axis. Then 1/C_e will be obtained as the slope and used to calculate the mass transfer coefficient (k) from the intercept.

F. Determination Of SPF Values

Dried banana peel extract of 96%, 70%, and 50% ethanol was dissolved in each ethanol concentration to the concentration of 300 ppm. SPF value was determined with firstly calculated the area under curve (AUC) of absorbances at 280-400 nm with 5 nm interval using the equation:

$$[AUC] = \frac{A_a + A_b}{2} \times (dP_{ab}) \quad (4)$$

with A_a is absorbance at wavelength a nm, A_b is absorbance at wavelength b nm, and dP_{ab} is difference in wavelength a and b. AUC of each wavelength segment is summed up to calculate the SPF value using the equation:

$$\log SPF = \frac{\sum AUC}{(\lambda_n - \lambda_1)} \quad (5)$$

with λ_n is the longest wavelength (400 nm), and λ_1 is the shortest wavelength (290 nm).

3. Result and Discussion

Based on Fig. 1 and Table 1, it can be concluded that there is an increase in the amount of yield along with the increasing amount of solvent used. This is in accordance with the principle of the gradient concentration that increase following the greater volume of solvent [1]. In addition, the greater the volume of solvent, the faster the diffusion process occurs and facilitate the mass transfer process [2].

Table 1. Extraction Yield Result

Material/Solvent Ratio (gr/mL)	Yield (%)		
	Ethanol Concentration (%)		
	96	70	50
1/30	10.4065	11.9175	15.2646
1/20	8.8401	9.9564	13.5078
1/10	5.7167	6.6717	11.3103

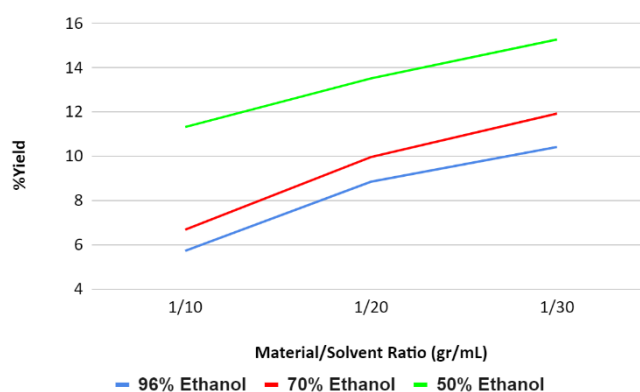


Figure 1. Effect of ethanol concentration and material/solvent ratio on extraction yield of banana peel

Meanwhile, the smaller concentration of solvent used, the greater the extract yield produced. According to previous studies, with variations in ethanol concentrations of 0% (100% water), 50%, and 100%, the highest yield was obtained at a concentration of 50% and the lowest at a concentration of 100% [3]. This result shows correlation between increasing polarity of ethanol with the addition of water volume, so that more polyphenolic compounds (not only flavonoids) are extracted [3]. In addition, the viscosity value of water is higher than ethanol, so ethanol has lower barrier mass transfer [3].

Based on the Table 2, in this study banana peel extract contained several types of compounds, such as flavonoids, triterpenoids, tannins, phenolic compounds, alkaloids, and saponins. This is due to the ability of these compounds to dissolve in polar solvents. These compounds are included in the antioxidant compounds that have many benefits. One of the benefit is for a sunscreen constituent, because of high photoprotective ability of these compounds.

Table 2. Phytochemical Test of Extract Sample

Phytochemical	Result	Examples of Compounds
Flavonoid	Red (+)	Quercetin, catechin, gallic acid [4]
Triterpenoid	Red (+)	Lupenone [5]
Tannin	Blackish green (+)	Tannin <small>Error! Reference source not found.</small>
Phenolic Compound	Black (+)	Chrysin, caffeic acid, cinnamic acid [7]
Alkaloid	Orange (+)	Phenylalanine [8]

Saponin	Stable foam (+)	Saponin <small>Error! Reference source not found.</small>
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The TFC value can be calculated using a standard calibration curve and the results are expressed in mg.QE (Quercetin Equivalent)/gr banana peel. The standard curves were made for 96%, 70%, and 50% ethanol concentrations respectively and the equations can be seen in Table 3 below.

Table 3. Standard Calibration Curve of Quercetin

Ethanol Concentration	Equation	R ²
96%	$y = 0.004x - 0.0103$	0.9915
70%	$y = 0.0048x - 0.0184$	0.9932
50%	$y = 0.005x - 0.0183$	0.9935

Based on Fig. 2 and Table 4, there is strong correlation between extract yield with the increasing amount of solvent during extraction. This is in accordance with the principle of the concentration gradient which gets bigger following increase of solvent amount [1]. In addition, the greater the volume of solvent, the faster the diffusion process occurs [2]. The amount of solvent volume will also affect the contact area between the material and the solvent. The more amount of solvent led to wider contact area and the distribution of the solvent [9]. Even distribution of solvent can increase yield and the amount of solvent will reduce the level of saturation of the solvent so that the components will be extracted perfectly [9].

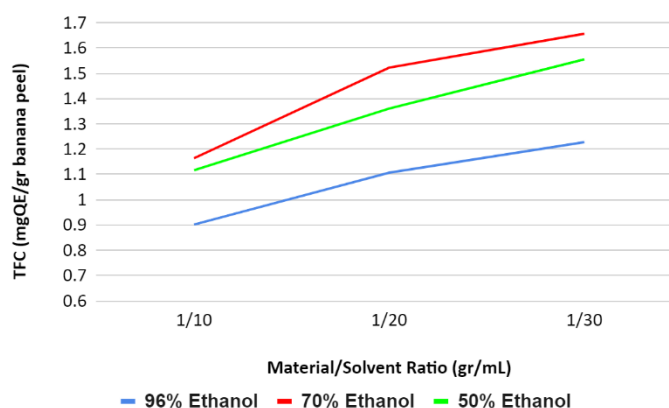


Figure 2. Effect of ethanol concentration and material/solvent ratio on TFC banana peel extract

Table 4. Total Flavonoid Content (TFC) on Banana Peel Extract

Material/Solvent Ratio (gr/mL)	TFC (mgQE/gr banana peel)		
	Ethanol Concentration (%)		
	96	70	50
1/30	1.2273	1.6559	1.5548
1/20	1.1068	1.5222	1.3604
1/10	0.9014	1.1639	1.1166

In addition, an increasing number of solvents will also increase the ability of the solvent to extract and increase the contact time that lasts between the material and the solvent [9]. Since the UAE uses cavitation principle for mass transfer, an increase in total volume led to higher extraction efficiency [10].

Based on Fig. 2, it is also known that the TFC value of 70% ethanol has the highest yield, followed by 50% ethanol and 96% ethanol. Quercetin solubility should increase with increasing ethanol concentration [11]. However, in this study, the lowest TFC value at 96% ethanol. Based on the phytochemical screening and from reference, it was found that the type of flavonoid in banana peel is catechin. Catechin is a flavonoid that belongs to the flavanol group, while the AlCl₃ method cannot detect flavonoids other than the flavone and flavanol groups [12].

The equation used to determine the value of the mass transfer coefficient (k) is a second-order reaction rate equation, with previous fittings and the highest R² value is obtained when the second-order equation is used.

In addition, the determination of the mass transfer coefficient with the first order cannot be used to describe all processes and can only describe processes that have a single mechanism [13]. There are two main mechanisms involved in extraction with UAE in plant samples. The first mechanism involves changes in plant

cell walls due to ultrasonic waves from the UAE. During this process, water molecules from the solvent are used assisted with ultrasonic energy which makes the plant matrix swell and then destroys it which makes the components of bioactive compounds extracted out. The second mechanism is the process of dissolving flavonoids in organic compounds with the help of ultrasonic waves, so that ethanol can extract the desired flavonoid compounds.

From (3), we get a graph plot between t/C_t vs. time (minutes) as follows.

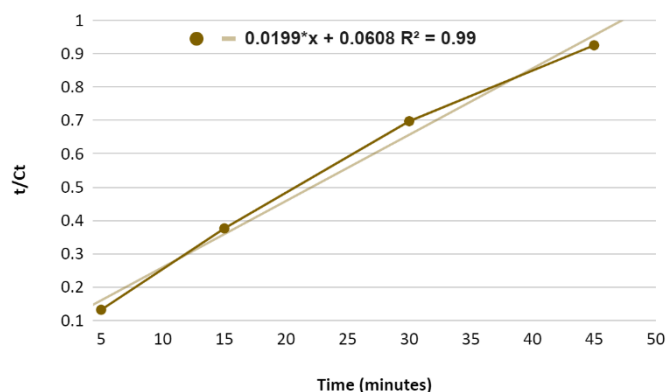


Figure 3. Mass transfer kinetic model of flavonoid extraction from banana peel

Fig. 3 shows the equation obtained from plotting t vs. t/C_t is $y = 0.0199x + 0.0608$ with $R^2 = 0.9901$. Through the calculation of the intercept value, the mass transfer coefficient value of $0.0151 \text{ mL}/\mu\text{gQE.min}$ was obtained. This number means that in 1 minute of extraction, to extract $1 \mu\text{g}$ quercetin is required 0.0151 mL 70% ethanol.

Table 5. SPF Value Results from Extracted Banana Peels

Ethanol Concentration (%)	SPF Value
96	7.6138
70	7.6566
50	8.9874

Based on the Table 5, Sun Protection Factor (SPF) value from extracts of 96%, 70% and 50% are 7.6138, 7.6566, and 8.9874. The SPF value of banana peel extract from solvent concentrations of 96% and 70% can be categorized as having extra protection and 50% solvent concentration can be categorized as having maximum protection based on the classification by Wilkinson & Moore [15].

Table 5 shows that the highest SPF value was obtained at 50% ethanol extract and the lowest SPF value was obtained at 96% ethanol extract. The SPF value obtained is comparable to the yield which also has the highest yield on 50% ethanol extract and the lowest on 96% ethanol extract. However, this result is not match with the TFC value where, the highest TFC value is in the 70% ethanol extract. There is possibility of other bioactive compounds besides flavonoids, which are more soluble in 50% ethanol resulting in high yield value. Thus, it can be said that the more bioactive compounds extracted, the higher the SPF value obtained.

Bioactive compounds that have a photoprotective role are generally soluble in polar solvents because they are included in the class of antioxidant compounds, especially phenolic compounds. Based on previous research, the highest SPF value of hazelnut peel ethanol extract was obtained in the ethanol extract which had the highest Total Phenolic Content (TPC) value, not in the extract with the highest TFC value [14]. This proves that the SPF value is not only depend on TFC, but also by the TPC value. Several photoprotective phenolic compounds in addition to the flavonoid group contained in the ethanolic extract of banana peels are p-coumaric acid, β -tocopherol [16]; ferulic acid [18]; chlorogenic acid [19]; carotenoids, coumarin [20].

The SPF value produced by natural ingredients often does not meet the standards commonly sold in the market. However, the incorporation of natural ingredients with synthetic ingredients as a constituent of sunscreens has been shown to increase the SPF value of the sunscreen. This is evidenced by previous research, the combination of 0.1% rutin (a type of flavonoid) with 6% benzophenone resulted in an increase in the SPF value from 24.3 ± 1.53 to 33.3 ± 2.89 when flavonoids were added [16].

The banana peel extracts were analysed using FTIR for identifying the chemical bond in the component. The FTIR result of chemical bond shows the possibility component. The analysis was carried out on all variations of

ethanol concentration (96%, 70%, and 50%) with a material/solvent ratio of 1/30 gr/mL and used quercetin as the standard.

Table 6. FTIR Results of Banana Peel

Bond	Wavelength Frequency (cm ⁻¹)	Banana Peel Extract Wave Number (cm ⁻¹)			Identification Result
		96% Ethanol	70% Ethanol	50% Ethanol	
O-H	3500-3200	3367.1030	3353.6040	3415.3140	Flavonoids, Saponins
C-H	3200-2800	2946.6970	2952.4820	2954.4110	Phenols
C=O	1800-1600	1658.4810	1650.7670	1646.9110	Flavonoids
N-H	1680-1550	1658.4810	1650.7670	1646.9110	Alkaloids
CH ₂	1480-1440	1452.1350	1454.0640	1457.9210	Triterpenoids
CH ₃	1480-1440	1452.1350	1454.0640	1457.9210	Triterpenoids
C=C aromatic	1465-1430	1452.1350	1454.0640	1457.9210	Flavonoids
C-O	1300-800	1112.7250	1110.7970	1108.8680	Flavonoids, Phenols, Tannins
C-O-H	1050-1000	1027.8730	1022.0870	1018.2300	Tannins
C-H aromatic	800-500	638.3225	673.0349	613.2524	Flavonoids
<i>Fingerprint Region of Flavonoid</i>	1300-900	1027.8730	1022.0870	1018.2300	Flavonoids

From Table 6, the three extracts have the main functional groups present in flavonoid compounds, namely O-H, C=O, C=C aromatic, and C-H aromatic [21]. In addition, there are also supporting functional groups that represent other compounds, namely triterpenoids, alkaloids, tannins, phenols, and saponins according to the results of phytochemical screening in this study.

4. Conclusion

Extraction of flavonoids from banana peels using Ultrasound-Assisted Extraction (UAE) method was carried out with variations in ethanol concentration and material/solvent ratio. The greater amount of solvent resulting in higher extract yield and Total Flavonoid Content (TFC). The highest yield and TFC was found at the ratio of material to solvent 1/30 gr/mL. And the smaller the solvent concentration, the greater the yield obtained which is at 50% ethanol. While the highest TFC value obtained at 70% ethanol and the lowest at 96% ethanol. The highest TFC in banana peels was obtained at 70% ethanol with a material/solvent ratio of 1/30 gr/mL, which was 1.6559 mg.QE/gr. The highest Sun Protecting Factor (SPF) value of banana peel extract was obtained at 50% ethanol, which was 8.9874 with maximum protection category. The mass transfer coefficient value of banana peel extraction was calculated on the variation with the highest TFC, and the mass transfer coefficient value was 0.0151 mL/μg.QE.min

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