

Determination of Total Flavonoid Content and Antioxidant Activity of Ethyl Acetate Extract from Pumpkin (*Cucurbita moschata*) Fruit Using Varied UAE Times with DPPH Free Radicals

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Abstract. Free radicals are unstable molecules that can cause damage to cells and body tissues, and increase the risk of chronic diseases. This study aims to determine the effect of variations in Ultrasonic-Assisted Extraction (UAE) extraction time on total flavonoid levels and antioxidant activity of pumpkin using the DPPH method. The purpose of this study was to determine the optimal extraction time to obtain maximum yield, the highest total flavonoid content, and the most effective antioxidant activity. The pumpkin fruit extraction method using the Ultrasonic-Assisted Extraction (UAE) method and ethyl acetate solvent, with variations in extraction duration, then an evaluation was carried out on the yield, total flavonoid content, and antioxidant activity of the extract produced using the DPPH method to determine the optimal extraction conditions. Results Ethyl acetate extraction of pumpkin fruit with an extraction time of 25 minutes produced the highest yield. Statistical analysis showed that the data at extraction times of 5 minutes and 15 minutes were normally distributed, while at extraction time of 25 minutes were not normally distributed. The homogeneity test showed that the data were homogeneous. Comparison of yields showed that there was no significant difference between 5-minute and 15-minute extraction times, but 25-minute extraction time showed a significant difference compared to both. The total flavonoid content in the ethyl acetate extract of pumpkin fruit was 6.204%, and its antioxidant activity was included in the weak category with an IC₅₀ value of 309.2 ppm

Keywords: Cucurbita Moschata, Total Flavonoids, Antioxidants, DPPH

INTRODUCTION

Global warming and climate change are affecting Indonesia. Global warming is a phenomenon that has occurred in recent years, resulting in a rise in the Earth's temperature (Ainurrohmah & Sudarti, 2022). Oxygen is essential for human life and plays a vital role in the living world; the majority of oxygen is utilized for energy production, while a small fraction remains as a byproduct of metabolic processes. These metabolic byproducts are known as free radicals or Reactive Oxygen Species (ROS). ROS are molecules with unpaired electrons, a characteristic that leads to instability (Rachmayani, 2015).

The causes of free radicals are categorized into two types: internal and external. One internal cause is aerobic metabolism—a vital biological process that invariably results in the formation of free radicals (Prasetyaningsih et al., 2022). Pumpkin (**Cucurbita moschata**) is a plant that grows in both lowland and highland regions; however, consumption levels among potential consumers remain very low. Pumpkins are rich in nutrients such as carbohydrates, proteins, and vitamins. Additionally, they contain bioactive compounds including phenolic compounds, vitamins, amino acids, carbohydrates, minerals, and flavonoids (Pujiastuti et al., 2022). Key advantages of Ultrasound-Assisted Extraction (UAE) include enhanced liquid penetration into cell walls, faster mass transfer rates, higher extraction yields, the use of lower temperatures, and reduced solvent volume requirements (Marlina et al., 2022).

Flavonoids are secondary metabolites that function as antioxidants, antifungals, and antibacterials (Hobir, 2020). Based on the background above, it is evident that no research has yet been conducted regarding extraction time variations in Ultrasound-Assisted Extraction (UAE); therefore, it is necessary to investigate optimal total flavonoid content by employing varying UAE durations and DPPH radicals.

METHODS

Type of Research

This study is an experimental investigation involving the determination of total flavonoid content and the assessment of antioxidant activity using the DPPH free radical scavenging assay of the ethyl acetate extract of pumpkin (*Cucurbita moschata*) fruit, employing varying extraction times via Ultrasound-Assisted Extraction (UAE).

Population and Sample

The sample used consisted of fresh pumpkin (*Cucurbita moschata* Duchesne) peels that were yellow-green in color, free from rot, and showed no signs of pest or disease damage; these were obtained from Rahtawu Village, Kudus, Central Java.

Equipment and Materials

The equipment used in the study included an analytical balance (Ohaus), a simplisia (dried plant material) blender (Getra), a moisture balance, a drying oven, dropping pipettes, volumetric pipettes (Pyrex), micropipettes, watch glasses, test tubes (Pyrex), a test tube rack, wooden clamps, a horn spoon, a spatula, a glass funnel, a stirring rod, a pot, a stove, a thermometer, a UV-Vis spectrophotometer (Biobest), quartz cuvettes, volumetric flasks (Pyrex), amber bottles, a Bunsen burner, and filtration cloth. The primary raw material was pumpkin fruit simplisia powder; other materials used included ethyl acetate, concentrated HCl, Dragendorff's reagent, Bouchardat's reagent, Mayer's reagent, analytical grade (p.a.) ethanol, Mg powder, NaOH, FeCl₃, AlCl₃, sodium acetate, distilled water, 2N HCl, quercetin, and DPPH.

Research Procedure

a. Preparation of Dried Simplisia Crude Drug Material)

To prepare the pumpkin fruit simplisia, 3.40 kg of pumpkin was peeled to remove unwanted parts such as the skin, seeds, and other impurities. The peeled pumpkin flesh was washed with clean running water and drained. It was then sliced thinly and dried at 50°C until completely dry to produce the simplisia. Subsequently, the drying loss was calculated using the following formula: (Anggreni et al., 2024). The dried pumpkin simplisia was then pulverized by blending until fine and sieved through a 40-mesh sieve to obtain a uniform and homogeneous powder (Anggreni et al., 2024). The moisture content of the pulverized simplisia was measured using a moisture balance. The moisture content of good-quality fruit simplisia must be <8% (Depkes RI, 1998).

b. Preparation of Pumpkin Fruit Extract

Ethyl acetate extraction of the pumpkin fruit was performed using the UAE (Ultrasound-Assisted Extraction) method with an ethyl acetate solvent at a 1:10 ratio (powder:solvent). Ten grams of pumpkin *simplisia* powder were placed in an Erlenmeyer flask, and 100 mL of ethyl acetate was added. Extraction was then carried out using the UAE method at a frequency of 40 kHz and a temperature of 40°C for durations of 5, 15, and 25 minutes, with three replicates performed for each duration. The resulting extract was filtered, and the residue was separated from the filtrate. The obtained filtrate was then evaporated using a water bath at 40°C. The concentrated extract yield was subsequently weighed; the resulting weight was used to calculate the percentage yield, and SPSS analysis was performed (Budiman et al., 2024; Rahmawati &

c. Phytochemical Screening

1). Alkaloid identification

$\text{Rendemen ekstrak (\%)} = \frac{\text{Bobot total ekstrak}}{\text{Bobot serbuk simplisia total}} \times 100\%$
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The alkaloid identification procedure in this study is based on the characteristics and phytochemical screening of pumpkin fruit as conducted by Arviani et al. (2023).

a) Mayer's reagent

Two grams of extract are placed in a test tube; 2 drops of 2N HCl are added, followed by 2 drops of Mayer's reagent. A positive result for alkaloids is indicated by the formation of a white or yellow precipitate.

b) Bouchardat's reagent

Two grams of extract are placed in a test tube; 2 drops of 2N HCl are added, followed by 2 drops of Bouchardat's reagent. A positive result for alkaloid compounds is indicated by the formation of a brown to black precipitate.

c) Dragendorff's reagent

Two grams of extract are placed in a test tube; 2 drops of 2N HCl are added, followed by 2 drops of Dragendorff's reagent. The presence of alkaloid compounds is indicated by the formation of an orange to red precipitate.

2). Flavonoid identification

a) Willstätter test

Two grams of pumpkin fruit extract are taken; a few drops of concentrated HCl and a small amount of Mg powder are added. Flavonoid content is indicated if the color changes to yellow (Arviani et al., 2023).

b) Bate-Smith test

Two grams of pumpkin fruit extract are taken; a few drops of HCl are added, followed by heating. Flavonoid content is indicated if the color changes to red (Arviani et al., 2023).

c) 10% NaOH test

Two grams of pumpkin fruit extract are taken, and a few drops of 10% NaOH reagent are added. A positive result for flavonoids is indicated by a color change to orange (Arviani et al., 2023).

3). Saponin Identification

Two grams of the pumpkin ethyl acetate extract filtrate were placed in a test tube 5 drops of hot water were added, and the mixture was cooled and shaken for 10 seconds. A foam layer 1 cm high persisting for 10 minutes meaning the foam did not disappear upon the addition of 2N HCl indicated the presence of saponins (Arviani et al., 2023).

3) Tannin Identification

One to two drops of FeCl₃ reagent were added to 2 grams of the extract filtrate; the presence of tannins is indicated by a color change to blue or greenish-black (Arviani et al., 2023).

4). Determination of Total Flavonoid Content

Determination of flavonoids in the ethyl acetate extract of pumpkin fruit

A 10 mg portion of the concentrated extract was weighed and then dissolved in analytical-grade (p.a.) ethanol to a volume of 10 mL (resulting in a solution concentration of 1000 ppm). A 1.0 mL aliquot of the extract solution was pipetted out; to this, 0.1 mL of 10% aluminum(III) chloride (AlCl₃) and 0.1 mL of 1 M sodium acetate were added. Analytical-grade ethanol was then added to bring the volume up to the mark (10 mL), and the mixture was shaken until homogeneous. Subsequently, the solution was incubated at room temperature for 26 minutes and measured using a spectrophotometer at a wavelength of 428 nm.

4) Antioxidant activity of pumpkin ethyl acetate extract using DPPH

A 1000 ppm solution of pumpkin ethyl acetate extract was prepared by weighing 10 mg of the extract and dissolving it in analytical-grade (p.a.) ethanol up to the 10 mL mark of a volumetric flask. Aliquots of 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL were pipetted out. From each concentration, a 0.5 mL sample was taken and mixed with 3.5 mL of 0.1 mM DPPH solution, followed by the addition of analytical-grade ethanol to bring the total volume to 10 mL in a volumetric flask. The mixture was incubated for 20 minutes in the dark, and the absorbance was subsequently measured at the maximum wavelength of 516 nm.

DATA ANALYSIS

1. Data analysis involved performing Normality and Homogeneity tests; the results determined whether to proceed with parametric or non-parametric testing.
2. The optimization of flavonoid content was determined using UV-Vis spectrophotometry; the flavonoid content—expressed as quercetin-equivalent concentration—can be calculated using a linear regression equation. Based on the study by Dewi et al. (2024) regarding the determination of total flavonoid content in pumpkin using the UV-Vis spectrophotometric method, the linear regression equation (in ppm) can be applied:

$$y = bx + a$$

Legend:

y = absorbance

x = flavonoid content (quercetin-equivalent concentration (ppm))

a = intercept (curve's intersection with the y-axis)

b = slope of the linear curve

The optimization data obtained can be used to determine the total flavonoid content in the sample by applying an equation based on the study by Indratmoko et al. (2021) regarding the optimization of testing conditions for total flavonoid compounds in plant extracts.

$$\frac{C \times V \times F_p \times 10^{-6} \times 100\%}{M}$$

Legend:

C = Quercetin equivalent concentration (ppm) V =

Total extract volume (ml)

F_p = Dilution factor

m = Sample weight (g)

3. Antioxidant activity using the DPPH method

The data analysis for the antioxidant activity test in this study follows the approach used to compare total flavonoid content and antioxidant activity in pumpkin extracts. Free - radical scavenging activity can be calculated using the equation from Pujiastuti et al. (2022).

Antioxidant activity is calculated using the following formula:

$$\% \text{Inhibisi} = \frac{\text{absorbansi kontrol} - \text{absorbansi bahan uji}}{\text{absorbansi kontrol}} \times 100\%$$

Note:

Blank absorbance = absorbance of solvent + DPPH;

$$\text{Sample absorbance} = \text{absorbance of solvent} + \text{DPPH} + \text{sample}.$$

RESULTS AND DISCUSSION

Preparation of Dried Pumpkin Peel

Five kilograms of pumpkin peel underwent wet sorting, washing, and slicing, followed by drying in a drying cabinet at 50°C. The results of drying the pumpkin peel (*Cucurbita moschata* Duchesne) are presented in Table 1.

Table 1. Shrinkage of Crude Drugs

Berat basah	Berat kering	Susut pengeringan	Serbuk
4.450 gram	340 gram	92%	300 gram

The drying process yielded 640 grams of dried *simplisia* (crude drug material), which was then pulverized using a blender and sieved through a 40-mesh screen to obtain powder of uniform particle size. Subsequently, the loss on drying was calculated. The loss on drying for the pumpkin peel was found to be 92%. Powder Moisture Content Analysis The results of the moisture content analysis are presented in Table 2.

Table 2. Results of Powder Moisture Content Test

Simplisia	Bobot serbuk simplisia (gram)	Kadar air (%)	Rata rata kadar air (%)
Daging buah	1,00	4,49	
labu kuning	1,00	4.58	4,51
	1,00	4.46	

This study, the moisture content of the crude drug material was tested in triplicate, yielding values of 4.49%, 4.58%, and 4.46%, with an average of 4.51%. These moisture content levels comply with the standard established by the Indonesian Ministry of Health (1985) specifically, a limit of <8% for fruit peel material.

The ethyl acetate extract of pumpkin fruit was prepared using the Ultrasound-Assisted Extraction (UAE) method with ethyl acetate as the solvent. UAE was selected for this study due to advantages over other methods, such as a shorter extraction time (which can increase extract yield), more effective solvent selection, and overall time efficiency.

The extraction of pumpkin fruit via UAE was conducted using a powder-to-solvent ratio of 1:10. Extraction was performed for durations of 5, 15, and 25 minutes at a temperature of 40°C and a frequency of 40 kHz (Marlina et al., 2022).

Table 3. Yield Results Obtained

Perlakuan (menit)	Replikasi	Berat Serbuk Simplisia	Ekstrak kental (gram)	Rendemen(%)	Rata Rata $\pm$ SD
5 menit	Replikasi 1	10 gram	1,09	10,9	10,4 $\pm$ 0,64
	Replikasi 2	10 gram	0,97	9,7	
	Replikasi 3	10 gram	1,07	10,7	
15 menit	Replikasi 2	10 gram	1,28	12,8	17,6 $\pm$ 4,25
	Replikasi 2	10 gram	1,91	19,1	
	Replikasi 3	10 gram	2,09	20,9	
25 menit	Replikasi 3	10 gram	3,06	30,6	32,1 $\pm$ 2,69
	Replikasi 2	10 gram	3,06	30,6	
	Replikasi 3	10 gram	3,51	35,1	

The extraction process yielded a viscous, brownish-orange extract with a characteristic odor. Using 10 grams of powder, the average extract yield was 10.4% at the 5-minute mark, 17.8% at 15 minutes, and 32.1% at 25 minutes.

The effect of extraction duration on the yield of pumpkin ethyl acetate extract can be assessed not only through calculations but also via SPSS analysis, which evaluates the extent to which extraction time influences the extract yield

Table 4. Normality

Perlakuan	%Rendemen Nilai sig	Keterangan
5 menit	0,298 (>0.05)	Normal
15 menit	0,407 (>0.05)	Normal
25 menit	0,000 (>0.05)	Tidak Normal

Data for the ethyl acetate extract of pumpkin fruit showed a normal distribution at the 5-minute mark (sig. <0.05), but the data at 15 minutes were not normally distributed; therefore, the analysis proceeded with the non-parametric Kruskal-Wallis test.

Tabel 5. Uji Nonparametrik Kruskal Wallis Test

Uji	Nilai Sig	Keterangan
<i>Kruskal Wallis</i>	0,039 (<0.05)	Terdapat pengaruh antara lama waktu ekstraksi

The Kruskal-Wallis test yielded a significance value of <0.05, indicating a significant difference between the extraction time and the yield of ethyl acetate extract obtained from pumpkin fruit.

The results regarding the identification of compounds contained in the ethyl acetate extract of pumpkin fruit (*Cucurbita moschata* Duchesne) are presented in Table 6

Table 6. Phytochemical Screening

No.	Uji	Hasil	Keterangan
1.	Alkaloid		
	a. Bouchardat	Endapan hitam	+
	b. Mayer	Endapan kuning	+
	c. Dragendof	Endapan jingga	+
2.	Flavonoid		
	a. Uji wilstater	Warna kuning	+
	b. Uji bate smith	Warna merah	+
	c. Uji NaOH	Warna orange	
3	Saponin	Buih tidak hilang	+
4	Tanin	Hijau kehitaman	+

Bouchardat Reagent Test

The test using Bouchardat reagent yielded a positive result for alkaloids; 2 g of concentrated extract was placed in a test tube, 2 drops of 2N HCl were added, followed by 2 drops of Bouchardat reagent, resulting in the formation of a white or yellow precipitate.

Mayer's Reagent

The test using Mayer's reagent yielded a positive result for alkaloids; 2 g of extract was placed in a test tube, 2 drops of 2N HCl were added, followed by 2 drops of Mayer's reagent, resulting in the formation of a yellow precipitate.

Dragendorff's Reagent

The test using Dragendorff's reagent yielded a positive result; 2 g of extract was placed in a test tube, 2 drops of 2N HCl were added, followed by 2 drops of Dragendorff's reagent, resulting in the formation of an orange-to-red precipitate.

Flavonoid Test: Wilstätter Test

A positive result for flavonoids was obtained by taking 2 g of pumpkin fruit extract, adding a few drops of concentrated HCl and a small amount of Mg powder, which caused a color change to yellow.

Bate-Smith Test

A positive result for flavonoids was obtained by taking 2 g of pumpkin fruit extract, adding a few drops of HCl, and heating the mixture; a color change to red occurred.

10% NaOH Test

A positive result for flavonoids was obtained by taking 2 g of pumpkin fruit extract and adding a few drops of 10% NaOH reagent, resulting in a color change to orange.

Saponin Test

A positive result for saponins was obtained by placing 2 g of the ethyl acetate extract of pumpkin fruit into a test tube, adding 5 drops of hot water, cooling the mixture, and shaking it for 10 seconds; this produced a foam layer 1 cm high that persisted for 10 minutes and did not disappear upon the addition of 2N HCl.

Tannin Test

A positive result for tannins was obtained by taking 2 g of the extract filtrate and adding 1–2 drops of FeCl₃ reagent, resulting in a color change to greenish-black.

Results of total flavonoid content analysis

The wavelength determination yielded a value of 428.0 nm and an absorbance of 0.381. The observed difference in wavelength could be attributed, in part, to the specific instruments used, as each instrument possesses varying levels of sensitivity and accuracy and determination of the optimal operating time yielded a result at the 1560 second with an absorbance of 0.509 than total flavonoid content of the ethyl acetate extract was calculated using linear regression based on a previously determined quercetin standard curve. The results regarding quercetin equivalence and flavonoid content are presented in Table 7.

Table 7. Total flavonoids				
Konsentrasi (ppm)	Absorbansi	Kesetaraan kuersetin (ppm)	Flavonoid total (%)	Rata rata Flavonoid Total $\pm$ SD
1000	0.502	62,42	6,242	6,204 $\pm$ 0,032
1000	0.498	61,85	6,185	
1000	0.498	61,85	6,185	

Antioxidant Activity of Pumpkin Ethyl Acetate Extract

The result obtained from the wavelength reading was 516.0 nm, with an absorbance of 0.207. The operating time result at the 2-minute mark showed an absorbance of 0.224. The absorbance values obtained from the antioxidant analysis of the pumpkin ethyl acetate extract were subsequently used to determine the percentage of inhibition-representing the scavenging activity of the antioxidant compounds against DPPH. The relationship between the percentage of inhibition and

the IC50 value is derived from the linear correlation between antioxidant activity and inhibition percentage; lower absorbance values correspond to higher inhibition values, indicating greater antioxidant activity in the extract (Paraeng et al., 2017). The results of the antioxidant analysis are presented in Tables 8 and 9

Table 8. DPPH & Quercetin Calculations

Replikasi	Log Konsentrasi (PPM)	Absorbansi	Inhibisi %	Persamaan regresi linier	IC50 (ppm)	Rata rata IC50 (PPM)		
I	1,3	0,790	3,304	y =	1,91	85,11		
	1,6	0,663	18,849	81,964x -				
	1,77	0,536	34,394	107,32				
	1,9	0,428	47,613	R ² = 0,9627				
	2	0,313	61,698					
II	1,3	0,751	8,078	y =	1,00		85,11	
	1,6	0,662	18,971	64,735x -				
	1,77	0,567	30,599	80,038				
	1,9	0,469	42,594	R ² = 0,9434				
	2	0,373	54,354					
III	1,3	0,715	12,484	y = 69,02x	1,89			85,11
	1,6	0,615	24,724	- 81,019				
	1,77	0,507	37,943	R ² = 0,9563				
	1,9	0,410	49,816					
	2	0,315	61,444					

Table 9. DPPH & Ekstrak Calculations

Table 3. DPPH & Ekstrak Calculations								
Replikasi	Log Konsentrasi (PPM)	Absorbansi	Inhibisi %	Persamaan regresi linier	IC50 (ppm)	Rata rata IC50 (PPM)		
I	100	0,698	14,565	y = 73,387x	2,53	309,02		
	200	0,582	28,763	- 136,01				
	300	0,486	40,514	R² = 0,9533				
	400	0,370	54,712					
	500	0,274	60,744					
II	100	0,651	20,318	y = 68,125x	2,49		309,02	
	200	0,557	31,823	- 120,13				
	300	0,465	43,084	R² = 0,9388				
	400	0,346	57,649					
	500	0,261	68,053					
III	100	0,615	24,724	y = 63,574x	2,45			309,02
	200	0,521	36,23	- 106,29				
	300	0,450	44,92	R² = 0,9318				
	400	0,330	59,608					
	500	0,247	69,767					

CONCLUSION

The ethyl acetate extract of pumpkin fruit tested positive for flavonoids, alkaloids, tannins, and saponins. The highest extraction yield was obtained at the 25-minute mark. The extract contained a total flavonoid content of 2.55% and exhibited moderate antioxidant activity, with a mean IC50 value of 353.18 ppm.

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Based on the research conducted, it is recommended that further studies be undertaken.

Future research could involve the specific identification of the types of flavonoids present in pumpkin, for instance, by utilizing chromatography techniques. Additionally, future studies could employ antioxidant activity analysis methods by comparing results obtained via the DPPH and ABTS assays. Furthermore, future research should aim to identify the specific compounds present in pumpkin and evaluate which of these compounds exhibits the strongest antioxidant activity.

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