Comparison of Total Flavonoid Content With 70% Ethanol and Ethyl Acetate Solvents Pumpkin (*Cucurbita moschata* Duchesne) Ultrasonic Assisted Extraction Method

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Abstract. Indonesia has a rich biodiversity that has potential for developing traditional medicines. One such plant was the yellow pumpkin (*Cucurbita moschata* Duchesne), especially its skin, which was often considered waste, even though it was rich in vitamins, phenolics, flavonoids, polysaccharides, and minerals. This studied aims to identify secondary metabolite compounds, determine total flavonoid levels, compare total flavonoid levels of pumpkin skin (*Cucurbita moschata* Duchesne) using 70% ethanol, ethyl acetate solvents and determine differences in total flavonoid levels using the UV-Vis spectrophotometry method and determine whether there are differences or not in total flavonoid levels of pumpkin skin (Cucurbita moschata Duchesne). This research was an experimental study using the UAE method with 70% ethanol, ethyl acetate solvents, and a phytochemical screening test was carried out. The data obtained were analyzed using the normality test and the nonparametric Kruskal-Wallis Test. Pumpkin skin (*Cucurbita moschata* Duchesne) positively contains flavonoids, alkaloids, tannins, and saponins. The water content contained in the simplicia powder is 7.32%. The total flavonoid content of the 70% ethanol extract was 3.69% and the total flavonoid content of the ethyl acetate extract was 4.68%. The normality test of the 70% ethanol extract and ethyl acetate was not normally distributed. The nonparametric Kruskal-Wallis Test resulted in a difference between the total flavonoid content of the 70% ethanol extract and ethyl acetate of pumpkin skin (*Cucurbita moschata* Duchesne). Because the sign results obtained were 0.046 <0.05.

Key words: [Yellow Pumpkin Skin, 70% ethanol, ethyl acetate, UV-Vis Spectrophotometry, total flavonoid content]

INTRODUCTION

Pumpkin (*Cucurbita moschata*) is a highly nutritious fruit with numerous health benefits. This plant is rich in carotenoids, which contain various water-soluble vitamins, phenolics, flavonoids, polysaccharides, minerals, and other vitamins that contribute to body health (Aukkanit & Sirichokworrakit, 2017). Pumpkin skin contains antibiotic compounds and has the potential to be a source of new antibiotics. Pumpkin skin is rich in secondary metabolites. Therefore, standardization is needed to ensure its quality and effectiveness (Kamarudin *et al*., 2014).

This study used the Ultrasonic Assisted Extraction (UAE) method. UAE is an extraction technique that utilizes acoustic cavitation, where ultrasonic waves produce small bubbles (cavitation) in a solvent at temperatures below the boiling point (Kanifah *et al.*, 2015). UAE works more efficiently, so the time required to extract compounds from the material is shorter, making it a faster and more effective method than traditional extraction techniques (Turrini *et al*., 2018).

Based on this background, researchers are interested in examining the total flavonoid content of pumpkin peel using polar solvents, namely 70% ethanol, and semi-polar solvents, namely ethyl acetate extract of pumpkin peel (*Cucurbita moschata* Duchesne) using the UV-Vis spectrophotometry method. This was done because no researchers have compared the total flavonoid content in 70% ethanol extract and ethyl acetate extract of pumpkin peel (*Cucurbita moschata* Duchesne), to select solvents in extraction to ensure efficiency and selectivity in extracting target compounds from raw materials.

METHODS

Population and Sample

The samples used were fresh, greenish-yellow pumpkin (*Cucurbita moschata* Duchesne) skins, free from rot and pests or disease, collected from the Tayu Traditional Market, Tayu District, Pati Regency, Central Java.

Tools and Materials

The tools used were analytical scales (*Ohaus*), dropper pipettes, measuring cups (*Pyrex*), test tubes (*Pyrex*), Erlenmeyer *(Pyrex*), UAE (*Ultrasonic Assisted Extraction*) (*Jinyuanbo* brand), sieve. 40 mesh (ABM), *rotary vacuum evaporator* (IKA *Laborthechnik*), blender (*Philips*), cuvette, dropper

pipette, glass beaker (*Pyrex*), Erlenmeyer (*Pyrex*), measuring flask (*Pyrex*), oven, porcelain cup, Whatman filter paper no. 4, label paper, UAE (*Ultrasonic Assisted Extraction*) (*Jinyuanbo* brand), sieve no. 40 mesh (ABM), *rotary vacuum evaporator* (IKA *Laborthechnik*).

The materials used were pumpkin skin (*Cucurbita moschata* Duchesne), distilled water, 70% ethanol, ethyl acetate, 5% $FeCl_3$ (*Merck)*, 1% NaOH (*Merck*), 2N HCl (*Merck*), Mayer's reagent, Wagner's reagent, Mg powder, ethanol p.a. (*Merck*), quercetin standard (*Sigma Aldrich*), sodium acetate (*Merck*), 10% $AlCl_3$ *(Merck*).

Research Procedure

1. Preparation of Dried Simple Drugs

Fresh pumpkin (*Cucurbita moschata* Duchesne) peel samples were collected, weighed at 5 kg, then wet sorted, drained, and cut into pieces for easy drying. Washing with running water was performed to remove microbes and impurities (Ilham et al., 2024). The pumpkin peels were then oven-dried at 50°C (Indrianingsih *et al*., 2019). Dried pumpkin peels were reweighed to calculate the percentage of drying loss. Powder was then prepared by blending and sieving through a No. 40 sieve, followed by weighing the resulting powder (Indriyanti *et al*., 2018). Moisture content of the pumpkin peels (*Cucurbita moschata* Duchesne) was determined using a moisture balance. One gram of fine pumpkin peel powder was placed into the moisture balance. The process was replicated 3 times, then the results were calculated as an average (Ramadhani *et al.*, 2020).

2. Preparation of 70% Ethanol and Ethyl Acetate Extracts from Pumpkin Peel

100 grams of dried pumpkin peel powder were weighed and added to 70% ethanol solvent in a 1:10 ratio. 100 grams of powdered plant material were placed in an Erlenmeyer flask, added to 400 ml of 70% ethanol solvent, then covered with aluminum foil. Extract was then performed using Ultrasonic Assisted Extraction (UAE) at a frequency of 40 kHz for 30 minutes at 40°C. This was repeated twice, and the filtrate was separated from the residue. The residue was re-extracted using 200 ml of solvent for 30 minutes following the same procedure for the ethyl acetate solvent (Puspita *et al.,* 2024). The liquid extract obtained was thickened with a rotary evaporator at a temperature of 40°C (Amelinda *et al*., 2018) and concentrated by heating using a cup above a water bath until no more liquid drips to obtain a thick extract (Gustandy & Soegihardjo, 2016). The yield formula was calculated. The yield formula is as follows:

$$\% \text{ yield} = \frac{\text{total weight extract}}{\text{total weight of powder}} \times 100\%$$

3. Phytochemical Screening

a. Flavonoid Identification

Wilstater Test

0.5 grams of thick extract were weighed into a test tube and dissolved in 10 ml of ethanol. 0.1 grams of magnesium powder and 5 drops of concentrated HCl solution were then added. A color change to orange indicates the presence of flavonoids in the extract (Asih, 2009).

Bate-Smith Test

0.5 grams of thick extract were weighed into a test tube, and 5 drops of concentrated HCl solution were added. The mixture was heated for 15 minutes in a water bath, and the color change was observed. If the color changed to red, the extract was considered positive for flavonoids (Asih, 2009).

NaOH Test

0.5 grams of thick extract were weighed into a test tube, and 5 drops of 10% NaOH solution were added. If the color changed to orange, red, or yellow, the extract contained flavonoids (Asih, 2009).

b. Alkaloid Identification

Weigh 0.5 grams of the thick extract into a test tube, dissolve it in 1 ml of 2N HCl and 9 ml of water, and divide it into three parts. A positive result for alkaloids is the addition of 1 ml of Mayer's reagent, which will form a white or yellowish-white precipitate. 1 ml of dagendrof reagent will produce an orange-red precipitate, and 1 ml of bouchardat reagent will produce a brown to blackish precipitate (Izzah *et al*., 2019).

c. Saponin Identification

0.5 grams of the thick extract will be dissolved in hot distilled water, HCl added and then shaken until foam forms, indicating a positive saponin (Minarno, 2016).

d. Tannin Identification

0.5 grams of thick extract were dissolved in ethanol, and then 1 ml of 1% $FeCl_3$ was added. If a blackish-green color formed, it indicated the presence of tannins in the extract (Halimu *et a*l., 2017).

4. Determination of Total Flavonoid Content in Yellow Pumpkin Peel

a. Preparation of Quercetin Standard Solution

Weigh 25 mg of quercetin into a 25 ml volumetric flask and dissolve it in ethanol p.a. to the mark. This results in a concentration of 1000 ppm (Sari *et a*l., 2019).

b. Determination of Maximum Wavelength (λ Max)

1 ml of 60 ppm quercetin solution was taken, added with 0.1 ml of 10% $AlCl_3$ and 0.1 ml of 1 M sodium acetate, then ethanol p.a. to the mark in a 10 ml volumetric flask. The maximum wavelength of quercetin was then calculated by running a standard quercetin solution at a wavelength range of 400-800 nm until a result indicating the highest maximum wavelength with the highest absorbance was obtained (Sari *et a*l., 2019).

c. Determination of Operating Time

The Operating Time (OT) was determined by taking 1.0 ml of 60 ppm quercetin solution, placing it in a test tube, adding 0.1 ml of 10% aluminum (III) chloride ($AlCl_3$), 0.1 ml of 1 M sodium acetate, and adding ethanol p.a. to the mark (10 ml). The absorbance of the solution was measured at the maximum wavelength obtained at 1-minute intervals for 1 hour. A stable absorbance value indicates the operating time (Sari *et al*., 2019).

d. Standard Curve Preparation

0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, and 1.2 ml of the 1000 ppm quercetin stock solution were pipetted. Ethanol p.a. was added to each pipette to a volume of 10 ml, resulting in concentrations of 40 ppm, 60 ppm, 80 ppm, 100 ppm, and 120 ppm. 1 ml of each quercetin standard series was pipetted, then 1 ml of 10% $AlCl_3$ and 5 ml of 1 M sodium acetate were added, and the mixture was allowed to stand for the specified operating time. Absorbance was determined using UV-Vis spectrophotometry at the maximum wavelength obtained (Asmorowati & Lindawati, 2019).

e. Determination of Total Flavonoid Content of Yellow Pumpkin Peel Ethanol Extract (*Cucurbita moschata* Duchesne).

The thick extract was weighed as much as 10 mg, then dissolved in ethanol p.a to 10 ml (solution concentration 1000 ppm). The ethanol extract test solution in a 1.0 ml pipette was added with 0.1 ml of 10% aluminum (III) chloride ($AlCl_3$), then 0.1 ml of 1 M sodium acetate, then ethanol p.a to the limit mark (10 ml), shaken until homogeneous. After that, the solution was incubated at room temperature for the operating time and measured on a spectrophotometer with the obtained wavelength (Sari *et al*., 2019).

DATA ANALYSIS

The data obtained from this study were primary data analyzed based on the absorbance from the standard curve, which was then used to create a calibration curve. Afterward, the total flavonoid content was calculated using the linear regression equation $y = bx + a$, generated from the standard calibration curve (Asmorowati & Lindawati, 2019).

Where:

y = absorbance value/regression line
x = concentration (%)
a + b = regression constant

After calculating the standard curve, the total flavonoid content was calculated. The total flavonoid content can be determined using the formula (Pujiastuti & Zeba, 2021).

$$F = \frac{V \times c \times Fp \times 10^{-3}}{g} \times 100\%$$

Description:
F : Total flavonoid content
c : Quercetin equivalent concentration (mg/L)
V : Volume of extract used (L)
Fp : Dilution factor
G : Sample weight (g)

In addition to flavonoid calculations, data analysis of flavonoid content of 70% ethanol and ethyl acetate extracts of pumpkin (*Cucurbita moschata* Duchesne) peels was also conducted using SPSS (Statistical Program for the Social Sciences) software. Total flavonoid data were analyzed using hypotheses, namely, data normality testing and the nonparametric Kruskal-Wallis test.

RESULTS AND DISCUSSION

A total of 5 kg of pumpkin skin was wet sorted, washed, chopped, and dried in a drying cabinet at a temperature of 50°C. The drying results of pumpkin skin (*Cucurbita moschata* Duchesne) are presented in Table 1.

Tabel 1. Penyusutan Simplisia

Simplicia	Wet Simplicia (gram)	Dried Simplicia (gram)	Drying shrinkage (%)	Colour
Pumpkin skin	5000 gram	638 gram	87, 24%	Yellowish green

Sumber: Data primer yang diolah (2025)

The drying process yielded 638 grams of dried herbal medicine, which was then ground using a blender and sieved through a 40-mesh sieve to obtain a uniformly sized herbal medicine powder. The next step was to calculate the drying loss. The drying loss test for pumpkin skin was 87.24%.

Table 2. Results of Powder Water Content Test

Replication	Result of water content (%)	Mean (%) ±SD
1	7,37	
2	7,26	7,32 ±0,15
3	7,06	

Sumber: Data primer yang diolah (2025)

The results of this study tested the water content of the simplex and replicated it three times, yielding consecutive results of 7.37%, 7.26%, and 7.06%, with an average of 7.32% ± 0.15%. This indicates that the water content of the three replicates meets the standards set by the Indonesian Ministry of Health (1985), which is <8%, which is the water content for the fruit skin.

The results of the 70% ethanol extract and ethyl acetate extract of pumpkin (*Cucurbita moschata* Duchesne) skin are shown in Table 3.

Table 3. Extraction Results

Powder of simplicia (gram)	Solvent	Weight Extract	Yield (%)	Terms	Information
100	Etanol 70%	23,56	23,56%	>8,3	fulfill terms
100	Etil asetat	3,82	3,82%	>8,3	Not fulfill terms

Sumber: Data primer yang diolah (2025)

The results of organoleptic observations of 70% ethanol extract and ethyl acetate extract can be seen in table 4.

Table 4. Organoleptic Test Results

Sample	Colour	Smell	Shape
70% ethanol extract	Blackish brown	Special	slightly sticky texture, easily washed off with water
Ethyl acetate extract	Blackish brown	Special	slightly sticky texture, easily washed off with water

The extraction process used in this study was the UAE method. The UAE method was chosen because it has the primary advantage of increasing solvent penetration into the material matrix through acoustic cavitation, a process that disrupts cell walls, accelerating the release of bioactive compounds (Julianto, 2019). Other advantages of this method include short extraction times, low energy consumption, and minimal solvent requirements (Kumoro, 2015). UAE results for 70% ethanol solvent yielded a thick, blackish-brown extract with a yield of 23.56%, while the ethyl acetate solvent yielded a thick, blackish-brown extract with a yield of 3.82%.

Phytochemical Screening

The results of the identification of compounds contained in the 70% ethanol and ethyl acetate extracts of pumpkin (*Cucurbita moschata* Duchesne) peel can be seen in Table 5.

Table 5. Phytochemical Screening Test Results

No	Identification	Reagent	Result	70% ethanol extract	Ethyl acetate extract
1.	Flavonoid				
	Wilstater	Mg powder, concentrated HCl	Orange	+	+
	NaOH	NaOH 10%	Orange	+	+
	Bate-Smith	concentrated HCl, waterbath	Red	+	+
2.	Alkaloid				
	Mayer	HCl 2 N, aquadest, mayer's reagent	Yellowish-white sediment	+	+
	Dagendrof	HCl 2 N, aquadest, dagendrof's reagent	Orange sediment	+	+
	Bouchardat	HCl 2 N, aquadest, bouchardat's reagent	Blackish-brown sediment	+	+
3.	Saponin	Hot aquadest, HCl	Stable foam forms	+	+
4.	Tannin	$FeCl_3$	Blackish-green	+	+

Description: (+) = Contains compounds
(-) = Does not contain compounds

Wilstater Flavonoid Test

The results of testing pumpkin (Cucurbita moschata Duchesne) peel extract in 70% ethanol and ethyl acetate solvents showed positive results for flavonoids. 0.5 grams of thick extract were added with 0.1 grams of magnesium powder and 5 drops of 10% NaOH. If a color change occurs in orange, red, or yellow, the extract contains flavonoids (Indriyanti et al., 2018).

NaOH Test

The 10% NaOH test showed that the 70% ethanol and ethyl acetate extracts of pumpkin peels turned orange (Khotimah, 2016).

Bate-Smith Flavonoid Test

The results of this study confirmed that the flavonoid compounds were red in the ethanol and ethyl acetate extracts of pumpkin peels. Concentrated HCl was added to hydrolyze and accelerate the hydrolysis reaction, indicating a positive result in this test by a red color change (Estikawati, 2019).

Tannin Test

This study, tannin identification in pumpkin skin, showed positive results, indicated by a blackish-green color for the 70% ethanol and ethyl acetate extracts (Tanaya et al., 2015).

Saponin Test

This study found a positive result for saponins due to the presence of persistent foam (Agustina et al., 2017).

Determination of Total Flavonoid Content in Pumpkin Peel

a. : Determination of Maximum Wavelength

UV-Vis spectrophotometry measurements yielded a wavelength of 429 nm with an absorbance of 0.381 nm, according to research by Sari et al. (2019). The maximum wavelength produced was 412 nm. Differences in wavelength results may be due to differences in the equipment used, as each instrument has varying sensitivity and accuracy (Suhartati, 2017). The wavelength results can be seen in Table 6.

Table 6. Maximum Wavelength

Wavelength	Ansorbance
426	0,379
427	0,380
428	0,381
429	0,381
430	0,381
431	0,381

b. Determining Operating Time

The measurement results showed a stable time at 32 minutes with an absorbance of 0.508. The operating time results can be seen in Table 7.

Table 7. Determination of Operating Time

Time (s)	Absorbance
1800	0.509
1860	0.509
1920	0.508
1980	0.508
2040	0.508
2100	0.508
2160	0.508
2220	0.508

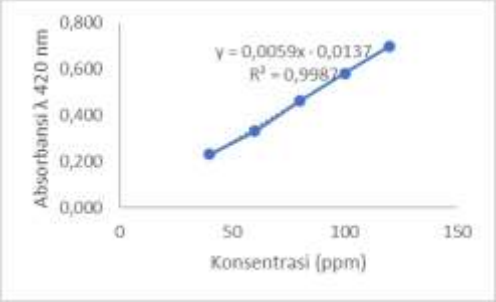
c. Standard Curve Determination Results

The results of the standard curve measurements are presented in Table 8.

Table 8. Results of the standard curve absorbance measurements of quercetin

Consentration (ppm)	Absorbance			Mean	SD
	R1	R2	R3		
40	0.223	0.213	0.26	0.232	0.025
60	0.335	0.327	0.335	0.332	0.005
80	0.473	0.478	0.434	0.462	0.024
100	0.577	0.632	0.540	0.583	0.046
120	0.749	0.741	0.614	0.701	0.076

The following graph shows the results of the standard curve of quercetin and the linear equation at a maximum wavelength of 429 nm, which can be seen in Figure 1.



d. Results of the study on total flavonoid levels

The total flavonoid levels of the 70% ethanol and ethyl acetate extracts were calculated using linear regression from the previously measured quercetin standard curve. The results of the quercetin and flavonoid equivalence levels are shown in Tables 9 and 10.

Table 9. Results of Determination of Total Flavonoid Content of Ethanol Extract

Consentration (ppm)	Replication	Absorbance	mg/L (C)	Level Flavonoid Total (%)
1000	1.	0,205	37,06	3,71%
	2.	0,204	36,89	3,69%
	3.	0,203	36,72	3,67%
Mean			36,89	3,69% ±0,02%

Table 10. Results of Determination of Total Flavonoid Content of Ethyl Acetate Extract

Consentration (ppm)	Replication	Absorbance	mg/L (C)	Level Flavonoid Total (%)
1000	1.	0,256	45,71	4,57%
	2.	0,266	47,40	4,74%
	3.	0,266	47,40	4,74%
Mean			46,83	4,68% ±0,10%

The total flavonoid results of the 70% ethanol extract and the ethyl acetate extract of pumpkin peel can be seen not only quantitatively through calculations but also using SPSS to compare the total flavonoid results of the 70% ethanol extract and the ethyl acetate extract by observing the sign value. The results of the normality test using SPSS can be seen in Table 11.

Table 11. Results of the Normality Test for Total Flavonoid Levels

Extract	Shapiro-wilk		
	Sig	P>0,05	Information
70% ethanol extract	1.000	Sig>0,05	Normally Distributed
Ethyl acetate extract	0.000	Sig<0,05	Not normally Distributed

The 70% ethanol extract of pumpkin peel >0.05 indicates normally distributed data, and the ethyl acetate extract <0.05 indicates abnormally distributed data, so the nonparametric Kruskal-Wallis test was continued**.** Nonparametric Kruskal-Wallis Test

Table 11. Nonparametric Kruskal-Wallis Test

Variable	Sig	P<0,05	Information
Flavonoid total	0,046	< 0,05	There is a significant difference

Based on the results of the Nonparametric Kruskal-Wallis Test, the total flavonoid content data using SPSS obtained a significance value of 0.046, meaning that if the significance value is <0.05, there is a significant difference in this study.

CONCLUSION

The results of secondary metabolites contained in the 70% ethanol extract and ethyl acetate with the phytochemical screening method are flavonoids, alkaloids, tannins, and saponins. The total flavonoid content contained in the 70% ethanol extract of pumpkin skin is 3.69% and the total flavonoid content contained in the ethyl acetate extract of pumpkin skin is 4.68%. There is a significant difference between the flavonoid content of the 70% ethanol extract and ethyl acetate of pumpkin skin (Cucurbita moschata Duchesne) because the resulting significance value is <0.05.

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