

**THE INFLUENCE OF HARVEST TIME AND DRYING TIME ON TOTAL FLAVONOID CONTENTS OF *PSIDIUM GUAJAVA FOLIUM*****Meilani Nur Rahmawati, Susilowati\*, Fany Putri Enjang Sesaningrum**

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\*[susilowati@stikesnas.ac.id](mailto:susilowati@stikesnas.ac.id)**ABSTRACT**

*Psidium guajava* L. have been used to treat or prevent various diseases such as diarrhea, flatulence, anti-inflammatory, antioxidant, antimicrobial and anticancer. Flavonoids are biomarker compounds responsible for their activity. However, harvest and post-harvest stages of plant may change flavonoid content. The present work investigates harvest time and drying time variations of quality and total flavonoids content of Guava leaves. Method: Guava leaves was collected in the morning (9 am), evening (3 pm), and dried for 24, 36 and 48 hours. Each simplisia was tested for macroscopic and loss drying contents. Total flavonoids in each extract were determined as quercetin by UV-Visible spectrophotometry, then comparative analysis was performed with One Way Anova. Total flavonoid levels harvested in the afternoon were higher (1.0887 mgQE/g) than in the morning (0.7200 mgQE/g), while drying time for 24 hours (0.24507 mg QE/g) showed the highest flavonoid levels compared to drying for 36 hours and 48 hours significantly. This indicates that guava leaves are best harvested in the afternoon and dried for 24 hours.

Keywords: flavonoid; guava; harvest and drying time

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**INTRODUCTION**

Common guava (*Psidium guajava* L.) is a popular medicinal plant in Indonesian society whose leaves are widely used as a traditional herbal concoction in various tribes and have been listed in the Indonesian traditional medicinal herb formulary. Apart from its many uses for treating diarrhea and flatulence, other benefits include anti-inflammatory, antioxidant, antimicrobial, and anticancer (Jumiarni & Komalasari, 2017). Quercetin is a flavonoid compound in guava leaves that provides benefits from its use and is, therefore, a biomarker for guava leaves (RI, 2017). Harvest time is a factor that influences the formation of flavonoids in plants. Different harvest times will affect the quality and quantity of compound content due to the sunlight. Light is vital for the biosynthesis process of growing plants. An increase or decrease in sunlight will influence the production of compounds, including flavonoids, since sunlight influences photosynthesis. The best harvest time is when plants contain the highest amounts of active compounds (Lu et al., 2019).

Post-harvest is also essential in processing guava leaves apart from the harvest stage. The main stage of the post-harvest process is drying, namely removing or eliminating most of the water by applying heat. Drying can reduce the water content of the material to reduce enzyme activity, which can damage the material and inhibit the growth of bacteria and fungi

(Syafriada, 2018). Additionally, the water removed can influence the physical condition and increase the flavonoid compound content in the material (Robbiyan et al., 2021).

Still, there has been no research regarding the influence of harvest time and drying time for guava leaves. In the drying stage, the temperature and drying time need to be considered since bioactive components, including flavonoids, are unstable to heating (Lu et al., 2019). According to (Handayani & Sriherfyna, 2016), the flavonoid content of guava leaves shows a significant decrease at drying temperatures of more than 50°C. This shows that making guava leaf simplicia is recommended at a temperature of 50°C. In addition, the time required for drying must be controlled to avoid exposure to excessive heat, which can damage and reduce the contents of flavonoids in the material (Supriningrum et al., 2018). Therefore, this study aims to determine the influence of differences in harvest time and drying time of guava leaves on the total flavonoid content.

## **METHOD**

### **Instruments and Materials**

This study used instruments such as an analytical balance (Ohaus PA214 sensitivity 0.0001 g), a pair of cuvettes (Hellma Analytic type No.100, 600 QG Light path lotum), UV-Vis spectrophotometry (Shimadzu UV-1280 No.A120654 ), S-300 electric stove, and glassware and water bath (Mettler). The materials used included guava leaves collected from Wonogiri in Central Java, quercetin (Sigma Aldrich), potassium acetate (Merck), H<sub>2</sub>SO<sub>4</sub>, AlCl<sub>3</sub> (Merck), NaOH, Aquadest, concentrated HCl, Mg powder, and methanol p.a. (Merck).

### **Research Variables**

1. An Independent variable is a variable whose variation influences other variables. This study's independent variables are harvest time (twice, 9 a.m. and 4 p.m. {Western Indonesian Time}) and drying time (24, 36, and 48 hours).
2. A controlled variable is an independent variable whose influence on the dependent variable is controlled by the researcher by making it a neutral impact. This study's controlled variables are the location where the plants grew, maceration, solvent, and quercetin concentration series.
3. The dependent variable is the research variable measured to determine the magnitude of the influence or the effect of the independent variable. This study's dependent variables are the quality of the simplicia and the total flavonoid content.

### **Research Stages**

#### **1. Preparing the Simplicia**

The guava leaves were identified and determined at the Research and Development Center for Medicinal Plants and Traditional Medicine (B2P2TOOT). The guava leaves were wet-sorted, washed with running water, and then dried in an oven at 50°C for varying drying times. The dried leaves were then crushed and sieved with a 40-mesh sieve.

#### **2. Organoleptic, macroscopic, and loss drying**

The organoleptic test was conducted by observing the shape, color, taste, and smell of the guava leaf simplicia. Then, the macroscopic test was conducted to directly determine the characteristics of simplicia and compare them with the general literature. In addition, the drying shrinkage percentage was determined using a moisture balance (Safrina et al., 2020).

#### **3. Making the extract**

A hundred grams of guava leaf powder for each sample was put into a maceration vessel and added with 750 ml of 70% ethanol solvent for 3-day soaking, then filtered using

flannel cloth and filter paper. The dregs obtained were soaked again in the remaining 250 ml solvent for two days with occasional stirring, then filtered and mixed with the menstruum resulting from the first soaking. Each maceration product was evaporated using a rotary evaporator, followed by a water bath at 50°C until a thick extract was obtained (Susilowati & Anggraini, 2018).

4. The qualitative analysis of the flavonoid (Hanani, 2015)
  - a. Wilstater test: the sample was added with two to four drops of concentrated HCl and added with small pieces of Mg. Positive results were marked orange, yellow, and red.
  - b. Alkaline reagent test: the sample was added with a few drops of NaOH solution. Positive results were marked bright yellow.
  - c. H<sub>2</sub>SO<sub>4</sub> reagent test: the sample was added with a few drops of H<sub>2</sub>SO<sub>4</sub> solution. Positive results were marked dark red and brownish black.
5. The quantitative analysis of the flavonoid
  - a. The making of the quercetin standard solution  
Ten milligrams of quercetin were dissolved in methanol to 25 mL until a stock standard solution of 1000 ppm was obtained. Next, 30, 35, 40, 45, and 50 ppm concentration series were made with methanol p.a. up to 5 mL (Susilowati; & Desi, 2022) .
  - b. The measurement of the total flavonoid (Shraim et al., 2021)  
Each test sample and standard solution were taken at 0.5 mL and added with 2 mL methanol p.a., 10% 0.2 AlCl<sub>3</sub>, and vortex mixing with a 3-minute equilibration time. Next, 0.2 ml CH<sub>3</sub>COONa was added, and the final volume was made to 5 ml using methanol p.a., then shaken until homogeneous. It was then left for 40 minutes, and the absorbance was measured using UV-Vis Spectrophotometry at a wavelength of 415 nm. This measurement was carried out in triplicate, and before the measurement, an optimization was conducted to determine the operating time and maximum wavelength.

## Data Analysis

1. Determining the total flavonoid content  
The flavonoid content was calculated using the quercetin linear regression curve equation. The absorption data obtained from determining the flavonoid content was entered into the calibration curve equation as  $y$ , thereby bringing the  $x$  value as the flavonoid concentration in the working sample solution. The results were expressed as the equivalent of a standard flavonoid solution using the reference standard quercetin to declare Quercetin Equivalent (QE) (Shraim et al., 2021).
2. The comparative analysis  
Total flavonoids from steeping fresh and dried leaves of clove mistletoe were carried out using the One-Way ANOVA test using SPSS. The flavonoid content was included as the dependent variable, and steeping water was the factor variable. Normality and Homogeneity tests of Variances were initially conducted to determine the data's normality and homogeneity. The data were normally distributed if the normality test result was  $<0.05$ , and if it was  $>0.05$ , then it was concluded that there was no real difference between variations in harvest time and drying time.

## RESULTS

Table 1 shows that, organoleptically, the color of the guava leaf simplicia harvested in the afternoon is darker than in the morning. At the same time, the drying time variation does not make a difference organoleptically or macroscopically. Drying losses in Table 1 show that harvesting in the afternoon produces smaller drying losses than harvesting in the morning. In contrast, the longer the drying time, the lower the drying loss compared to other dryings. The

results of the paired samples test from the drying loss comparison produced a sig value of 0.076 (>0.05), indicating that the drying loss of guava leaves from various treatments is not significantly different.

Table 1.  
Organoleptic, macroscopic and drying loss test of dried Guava Leaf in Harvest Time and Drying Time

Testing	Criteria <sup>(2)</sup>	Harvest time			Drying time	
		Morning (9 am)	Evening (3 pm)	24 hours	36 hours	48 hours
Organoleptic						
Color	Green	Dark green	Brownish green	Green	Green	Green
Smell	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
Flavor	Chelate	Chelate	Chelate	Chelate	Chelate	Chelate
Macroscopic						
Leaf type	Single	Single	Single	Single	Single	Single
Stem length	0, 5 – 1 cm	0, 8 cm	0, 7 cm	0,7 cm	0,8 cm	0,7 cm
Leaf blade length	5- 13 cm	±10 cm	±10,5 cm	11,5 cm	10,4 cm	10,3 cm
Wide	3 – 6 cm	±4,6 cm	±4,7 cm	6 cm	4,7 cm	5 cm
Shape	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded
	protruding	protruding	protruding	protruding	protruding	protruding
Leaf surface	Slippery	Slippery	Slippery	Slippery	Bit rough	rough
Bone leaves	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate	Pinnate
Drying loss	<10%	9,806%	8,914%	9, 182%	9,018%	8,288%

Table 2.  
Organoleptic and yield of Guava Leaf Extract Variations in Harvest Time and Drying Time

Testing	Criteria <sup>(2)</sup>	Harvest time			Drying time	
		Morning (9 am)	Evening (3 pm)	24 hours	36 hours	48 hours
Organoleptic						
Color	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Smell	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
Flavor	Chelate	Chelate	Chelate	Chelate	Chelate	Chelate
Form	Thick extract	Thick extract	Thick extract	Thick extract	Thick extract	Thick extract
Yield	Not less than 12,3%	20,335%	20,545%	38,8%	38,7%	43,3%
Flavonoid						
Wilstater cyanidin	+	+	+	+	+	+
Alkali test	+	+	+	+	+	+
Acid test	+	+	+	+	+	+

Table 2, the results of the extracts produced from various variations of simplicia production show that there are no organoleptic differences. However, the extract yield in making simplicia with the longest drying time (48 hours) shows the highest yield. Furthermore, All of the extracts containing flavonoids. Based on the comparison of the paired samples test, a sig value of 0.013 (<0.05) is produced, indicating that differences in the treatment of making guava leaf simplicia influence the extract yield.

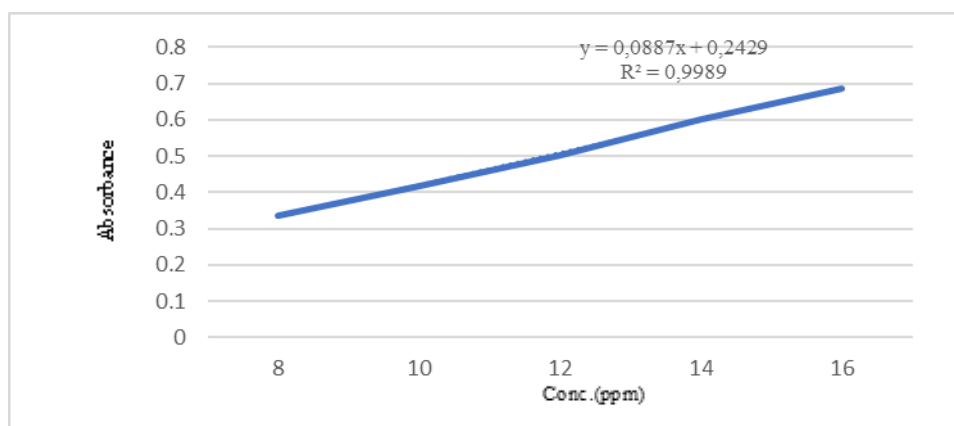


Figure 1. Linear regression curve of concentration versus absorbance of quercetin in UV-Visible Spectrophotometry

Figure 1 shows the linear regression curve between quercetin contents and absorbance produced by UV-visible spectrophotometry. The total flavonoid content in guava is determined at a wavelength of 426 nm with an operating time of 30 minutes. The standard curve is determined using concentrations of 8, 10, 12, 14, and 16 ppm, considering the absorption range between 0.2 and 0.8 to avoid photometric errors.(Saputri et al., 2022) The linear correlation between the absorbance of quercetin concentration is shown by the correlation coefficient (r) value, namely 0.9989. Figure 1 shows the linear regression equation for quercetin,  $y = 0.0887x + 0.2429$ , which is then used to calculate the total flavonoid content of guava leaf extract with various treatments.

Tabel 3.  
Flavonoid total of Guava Leaf Extract Variations in Harvest Time and Drying Time

Harvest condititon		Flavonoid total (mg QE/gram)	The average of Flavonoid total (mg QE/gram)	%KV
Harvest time	Morning (9 am)	0,7187	0,7200*	0,212%
		0,7197		
		0,7217		
	Evening (3 pm)	1,0867	1,0887*	0,243%
		1,0877		
		1,0917		
Drying time	24 hours	0,2446	0,24507*	0,1893%
		0,2449		
		0,2457		
	36 hours	0,2135	0,21397*	0,1720%
		0,2140		
		0,2144		
	48 hours	0,2225	0,22303*	0,2018%
		0,2230		
		0,2236		

\*= sig < 0,05 shows that there were significant differences between treatments

Table 3 shows that the flavonoid content in all variations of simplicia is significantly different. Harvesting in the afternoon provides higher content compared to harvesting in the morning. Meanwhile, the 24-hour drying results in the highest flavonoid content compared to other drying times. The reaction for the formation of the flavonoid and AlCl<sub>3</sub> complex can be seen in Figure 2.

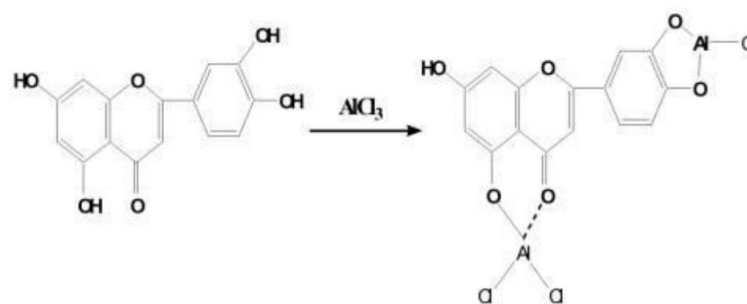


Figure 2. Formation of the Quercetin-aluminum chloride complex compound (Shraim et al., 2021)

## DISCUSSION

The guava leaves in this study, harvested in the morning and afternoon, were wet sorted to separate the leaves from dirt or unwanted foreign materials so they do not influence the following process. The guava leaves are then washed using running water to dissolve dirt that may still be attached to the leaves after the wet sorting process. Next, the drying is carried out at a temperature of 50°C with varying drying times of 24, 36, and 48 hours. The drying aims to reduce the water content in guava leaves so they last a long time in storage and are not easily damaged. Several dried leaves *simplicia* are then taken from various variations for organoleptic, macroscopic, and drying loss tests. The remaining *simplicia* is powdered with a blender, sieved, and weighed 100 grams for each drying time.

Table 1 shows that the color of the guava leaves harvested in the morning is dark green, and those harvested in the afternoon are brownish green. This is because leaves picked in the morning have not had assimilation yet. In contrast, the leaves picked in the afternoon have had assimilation. Assimilation is the complex formation of simple compounds in the presence of sunlight and can be said to be one of the processes of photosynthesis (Ibrahim et al., 2011). According to (Gustina, 2017), perfect photosynthesis in plants occurs in the afternoon, when there is an increase or decrease in sunlight, influencing the production of flavonoids. In addition, the surface of the leaves of the 48-hour drying is rougher compared to other treatments. This shows that the longer process of evaporation of water in the leaves due to the long drying time can influence the texture of the resulting *simplicia*.

The different treatments in making guava leaf *simplicia* do not influence the organolepticity of the extract but have an impact on the yield or biomass of the extract produced. The required drying loss of guava leaves is no more than 10% (Kemenkes RI, 2017); therefore, the results of the drying loss of all treatments meet the requirements. The leaf samples' lowest percentage of drying loss is produced within 48 hours of drying due to the longer drying time that causes more evaporation of the water content in the leaves. Yield compares the resulting extract's final weight with the *simplicia*'s weight. The higher the yield of the extract, the higher the content of substances attracted to the raw material (Wijaya et al., 2018). The treatment with 48-hour *simplicia* drying shows the highest yield (43.3%). This result is in line with research by (Rusmawati et al., 2021), which shows that the shorter the drying time for *simplicia*, the lower the extract yield obtained will be; in shorter drying, the water content that is still high will cause the texture of the leaves to become rigid and difficult to destroy, and thus many large particles considered residue, can cause the low yield obtained.

Harvesting in the afternoon (20.545%) also shows a higher extract yield than harvesting in the morning (20.335%). This is due to the amount of light captured in the photosynthesis process

that is directly proportional to the biomass, which shows its dry weight. The high dry weight reflects the biomass in the plant tissue (Chikmawati et al., 2013). Therefore, the low light intensity in the morning will influence the biomass and yield of guava leaf extract.

Table 3 shows that all guava leaf simplicia with different treatments positively contain flavonoids. In the Wilstater Cyanidin test, samples containing flavonoids will experience a color change from orange-yellow to red. (Hanani, 2015) In this test, HCl is added to hydrolyze the flavonoids into their aglycos. The hydrolysis process causes O-glycosyl to become an aglycone (not bound to sugar) so that H<sup>+</sup> is released and O is bound by Mg, which reduces the flavonoids to give a red, yellow, or orange color (Baud et al., 2014).

In the alkaline reagent test, the guava leaf extract (*Psidium guajava* L.) is positive for containing flavonoids since the entire sample changes to yellow after being dripped with NaOH. This is due to the decomposition of the base into yellow acetophenone molecules due to the breaking of bonds in the isoprene structure. (Kusnadi dan Devi, 2017) The addition of concentrated H<sub>2</sub>SO<sub>4</sub> reconfirms the qualitative test of flavonoids. The results change the color of the sample from blackish brown to dark red. This shows an oxidation-reduction reaction between concentrated H<sub>2</sub>SO<sub>4</sub> and flavonoids, which causes the formation of complex compounds that give the sample a dark red to blackish-brown color (Hanani, 2015).

The flavonoid content is determined to see the influence of different preparations for simplicia on the flavonoid content, which is the biomarker compound in guava leaves. The content is determined using the UV-visible spectrophotometric method since flavonoid has chromophore and autochrome groups. This study uses the aluminum chloride method, which is designated as quercetin. The principle of determination begins with forming a stable complex between aluminum chloride and the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom of the flavanol. In addition, the formation of a labile complex at the ortho hydroxyl in Ring B. The quercetin standard used is a flavonoid from the flavanol group, which has a keto group on the C-4 atom, a hydroxyl group on the neighboring C-3 and C-5 atoms, and an ortho hydroxyl group on the C-4 atom in Ring B, and thus a complex reaction between quercetin and AlCl<sub>3</sub> can be formed, as shown in Figure 2 (Shraim et al., 2021) The formation of the quercetin aluminum chloride complex is indicated by a yellow solution, resulting in a shift in wavelength towards visible light and the addition of sodium acetate, which functions to stabilize complex compounds (Makuasa & Ningsih, 2020).

The difference in harvest time for guava leaves influences the total flavonoid content. The afternoon harvest (1.0887 mgQE/gram ± 0.243%) shows higher total flavonoid content than the morning harvest (0.7200 mgQE/gram ± 0.212%). This study's results are in line with research by Allasady (Allasady et al., 2022), which shows that the afternoon harvest time for celery and parsley leaves at 3 p.m. shows that the phenolic group content, including flavonoid, is higher than when harvested in the morning, at 9 a.m. and 12 p.m. The amount of secondary plant metabolite content, including flavonoids, depends on factors such as plant age and abiotic environment, light intensity, water stress, pollution, and nutrient supply (Chikmawati et al., 2013). The higher light intensity received by the plant will influence the stress felt by the plant, increasing the ability to produce secondary metabolites.

The difference in drying time for guava leaf simplicia also influences the total flavonoid content. The shorter the drying time, the greater the flavonoid content in the sample. This study's results align with research by Nathaniel et al., (2020), which shows that a longer drying time results in a significant decrease in the total flavonoid and phenolic content of

*rambusa (passiflora foetida)* leaf tea bags. In addition, the length of drying time influences the entire flavonoid content. Longer exposure to heating can cause damage to several phytochemical contents, which influence the integrity of cell walls and cause degradation of several components, including flavonoids. Chemical reactions can also influence the decrease in plant flavonoid content. Flavonoid degradation can occur through chemical reactions, including oxygen, enzymes, and light (Rabeta & Vithyia, 2013). Short-time drying will reduce the activity of flavonoid enzymes against degrading enzymes, such as polyphenol oxidase, so that it can increase the total flavonoid content (Rababah et al., 2015). The results of this study recommend that the process of making guava leaf simplicia should be carried out in the morning, with 24-hour drying and at a temperature of 50°C.

## CONCLUSION

Guava leaf simplicia (*Psidium guajava* L.) at various variations in harvest time and drying time meet standard parameter values in the Indonesian Herbal Pharmacopoeia related to organoleptic, macroscopic, and drying loss. It is recommended to make guava leaf simplicia during harvest in the afternoon and with a 24-hour drying time since it shows the highest total flavonoid content.

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