

## Extraction and Determination of Total Phenolic and Flavonoid in Kapok Leaves (*Ceiba pentandra L.*) using Ethanol as Solvent

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**Abstract:** Kapok (*Ceiba pentandra L.*) is traditionally used in medicine. Based on the phytochemical screening, the leaves contain phenolic compounds, including flavonoids and tannins. The method used to determine the total phenol content was the Folin-Ciocalteu method using gallic acid as standard solution. The linear regression equation obtained on measured at 765 nm is  $y = 0.0054x + 0.0272$  with determination ( $r$ ) and correlation coefficient ( $R^2$ ) of 0.9994 and 0.9997. Total phenolic content in ethanol extracts is 89.185  $\mu\text{g/ml}$  calculated as gallic acid. Meanwhile, determination of flavonoid content used comparison standard of quercetin used the  $\text{AlCl}_3$  method. Measuring at a wavelength of 510 nm gives a linear regression equation  $y = 0.122 + 0.003x$  with  $r = 0.9984$  and  $R^2 = 0.9960$ . Flavonoid content in kapok leaves is 1.6287% w/w calculated as quercetin.

**Keywords:** phenolic, flavonoid, extract, ethanol, *Ceiba pentandra L.*

**Abstrak:** Kapok (*Ceiba pentandra L.*) secara tradisional digunakan dalam pengobatan. Berdasarkan skrining fitokimia, daunnya mengandung senyawa fenolik antara lain flavonoid dan tanin. Metode yang digunakan untuk menentukan kadar fenolik total adalah metode Folin-Ciocalteu dengan menggunakan asam galat sebagai larutan baku. Persamaan regresi linier yang diperoleh pada panjang gelombang 765 nm adalah  $y = 0,0054x + 0,0272$  dengan koefisien determinasi ( $r$ ) dan korelasi ( $R^2$ ) berturut – turut sebesar 0,9994 dan 0,9997. Kandungan total fenolik adalah 89,185  $\mu\text{g/ml}$  dihitung sebagai asam galat. Sedangkan untuk penentuan kadar flavonoid digunakan standar pembandingan quersetin menggunakan metode  $\text{AlCl}_3$ . Pada pengukuran panjang gelombang 510 nm menghasilkan persamaan regresi linier  $y = 0,122 + 0,003x$  dengan  $r = 0,9984$  dan  $R^2 = 0,9960$ . Kandungan flavonoid daun kapok sebesar 1,6287% b/b dihitung sebagai kuersetin.

**Kata kunci:** fenolik, flavonoid, ekstrak, etanol, *Ceiba pentandra L.*

### INTRODUCTION

Kapok is a tropical plant in the Malvaceae Family known as Kapas Jawa or Kapok Jawa and it is traditionally used to fill mattresses. Based on the chemical content, it has many advantages, and the community can use almost all parts to treat various diseases (Pratiwi 2014).

Kapok leaves treat intestinal inflammation, fever, cough accompanied by phlegm, diarrhea, blood sugar, high blood pressure, and wound. Furthermore, young kapok leaves, flowers, and fruits can be consumed as vegetables. The root and bark are also effective in treating diseases of the stomach, spleen, antitussive, preventing asthma, stimulating uterine contractions, accelerating the birth of a baby, abortivum, reducing menstrual bleeding, facilitating blood coagulation, and stimulating the release of breast milk (Diana *et al.* 2013).

Based on phytochemical screening, the leaves contain phenolic compounds, reducing sugars, polyuronoids, and plobatins (Asare & Oseni 2012). Meanwhile, the young leaves contain flavonoids,

alkaloids, tannins, saponins, phytate, oxalate, trypsin inhibitors, and hemagglutinins (Apriliani *et al.* 2016).

Phenolic compounds are secondary metabolites with one or more hydroxyl groups on the aromatic ring. This compound helps prevent oxidation reactions from occurring and have bactericidal, antiseptic, and anthelmintic properties. Phenolic are abundant in plants and widely distributed in plant parts. Meanwhile, flavonoids are one of the chemical compounds derived from plants and are classified as secondary metabolites. These compounds are widely distributed in plant angiosperms, gymnosperms, and pteridophytes. Flavonoids provide physiological and pharmacological effects on living things. In plants, they work as dyes, growth regulators, disease agents, and markers in plant classification (Diana *et al.* 2013).

According to Susanti (2021), kapok leaves infusion's total antioxidant and phenolic activities are  $31.57 \pm 1.55$  mg AA/g DW and  $25.14 \pm 2.71$  mg GAE/g DW, respectively. According to Taeri *et al.* (2018) using 70% ethanol as a solvent, the antioxidant and

flavonoid activities of kapok leaves are  $IC_{50}$  of 59.296 ppm and 12.69%, respectively. Therefore, this study aimed to determine phenolics and flavonoids total in ethanol extracts of kapok leaves using reference solutions of tannic acid and quercetin using the Folin-Ciocalteu and the  $AlCl_3$  method.

## MATERIALS AND METHODS

### Materials

Ethanol 96%, Folin-Ciocalteu reagent,  $Na_2CO_3$ , gallic acid, quercetin,  $AlCl_3$ ,  $CH_3COOK$  p.a, HCl p.a, magnesium powder,  $FeCl_3$ , and aquadest.

### Sample Processing

Kapok leaves are washed, air-dried without direct sunlight, chopped, and stirred homogeneously. It was then macerated with 96% ethanol for  $3 \times 3$  days and filtered. To evaporate the solvent, used a rotary evaporator to obtain a thick extract and weighed.

### Flavonoid Qualitative Test

Flavonoid examination takes 1 ml of kapok leaves liquid extract plus a few drops of HCl p.a and Mg powder. Positive results are indicated by the appearance of a yellow to red color (Harborne, 1987).

### Preparation of Gallic Acid Calibration Curve (Pourmorad *et al.* 2006)

Gallic acid with concentration 1000  $\mu\text{g/ml}$  is pipetted at 0.4 ml, 0.6 ml, 0.8 ml, 1 ml, and 1.2 ml, and diluted with aquadest to 10 ml to obtain concentrations of 40, 60, 80, 100, and 120  $\mu\text{g/ml}$ . Each concentration is pipetted at 0.5 ml, added with 5 ml of Folin-Ciocalteu reagent 1:10 and 4 ml of 1M  $Na_2CO_3$  solution, and then left for 15 minutes. Absorption is measured using a spectrophotometer at a wavelength of 765 nm.

### Determination of Total Phenolic Content in the Ethanol Extract of Kapok Leaves with Folin-Ciocalteu Method (Pourmorad *et al.* 2006)

50 mg of extract was dissolved using 96% ethanol to 50 ml. Pipette a total of 5 ml into a 25 ml volumetric flask and fill to the limit mark with aquadest. In addition, pipet 0.5 ml into a 10 ml volumetric flask, then add 5 ml of Folin-Ciocalteu reagent 1:10, shaken and added 4 ml of 1 M  $Na_2CO_3$  solution and incubated for 15 minutes. Absorbance is measured on a spectrophotometer at a wavelength of 765 nm.

### Preparation of Quercetin Calibration Curve (Zou *et al.* 2004)

About 1, 1.25, 1.5, 1.75, and 2 ml of quercetin standard solution (1000  $\mu\text{g/ml}$ ) is pipetted, then diluted with ethanol to 10 ml to obtain 100, 125, 150, 175, 200  $\mu\text{g/ml}$ , respectively. Each concentration is pipetted 0.8 ml, added with 4 ml of aquadest and 0.3 ml of 5%  $NaNO_2$ , and then left for 6 minutes. Furthermore, 0.3 ml of 10%  $AlCl_3$  is added, then left

for 6 minutes, added with 4 ml of 4% NaOH, diluted to 10 ml, and left for 15 minutes. Absorbance was measured using a UV-Vis spectrophotometer at  $\lambda$  510 nm and repeated three times. The average is calculated, and a calibration curve relates the quercetin concentration to the absorbance.

### Determination of flavonoid content in ethanol extracts of Kapok leaves (Zou *et al.* 2004)

Phenolic and flavonoid content is determined using linear regression equations as shown in equation (1).

$$\hat{y} = a + bx \dots (1)$$

where:

$\hat{y}$  = estimated value of dependent variable

a = intersection point of regression on the y-axis

b = gradient of regression line

x = value of independent variable (Sugiyono, 2002).

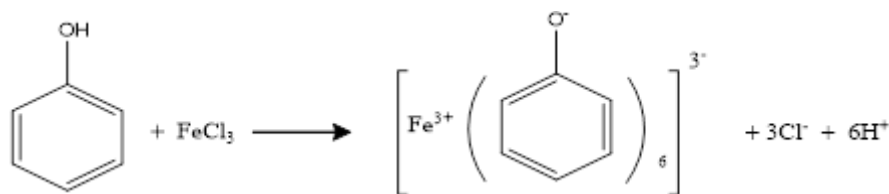
## RESULTS AND DISCUSSIONS

In this research, the sample used is kapok leaves obtained in the Tikalak X Koto Singkarak area, Solok Regency, West Sumatra Province in December 2021. Before processing, the leaves were washed to remove on surface leaves dirt and allowed to air dry without exposure to the sun to avoid damaging the active ingredients. The dried leaves were chopped, and the active substance was pulled completely by the solvent (Nofita *et al.* 2020).

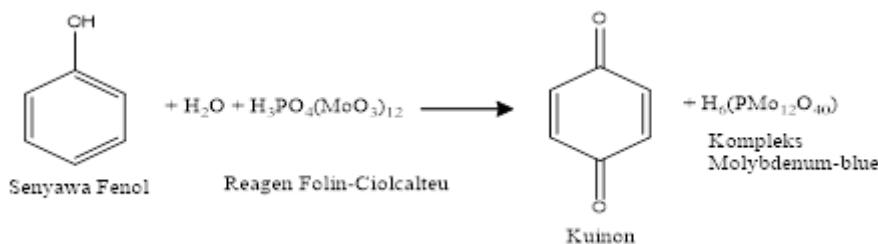


**Figure 1.** Kapok leaves

The extraction method used is maceration as it is very easy and good at extracting heat-intolerant compounds. The principle of maceration is the occurrence of diffusion of the solvent into plant cells containing active compounds and causes the osmotic pressure inside the cell to be different from that outside the cell. Finally, the active compounds are driven out of the cell (Marjoni *et al.* 2018). Simplicia soaking was used to lyse the kapok leaves cell wall, and the bioactive compounds in the sheet were dissolved in the solvent used (Tiwari, *et al.* 2011). Maceration was conducted using ethanol, a common solvent that is safe. According to Kate (2014), other advantages of ethanol are non-toxic, neutral, harmless to the environment, and small boiling points. A rotary evaporator was used to evaporate the solvent, and the thick extract obtained from 100 g



**Figure 2.** Chemical reaction of phenolic compounds with FeCl<sub>3</sub> (Arum *et al.*, 2013).



**Figure 3.** Reaction of phenol compounds with folin ciocalteu reagents (Nofita & Nurlan, 2020)

of kapok leaves was 12.7 g.

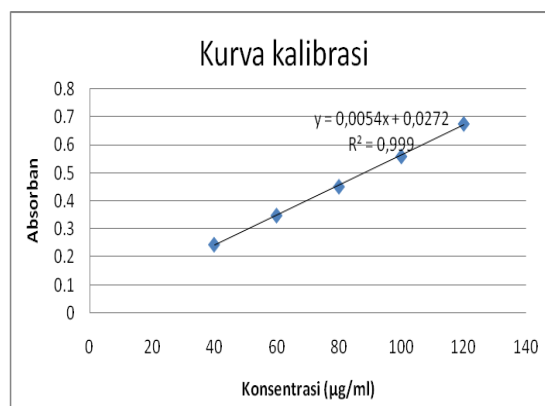
#### Determination of Phenolic Content

Quantitative analysis is an analysis to determine the absolute or relative amount of an element in the sample (Nofita, 2022). Before quantitative analyzes to determine the content of phenolic and flavonoids, initial tests were performed to determine the levels of these compounds. A phenolic test is carried out using a FeCl<sub>3</sub> reagent, and a positive sample contains phenolic when the stain is green, red, purple, blue, or strong black (Harborne 1987). The result showed a blackish green color hence kapok leaves positively contain phenolic compounds. The chemical reaction is shown in Figure 2.

The method used to determine total phenolic content is the Folin Ciocalteu method. This method uses a small amount of the specific and sensitive reagent with phenolic compounds (Waterhouse 2006). Folin ciocalteu reagent is yellow and will turn into a complex blue solution when it reacts with phenolic compounds after adding sodium carbonate as depicted in Figure 3. This complex compound is absorbed by the spectrophotometer at a maximum wavelength of 765 nm. 1M Na<sub>2</sub>CO<sub>3</sub> used functions as a regulator of alkaline conditions. This is because the reaction for the formation of complex compounds between phenolics and folin ciocalteu takes place in an alkaline conditions, forming phenol ions by proton dissociation (Apsari & Susanti 2011).

Gallic acid was chosen as a standard because it is a more economical and stable natural phenolic compound than other phenolic compounds. These compounds are derived from hydroxybenzoic acid and are classified as simple phenolic acids. This

compound available in a pure and stable form (Mariska 2009).



**Figure 4.** Standard curve of gallic acid

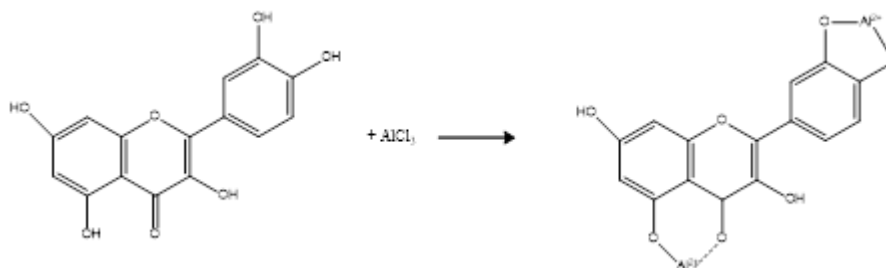
A calibration curve is designed to obtain a linear regression equation with make standard series. The regression equation  $\hat{y} = 0.0272 + 0.0054x$  with a determination and correlation coefficient of 0.9994 and 0.9997 can be observed in Figure 4. Total phenolic in ethanol extract of kapok leaves is 89.185 µg/ml, calculated as gallic acid. Based on Table 1, phenolic content in kapok leaves using the same solvent and method gives higher yields than the bark.

#### Determination of Flavonoid Content

A qualitative test of flavonoids is carried out using an HCl reagent and Mg powder. Based on the results, kapok leaves contain flavonoid compounds, as indicated by a reddish-orange color. This result is supported by Taeri *et al.* (2018) proving that kapok leaves contain flavonoids, alkaloids, saponins, tannins, phenolics, and triterpenoids. Meanwhile

**Table 1.** Comparison of phenolic content in certain kapok plants

Plant part	Solvent	Method	Content	Literature
Bark	Ethanol	Folin-Ciocalteu	43.063 $\mu\text{g/mL}$	Taeri <i>et al.</i> 2018
Leaves	Ethanol	Folin-Ciocalteu	89.185 $\mu\text{g/mL}$	This research

**Figure 5.** Reaction of complex formation between  $\text{AlCl}_3$  and flavonols (fadillah *et al.* 2017)**Table 2.** Comparison of flavonoid content using different solvents

Plant Part	Solvent	Method	Content (%)	Literature
Leaves	70% ethanol	$\text{AlCl}_3$	12.690	Fauziah & Sari, 2020
Leaves	96% ethanol	$\text{AlCl}_3$	1.6287	This research

Diana *et al.* (2013) stated that the flavonoid compounds belong to the flavone group. Quercetin is a flavonoid with a flavonol group with keto at C-4 and hydroxy at C-3 or C-5 atoms, next to flavones and flavonols, and thus used as a standart solution to determine total flavonoid content (Azizah dkk. 2014).

The principle of determining flavonoid content is to react flavonoids with  $\text{AlCl}_3$  to form a yellow complex compound, as shown in Figure 5. Absorption is measured at a wavelength of 510 nm, shifting to more yellowish visible light. Additionally,  $\text{AlCl}_3$  reacts with the keto and OH groups of flavones or flavonols to form stable complexes. Sodium nitrit and sodium hidroksida compounds form a complex system of  $\text{NaNO}_2\text{-AlCl}_3\text{-NaOH}$ . It has a special color due to the reaction of aluminum ions and flavonoids in alkaline medium to form complex compounds (Zhu *et al.* 2010).

The resulting regression equation is  $y = 0.122+0.003x$  with correlation and determination coefficients of 0.9984 and 0.9960 respectively as illustrated in Figure 6. The flavonoid content in kapok leaves is 1.6287% w/w calculated as quercetin. The flavonoid content of kapok leaves using 96% ethanol solvent is lower than that of 70% ethanol solvent (Table 2). This indicates that 70% ethanol is a more suitable solvent for dissolving flavonoid compounds.

## CONCLUSION

The research concluded that the ethanol extract of kapok leaves has phenolic content of 89.185g/ml calculated as tannic acid and and flavonoid of 1.6287% calculated as quercetin.

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