

# Flavonoid content analysis of ethanol extract of kluwih (*Atrocarpus camansi*) fruit seeds by UV-Vis spectrophotometry

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**ABSTRACT:** Kluwih is traditionally used to lower cholesterol levels, blood pressure and help treat diabetes. Kluwih (*Arocarpus camansi*) is one of the plants that contains various types of secondary metabolites such as alkaloids, phenolics, steroids, and flavonoids. The content of these metabolites plays a major role in its pharmacological activities. This study aims to determine the flavonoid levels contained in the ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*). This study used UV-Vis spectrophotometric method with potassium acetate reagent 120Mm and aluminum chloride 2% to form a yellow color solution and as a comparison used quercetin. The results showed that the average flavonoid content of ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*) was  $6.750 \text{ mg} \pm 1.35 \text{ QE/g}$  extract.

**KEYWORDS:** Kluwih; *Atrocarpus camansi*; flavonoids; spectrophotometry UV-Vis.

## 1. INTRODUCTION

Traditional medicine using herbs is still an option to be used as a treatment (Yigit, 2017). When compared to chemical drugs, traditional medicine has a slower ability, but the use of herbs as the main raw material makes traditional medicine have less side effects (Vigneshwaran et al., 2014). Empirically, people process kluwih fruit as a vegetable, boiled or roasted seeds are eaten as snacks. Kluwih seeds are also used as traditional medicine to treat cholesterol, lower blood pressure, while kluwih leaves are used as diabetes medicine.

Previous research stated that ethanol extract of kluwih seeds in doses of 100, 200, and 400 mg/kgBW can reduce blood glucose levels of DM male Wistar rats induced by streptozotocin (Setyawati, 2023). Kluwih fruit seeds have a total phenolic content in TPC methanol extract of 22.1 mg GAE/g and in water extract of kluwih fruit seeds of 5.22 mg GAE/g. (Rabeta and Syafiqah, 2016) and flavonoid content of water extract of kluwih fruit seeds amounted to  $400.858 \pm 40.328 \text{ mg CE } 100 \text{ g}^{-1} \text{ DW}$  (Vianney, 2020).

Kluwih water extracts (leaves and some parts of the fruit such as skin, flesh and seeds) have antioxidant activity against DPPH radicals with  $IC_{50}$  values ranging from 80 - 250  $\mu\text{g/mL}$ . Kluwih seed and leaf water extracts showed strong antioxidant activity (50-100  $\mu\text{g/mL}$ ), kluwih fruit skin had moderate activity (101-150  $\mu\text{g/mL}$ ), and kluwih fruit flesh had low activity ( $>150 \mu\text{g/mL}$ ) (Vianney, 2020).

Based on the description above, this research was conducted to analyze the flavonoid content of ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*) so that this research can provide scientific data on the utilization of kluwih fruit seeds (*Atrocarpus camansi*). The flavonoid compound group is one of the secondary metabolite contents that have pharmacological effects.

## 2. EXPERIMENTAL SECTION

### 2.1. Sample collection

Seeds are separated from the ripe kluwih (*Atrocarpus camansi*) fruit and cleaned with water, then thinly sliced and dried. The dried kluwih fruit seeds were pulverized using a blender (Arif, 2018). Kluwih fruit seed powder was extracted by maceration using 96% ethanol. Let stand for 5 days at room temperature and stirred every day for 5 minutes. After 5 days of soaking, the supernatant was filtered and remacerated. The liquid extract was evaporated using a rotavapor (Setyawati, 2023).

### 2.2. Qualitative analysis

Ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*) was added with 5 drops of  $\text{FeCl}_3$ . The formation of green or blue green color indicates the presence of flavonoid compounds (Harbone, 1987).

### 2.3. Standard solution and reagents

Ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*) was dissolved in 5 mL of 96% ethanol, the sample was taken 2 mL, 0.1 g Mg powder was added and 10 drops of concentrated HCl was added from the tube wall and shaken slowly. If a red or orange color is formed, it positively indicates the presence of flavonoids (Hanani, 2015). Potassium acetate was weighed as much as 0.294 mg and dissolved with distilled water up to 25 mL. Aluminum chloride ( $\text{AlCl}_3$ ) was weighed as much as 2 grams and dissolved with distilled water up to 100 mL.

## 2.4. Quantitative analysis

### 2.4.1. Preparation of quercetin standard curve

Quercetin standard was weighed as much as 10 mg of standard and dissolved in 10 mL of ethanol. The stock solution was pipetted as much as 1 mL and the volume was sufficient to 10 mL with ethanol to obtain a concentration of 100 ppm. From the 100 ppm quercetin standard solution, then made a concentration series of 7.5; 10; 12.5; 15; 17.5; 20 and 22.5 ppm. From each concentration of quercetin standard solution was pipetted 1 mL and then added 1 mL of AlCl<sub>3</sub> 2% and 1 mL of potassium acetate 120mM, the solution was incubated for 1 hour at room temperature. The absorbance was determined using a Uv-Vis spectrophotometer at a maximum wavelength of 430 nm (Stankovic, 2011).

### 2.4.2. Determination of flavonoid content of kluwih

The sample extract was weighed as much as 15 mg, dissolved in 10 mL of 96% ethanol. The solution was pipetted 1 mL and added 1 mL of 2% AlCl<sub>3</sub> solution and 1 mL of 120 mM potassium acetate, incubated for one hour at room temperature. Absorbance was determined using UV-Vis spectrophotometer method at a wavelength of 430 nm. Three replicates were performed.

## 3. RESULTS AND DISCUSSION

In this study, the maceration method was used to avoid damage to the components contained in the sample. From 1000 g of extracted simplisia, 17.77 g of thick extract was obtained with a percent yield of 1.777%.

**Table 1.** Qualitative test results of ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*)

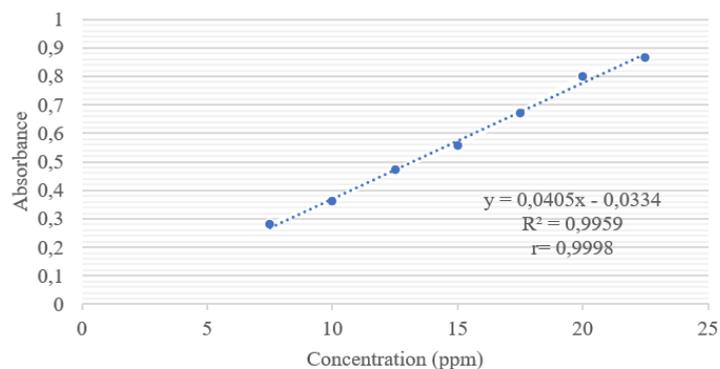
Sample	Reagents	Color Change	Library
Ethanol extract of kluwih fruit seeds	FeCl <sub>3</sub>	Green (+)	Green or Blue green (Harborne, J.B 1987)
( <i>Atrocarpus camansi</i> )	HCl concentrate + Mg	Orange (+)	Red or orange (Hanani, 2017)

Qualitative tests are carried out to determine the chemical components in plants, using color reactions using certain reagents (Melati & Parbuntari, 2022). In the qualitative test, the seeds of kluwih fruit (*Atrocarpus camansi*) were positive for flavonoids. Qualitative analysis using FeCl<sub>3</sub> reagent is positive if there is a green or blue green color change. The addition of FeCl<sub>3</sub> causes a reaction between the hydroxyl groups contained in flavonoids with Fe<sub>3</sub><sup>+</sup> ions in FeCl<sub>3</sub> solution resulting in a color change to green or blue green (Harbone, 1987). Qualitative analysis using magnesium powder and concentrated HCl is positive if a red or orange color is formed (Hanani, 2015). The addition of hydrochloric acid and magnesium causes a reduction reaction at the core of benzopyrone to form flavilium salts. The reaction causes an orange color, magnesium powder and concentrated hydrochloric acid react to form H<sub>2</sub> gas bubbles (Yasser *et al.*, 2022).

Analysis of flavonoid content of ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*) was carried out using a UV-Vis spectrophotometer because flavonoid contains aromatic compounds that are conjugated so that they show strong absorption bands in the ultraviolet light spectrum and visible light spectrum (Aminah, *et al.*, 2017).

Determination of flavonoid content was carried out using quercetin as a comparator, and aluminum chloride as a reagent where the addition of aluminum chloride causes the formation of complexes between aluminum chloride with ketone groups at C-4 atoms and hydroxyl groups at adjacent C-3 or C-5 atoms so that there is a shift in wavelength towards longer and into the visible wavelength range characterized by a solution producing a more yellow color (Chang, *et al.*, 2002). Then potassium acetate is added to maintain the stability of the wavelength in the visible region so that the reaction lasts longer (Kurniawati, *et al.*, 2024).

Furthermore, the concentration series solution and the test solution were measured at the maximum wavelength. The reason it is measured at the maximum wavelength is because the maximum wavelength has maximum sensitivity where the change in absorbance for each unit of concentration is the greatest, besides that at the maximum wavelength the shape of the absorbance curve fulfills the Lambert-Beer law (Fawwaz *et al.*, 2023).



**Figure 1:** Standard curve of quercetin standard solution

Determination of levels using a calibration curve with 7 series of quercetin concentrations, namely 7.5; 10; 12.5; 15; 17.5; 20; and 22.5 ppm. Each concentration was pipetted 1 mL then added 1 mL of 2% aluminum chloride and 1 mL of 120mM potassium acetate then incubated for 1 hour with the aim that the reaction between quercetin standard solution and the reagent can take place perfectly. Then measured each concentration series at the maximum wavelength.

Furthermore, sample measurements were made in three replicates where the sample solution was added with 2% aluminum chloride and 120 mM potassium acetate and incubated for 1 hour then measured at the maximum wavelength.

**Table 2.** Measurement results and calculation of flavonoid content of ethanol extract of kluwih (*Atrocarpus camansi*) fruit seeds.

Replication	Sample weight (g)	Absorbance	Flavonoid content (mg QE/g extract)	Average flavonoid content (mg QE/g extract)
1	0.0152	0.477	7.205	6.750
2	0.0151	0.353	5.225	
3	0.0154	0.521	7.817	

Based on the table above, the results obtained flavonoid content of ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*) amounted to 6.750 mgQE/g extract. When compared to the flavonoid content in other atrocarpus genus such as jackfruit seeds containing flavonoids of 4.8819 mgQE/g extract and cempedak (*Atrocarpus integer*) does not contain flavonoids in its fruit seeds, kluwih fruit seeds have a greater flavonoid content compared to other atrocarpus genus.

#### 4. CONCLUSION

Based on the results of the research that has been done, it can be concluded that the analysis of flavonoid levels in ethanol extracts of kluwih fruit seeds (*Atrocarpus camansi*) in qualitative tests identified as containing flavonoid compounds that have been measured using UV-Vis spectrophotometry contains levels of  $6.750 \pm 1.35$  mgQE/g extract.

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