



Quantification of Flavonoid Compounds in Keji Beling Leaves (*Strobilanthes Crispa* (L.) Blume) Extracted by Ultrasonic Assisted Extraction (UAE) and Maceration Using TLC Densitometry

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Abstract

Keji Beling leaves (*Strobilanthes crispa* (L.) Blume) contain secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and polyphenols. These secondary metabolites have antioxidant, antidiabetic, and anticancer activities. In this study, flavonoid identification was tested using thin-layer chromatography (TLC), followed by determination of the flavonoid content in Keji Beling leaf extract (*Strobilanthes crispa* (L.) Blume) using the densitometry method. The extraction methods used were Ultrasonic Assisted Extraction (UAE) and maceration using 70% ethanol solvent, yielding extracts with a yield of 15.143% and 11.092%, respectively. Quantification of flavonoid compounds using quercetin standard showed that the average flavonoid content of keji beling leaf extract using the Ultrasonic Assisted Extraction (UAE) method was 0.057 mg/mL and using the maceration method was 0.156 mg/mL. This research is different from previous research which used the visible spectroscopy method, because the determination of flavonoid levels used the TLC densitometry method and the study provide an overview that different extraction methods show different flavonoid levels in the keji beling extract. This study aims to determine the flavonoid content of keji beling extract using the TLC densitometry method.

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INTRODUCTION

Keji beling (*Strobilanthes crispa* (L.) Blume) is a well-known herbal plant in Indonesia. Keji beling (*Strobilanthes crispa* (L.) Blume) is a plant native to Madagascar. Keji beling is also commonly found in Indonesia and grows abundantly. This plant is commonly known as binalu api (Malay), reundeu beureum (Sundanese), sambang geteh, sarap (Javanese), and lire (Ternate). Its leaves are widely used as traditional medicine (Saparinto & Rini, 2016). Keji beling (*Strobilanthes crispa* (L.) Blume) is a wild plant that grows in forests, riverbanks, cliffs, and is often planted as a hedge in home gardens (Dalimartha 2006, 39).

This plant is known and used by the community as a traditional medicine because it contains various secondary metabolite compounds. The main claimed efficacy of keji beling herbal medicine is to treat kidney stones, but it is also used to treat diabetes, gallstones, and tumors (Adibim et al., 2017; Nuraqidah et al., 2025). This plant is known to contain bioactive compounds such as flavonoids, tannins, and saponins that have pharmacological activity (Hasnaeni, et al., 2025; Salsa Dinurrosifa, 2022). Several studies determined the flavonoid content of keji beling, obtained different flavonoid content with different extraction and content determination methods (Mahyantika et al., 2025; Ghasemzadeh, A et al., 2025; Sitorus, R et

al., 2024). Flavonoids, a group of polyphenolic compounds, have potential as antioxidants, anti-inflammatories, and antidiabetics (Larasati & Putri, 2021; Haida, Z et al., 2020). Flavonoids have a basic framework of 15 carbon atoms consisting of two benzene rings connected by a propane chain of three carbon atoms. The basic structure of flavonoids is a C₆-C₃-C₆ carbon framework (Estiasih 2022). Flavonoids function as anti-inflammatory, antitumor, antiviral, anti-allergic, and anticholesterol agents (Ariani 2021).

In an effort to improve the quality and quantity of bioactive compounds in keji beling leaves, efficient extraction is essential. In this study, two types of extraction methods were used, namely UAE and maceration. The UAE (Ultrasonic Assisted Extraction) method is a technique that can accelerate the extraction process by utilizing ultrasonic waves. These waves are capable of breaking down cell walls, allowing the compounds to escape and dissolve into the extraction solvent. The ultrasonic method has been proven effective in increasing extraction yields by utilizing ultrasonic waves to break down cells and increase solvent penetration into the sample matrix (Rifkia & Revina, 2023; Kartika et al., 2024).

The maceration method was chosen because flavonoids are phenolic compounds with a conjugated aromatic system that is easily damaged at high temperatures, and the maceration method can avoid damage to the compound components due to heating (Sa'adah et al., 2017; Hasnaeni et al., 2023). To determine the quantification of flavonoid compounds in keji beling leaf extract (*S. crispa*) using Thin Layer Chromatography Densitometry (TLC-Densitometry). This method is one of the analytical techniques that can be used to quantify flavonoid compounds. This method combines the TLC separation technique with densitometry detection, which allows the identification and quantification of compounds based on the color intensity on the thin layer chromatography (Ika Salsabillahi Topanni et al., 2024). The research problem is whether there is a difference in the flavonoid content of keji beling extract extracted using the maceration and UAE methods.

METHOD

Tools and Materials

The equipment used includes an analytical balance, Erlenmeyer flasks, measuring cups, stirring rods, funnels, porcelain dishes, dropper pipettes, Ultrasonic-Assisted Extraction (UAE), maceration containers, water baths, chambers, ovens, 254 nm and 366 nm UV lamps, aluminum foil, filter paper, KLT plates, silica gel, capillary tubes, micropipettes, vacuum rotary evaporators. Keji beling leaf extract (*Strobilanthes crispa* (L.) Blume), 70% ethanol, distilled water, ethyl acetate p.a (C₄H₈O₂), aluminum chloride (AlCl₃), N-hexane p.a (C₆H₁₄), ethanol p.a (C₂H₆O), quercetin.

Sample Preparation

Samples of keji beling leaves (*Strobilanthes crispa* (L.) Blume) that have been collected are sorted to separate unused parts or impurities. They are then washed with running water and drained, then dried by airing at room temperature and not exposed to sunlight. The dried samples are ready for extraction. (Rafsanjani, R et al., 2024; Fardiyah, Q et al., 2020).

Extraction

Ultrasonic-Assisted Extraction (UAE) :

100 grams of crushed keji beling leaves (*Strobilanthes crispa* (L.) Blume) were placed in a beaker. Enough 70% ethanol solvent was added to cover the leaves, then extracted for 60 minutes at a temperature of 65o C and repeated 3 times. The extract was then filtered using

filter paper. The extract obtained was then evaporated using a vacuum evaporator at a temperature of 50-60°C. (Hasnaeni et al., 2023).

Maceration

A total of 200 grams of chopped keji beling leaves were then macerated using 70% ethanol solvent until submerged for 3x24 hours. The ethanol extract was collected by filtering. The liquid extract was evaporated using a vacuum rotary evaporator until a thick extract was obtained. (Nurjannah, I et al., 2022; Hasnaeni et al., 2025).

Quantitative Analysis of Flavonoid Content

Identification by Thin Layer Chromatography (TLC)

The ethanol extract of keji beling leaves and the quercetin reference were each dissolved in sufficient amounts of p.a ethanol, then spotted on a TLC plate and eluted using an eluent/eluting liquid (n-hexane:ethyl acetate) (5:5). Observed using a UV lamp at 254 nm and 366 nm.

Sample Solution Preparation

Weigh 500 mg of keji beling leaf ethanol extract (*Strobilanthes crispus* (L.) Blume) and dissolve in 1 mL of p.a ethanol.

Quercetin Standard Preparation

Quercetin was weighed at 2.5 mg, then dissolved with p.a ethanol at a concentration of 1000 ppm (1 mL). From the 1000 ppm quercetin standard solution, 5 series of quercetin reference concentrations were made, namely 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.3125 mg/mL, and 0.1562 mg/mL. Each concentration was pipetted 0.5 mL and then made up to 1 mL with ethanol p.a.

Densitometric Thin Layer Chromatography

The quercetin standard solution at each concentration and the extract sample were spotted three times using a micropipette (1 µl) on a densitometry TLC plate, then eluted using an eluent (n-hexane:ethyl acetate) (5:5). Observe under a UV lamp at 254 nm and 366 nm. Measured using a Densitometer. (Sharma1, A., 2020).

RESULTS AND DISCUSSION

The concentrated ethanol extract of keji beling leaves obtained was then calculated by comparing the extract obtained during the extraction procedure with the initial simplisia obtained. The yield of the extract obtained is one of the quality factors in an extract. The higher the yield value obtained, which indicates a higher extract value, the higher the yield value obtained and calculated in percent (%). Based on the results, the extract yield obtained was quite high, namely 15.143%, while the requirement for thick extract yield is a value of not less than 10%. Several factors can affect extract yield, one of which is the extraction solvent used (Elvansi & Vifta, 2022).

Tabel 1. Extraction Results and Yield Percentage (%) of Keji Beling Leaves (*Strobilanthes crispus* (L.) Blume) *Ultrasonic-Assisted Extraction* (UAE) Method

Extraction Method	Sample Weight (g)	Extract Weight (g)	Extraction Results (%)
UAE	100	15,143	15,143
Maserasi	200	22,186	11,093

The solvent used was 70% ethanol because it is polar, volatile, and a universal solvent capable of extracting polar, semi-polar, and non-polar compounds, resulting in a greater number of compounds being successfully extracted (Marwati et al., 2024). Ethanol is a solvent that has

the ability to penetrate cell walls, enabling cell diffusion and attracting bioactive compounds more quickly (Yulianti et al., 2021). 70% ethanol has greater polarity than 96% ethanol, due to its higher hydroxyl group ($-OH$) content, making it more effective in dissolving polar compounds (Nurhasanah et al., 2024).

Identification of flavonoid compounds using Thin Layer Chromatography (TLC). Identification of these compounds was performed using n-hexane:ethyl acetate (5:5) as the mobile phase, which can separate the compounds contained in keji beling leaves. This eluent was used because it has different polarities. Thin Layer Chromatography (TLC) is an analytical method used for rapid separation based on the principles of adsorption and partition (Ahmad et al., 2020). Elution is the stage where components are carried by the eluent during the process. The resulting chromatogram profile is observed under UV light at 254 and 366 nm. The results of the flavonoid compound identification test on keji beling leaves (*Strobilanthès crispà* (L.) Blume) can be seen in the image below.

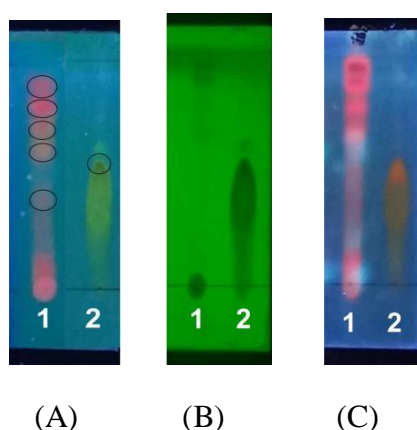


Figure 1 . Profile of KLT Extract of Keji Beling Leaves (*Strobilanthès crispà* (L.) Blume) and Quercetin, (A) Observed at UV 366 nm, (B) Observed at UV 254 nm, (C) Sprayed with $AlCl_3$ reagent, (1) Keji beling extract, (2) Quercetin standard.

Flavonoids are identified using thin layer chromatography (TLC) to observe their spot profiles. Observation of spots using ultraviolet light is used for colorless compounds because some organic compounds can fluoresce when exposed to ultraviolet light at short wavelengths (254 nm) or long wavelengths (366 nm) (Lintang et al., 2024).

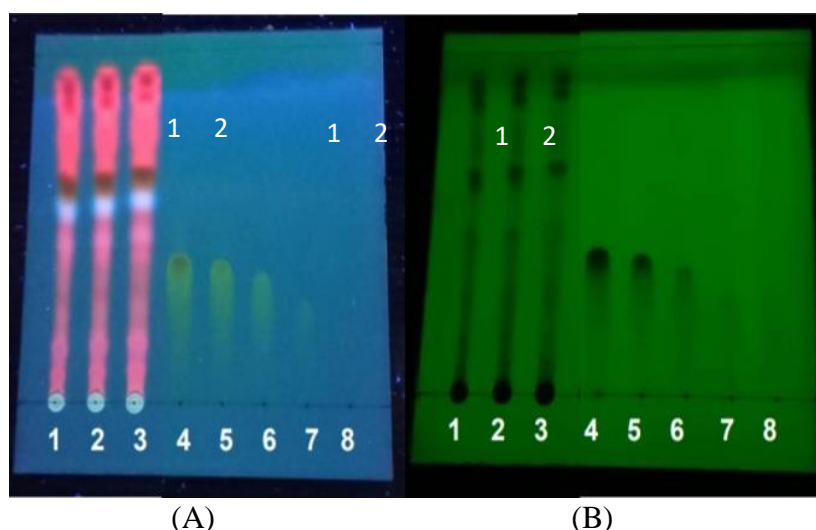


Figure 2. Profile of KLT Densitometry of Keji Beling Leaf Extract and Quercetin UAE extraction results, (A) Observed at UV 366 nm, (B) Observed at UV 254 nm, (1,2,3) Keji beling extract, (4,5,6,7,8) Quercetin standard.

Identification is also carried out using specific reagents for flavonoids. In the identification of flavonoid compounds, the reagent used is $AlCl_3$, where a sample that is positive for flavonoids will produce an intense yellow color similar to the quercetin standard, which indicates a positive result for flavonoids (Puspa Yani et al., 2023). Identification using the $AlCl_3$ reagent showed that the keji beling leaf extract was positive for flavonoids, indicated by the presence of yellow spots. The R_f value is a parameter that indicates the position of the spot in the stationary phase.

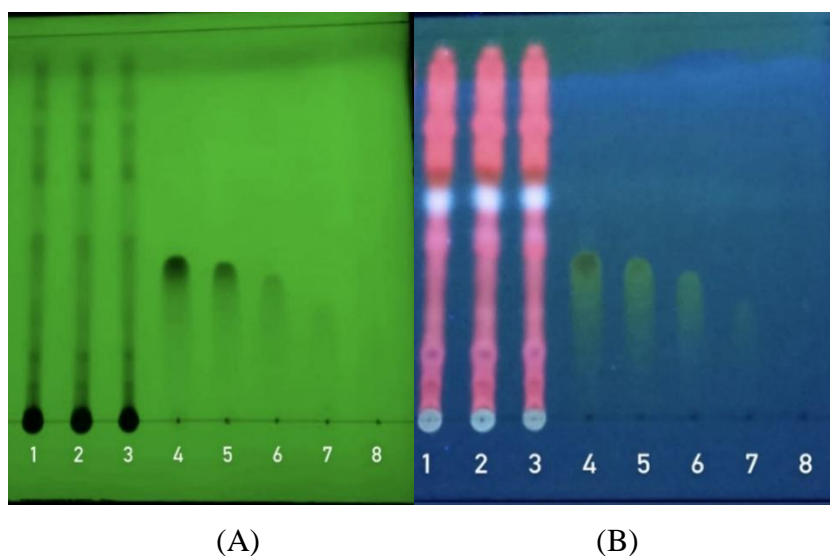


Figure 3. Profile of KLT Densitometry of Keji Beling Leaf Extract (*Strobilanthes crispus* (L.) Blume) and Quercetin Using the Maceration Method, (A) Detection with UV-254 nm, (B) Detection with UV-366 nm, (1,2,3) Keji Beling extract, (4,5,6,7,8) Quercetin standard.

Thin Layer Chromatography (TLC) Densitometry is a method used to determine the concentration of a compound in a sample. Thin Layer Chromatography Densitometry is a chromatography technique in which an adsorbent is coated on a plate as a stationary phase, and a chromatogram is formed when the mobile phase passes through the adsorbent (Ika Salsabillahi Topanni et al., 2024). When using TLC densitometry equipment, which has high sensitivity for analyzing detected compounds, the same volume of extract is spotted (Savitri & Megantara, 2019). Sample preparation is carried out three times to obtain optimal absorbance, minimize errors in analysis measurements, and obtain more accurate data (Rice et al., 2025).

Tabel 2. Area Under the Curve (AUC) Measurement Results Using KLT Densitometry with the Ultrasonic-Assisted Extraction (UAE) Method

Sampl	Concentration(mg/mL)	AUC
X I	500	3159,0
X II	500	4430,3
X III	500	3202,6
Quercetin 1	2,5	45041.9
Quercetin 2	1,25	26102.7
Quercetin 3	0,625	18701.2
Quercetin 4	0,3125	7266.5
Quercetin 5	0,1562	2492.2

The measurement of the maximum wavelength of quercetin aims to determine the wavelength at which maximum absorption is reached. Quercetin was chosen as the standard solution because it is a flavonol flavonoid that has a keto group at (C-4) and a hydroxyl group at atom (C-3) or (C-5) adjacent to flavon and flavonol. The maximum wavelength of quercetin is

determined by reading the absorption of the quercetin standard solution in the wavelength range of 400-800 nm (Puspa Yani et al., 2023).

Tabel 3. Area Under the Curve (AUC) Measurement Results Using KLT Densitometry Maceration Method

Sampel	Concentration(mg/mL)	AUC
X I	500	6492.2
X II	500	6092.8
X III	500	4767.4
Quercetin 1	2,5	45041.9
Quercetin 2	1,25	26102.7
Quercetin 3	0,625	18701.2
Quercetin 4	0,3125	7266.5
Quercetin 5	0,1562	2492.2

Tabel 4. Measurement Results of Average Flavonoid Content in Keji Beling Leaf Extract (*Strobilanthes crispa* (L.) Blume) Using KLT Densitometry and Ultrasonic-Assisted Extraction (UAE) Method

Sample Extract	Flavonoid Content (mg/mL)	Average Level (mg/mL)
1	0,035	0,057
2	0,076	
3	0,060	

Tabel 5. Hasil Pengukuran Kadar Rata-Rata Flavonoid Daun Keji Beling (*Strobilanthes crispa* (L.) Blume) Menggunakan KLT Densitometri Metode Maserasi

Sample Extract	Flavonoid Content (mg/mL)	Average Level (mg/mL)
1	0,197	0,156
2	0,173	
3	0,098	

Measurements using densitometry yielded AUC values. The data were processed using Microsoft Excel to produce a calibration curve showing the relationship between concentration and linear regression equation. A standard curve was created to obtain a linear regression equation. The linear regression equation was created by measuring the AUC values of different concentrations of quercetin. The standard curve shows the correlation between the increase in concentration and the increase in area in the form of AUC. As the concentration increases, the measured AUC will increase in proportion to the increase in concentration, so that the relationship formed is a proportional relationship (Oktaviani, 2021).

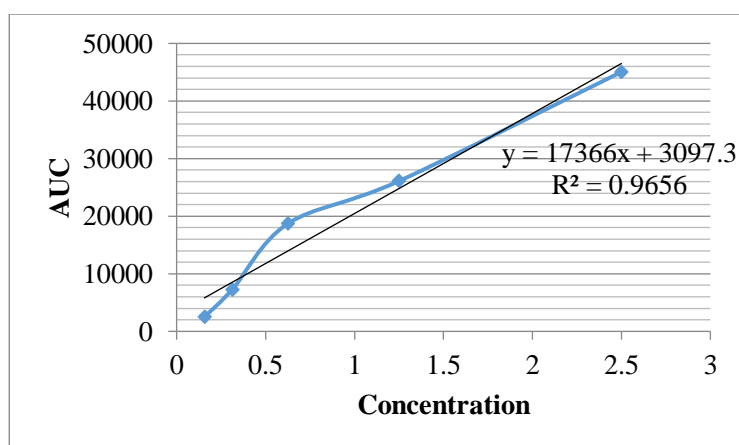


Figure 4. Standard Curve of Quercetin in Ultrasonic-Assisted Extraction (UAE) Method

Based on the standard curve values, the linear regression equation obtained is $y = 17366x + 3097.3$ with a correlation coefficient (R^2) = 0.9656 obtained in this study, indicating good linearity, so that it can be used to determine the flavonoid content of keji beling leaves (*Strobilanthes crispa* (L.) Blume), so that the linear regression equation can be used to calculate the compound content. Based on the results of determining the quercetin content using KLT densitometry, the average flavonoid content in keji beling leaf extract (*Strobilanthes crispa* (L.) Blume) was obtained at 0.057 mg/mL.

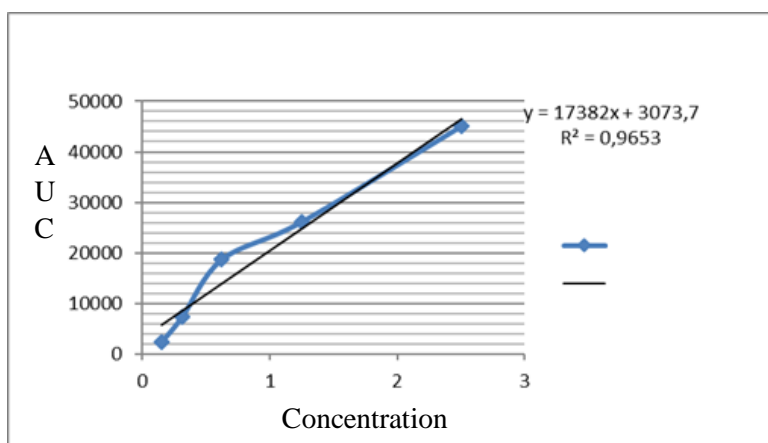


Figure 5. Standard Curve of Quercetin Using the Maceration Method

Based on the standard curve values, the linear regression equation obtained is $y = 17382x + 3073.7$ with an R^2 value of 0.9653. An R^2 value close to 1 indicates excellent linearity, showing that the linear calibration curve has a relationship between concentration and area, so that the linear regression equation can be used to calculate the compound content. Based on the results of determining the quercetin content using KLT densitometry, the average quercetin content in keji beling leaf extract was 0.156 mg/mL. Several studies have been conducted to determine flavonoid levels, each using a different method so that the flavonoid levels are also different. Mahyantika et., 2025 determined the levels of keji beling flavonoids using the UV-vis spectroscopy method and obtained levels total flavonoids of 144.347 ± 134.854 mg QE/g extract. Haida et al., 2020 determined total polyphenols and phenolic acids content were conducted using the Folin-Ciocalteu method and the results were ranged between 5.71 to 10.80 mg GAE g⁻¹ DW.

CONCLUSION

Identification of flavonoid testing conducted using TLC densitometry concluded that the average flavonoid content of keji beling leaves (*Strobilanthes crispa* (L.) Blume) extracted using Ultrasonic Assisted Extraction (UAE) was 0.057 mg/mL, while that extracted using maceration was 0.156 mg/mL.

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