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Effect of Particle Size and Extraction Time on Total Flavonoid Level of *Artemisia vulgaris* Ethanol Extract

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ABSTRACT

Artemisia vulgaris, also referred to as Daun Baru Cina, is a herbaceous species within the Asteraceae family. *A. vulgaris* is a traditional remedy for numerous ailments, exhibiting anti-inflammatory and antioxidant properties due to its secondary metabolites, specifically flavonoids. The extraction method is essential for extracting the desired chemical from natural sources intended for therapeutic use. The extraction process is affected by several elements, including the particle size of simplicia powder and the duration of extraction. Objective: This study aims to test the effect of differences in particle size of powdered simplicia and extraction time of *A. vulgaris* leaves on total flavonoid levels, using 70% ethanol solvent. Researchers used a quantitative approach using a descriptive research design. In this study, a qualitative test was carried out in the form of phytochemical screening followed by a quantitative test to determine the total flavonoid levels of *A. vulgaris* leaves. Various particle sizes (40 mesh and 80 mesh) and extraction times (12 hours and 36 hours) were used in this study. Using ultraviolet-visible spectrophotometry, the Total Flavonoid Level (TFC) of the ethanol extract of *A. vulgaris* leaves was determined. Results: The TFC value obtained for the optimal particle size was 40 mesh, with an extraction time of 12 hours, which was 72.073±1.126 mgQE / g extract. While 80 mesh, with an extraction time of 36 hours, gave a TFC value of 70.169±0.480 mgQE / g extract. In addition, 70% ethanol extract of *A. vulgaris* leaves contains secondary metabolites of phenolic alkaloids, tannins, saponins, and steroids. Conclusion: The study concludes that the quantity of simplicia powder particles and the extraction duration influence the total flavonoid concentrations in *A. vulgaris*.

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1. Introduction

Products originating from nature can be obtained from various sources, such as plants, animals, and microorganisms, which contain a large number of bioactive compounds (Abiri et al., 2018). This is because bioactive compounds in plants can treat many diseases. In particular, plants have the potential to be utilized as therapies for a variety of diseases due to the presence of numerous bioactive substances within them. According to research by Rivai et al., (2020), the term

"traditional medicine" is widely used to refer to treatments that result from plants. According to Judzentiene & Garjonyte, (2016), an excellent possibility exists for medicinal plants to be utilized as alternative medications due to the fact that they are non-toxic and have a limited number of adverse effects. According to Anibogwu et al., (2021), the variety of *Artemisia vulgaris* is a type of Artemisia species that is extensively distributed over the continents of Africa, North and South America, Asia, and Europe (Anibogwu et al., 2021). *A. vulgaris* has been proven to show effects as an antioxidant (Budiana et al., 2017a; Febrina et al., 2017), hypolipidemic (Khan & Khan, 2015), hepatoprotective and cytotoxic (Erel et al., 2011), antibacterial (Sari et al., 2021), estrogenic, antispasmolytic, and analgesic (Obistioiu et al., 2014; Pandey et al., 2021). The *A. vulgaris* type is less explored because it contains lower levels of bioactive compounds compared to other types of Artemisia (Ekiert et al., 2020). However, the use of natural ingredients can be used as a preliminary study and developed into natural medicinal preparations.

A. vulgaris contains bioactive compounds such as flavonoids, phenolics (Dhifallah, 2022), essential oils (Bora & Sharma, 2011; Judzentiene & Garjonyte, 2016; Obistioiu et al., 2014), sterols, carotenoids and coumarins (Trifan et al., 2022). Flavonoids are one of the most studied types of secondary metabolites and provide various biological activities such as anti-inflammatory, anticancer, and antioxidant (Reungoat et al., 2021). The content of secondary metabolites such as flavonoids obtained can be influenced by several factors, such as extraction method, type of solvent, particle size, and length of extraction time (Syamsul et al., 2019). It has been asserted in the research that was reported by Malau et al., (2021); Tiwari et al., (2023) and Xiao et al., (2014) that the flavonoid content in *A. vulgaris* and *A. lactiflora* can be affected by the concentration of ethanol dissolution and the extraction time. There is still no research on how the particle size and maceration time of a 70% ethanol extract of *A. vulgaris* affect the total amount of flavonoids. This research was conducted to evaluate the effect of variations in the particle size of simplicia powder and the maceration time of a 70% ethanol extract of *A. vulgaris* on the total flavonoid content as a candidate for traditional medicine.

2. Methods

2.1 Preparation of simplicia

A. vulgaris L. leaves were obtained from CV. Merapi Farma Herbal, Sleman, Yogyakarta. Dried *A. vulgaris* leaves were cut into small pieces, crushed, and sieved at 40 and 80 mesh to ensure they were passed the 40 mesh sieve but not the 80 mesh sieve, but also produced powder that was passed the 80 mesh sieve.

2.2 Preparation of extracts

The sample powder was sieved and found to weigh 200 g. After undergoing the particle size variation treatment, it was extracted with 500 mL of 70% ethanol solvent at a ratio of 1:2.5. The sample powder was macerated at 25°C for 12 and 36 hours before being filtered using a Buchner funnel. The filtrate obtained from the maceration results is evaporated until a thick extract is achieved. The yield % is determined by weighing the thick extract and applying the formula (Rahmawati et al., 2022).

$$\%\,\text{Yield} = \frac{\text{Extract weight (g)}}{\text{Sample powder weight (g)}} \times 100\% \quad (1)$$

2.3 Phytochemical screening

Secondary metabolite compounds can be identified qualitatively, using the phytochemical screening method. Flavonoids, phenolics, alkaloids, tannins, saponins, and steroids are among the secondary metabolite compounds often found in plants.

2.3.1 Flavonoids

2 mL of 70% ethanol extract of *A. vulgaris* leaves were heated for 5 minutes; 0.2 g magnesium and 5 drops of concentrated H_2SO_4 are added. A positive result for flavonoids is obtained when the color changes from yellow to red (Leswara et al., 2024).

2.3.2 Phenolic

A total of 2 mL of 70% ethanol extract from *A. vulgaris* leaves was reacted with 2 drops of 5% $FeCl_3$. The color change should be observed; if a blackish-green color was formed, the presence of phenolic compounds in the extract was indicated. This reaction is characteristic of phenolic compounds, known for their antioxidant properties and potential health benefits (Leswara et al., 2024).

2.3.3 Alkaloids

5 mL of 70% ethanol extract of *A. vulgaris* leaves was taken, 5 mL of 2 N HCl was added, then it was divided into 2 in a test tube. 3 drops of Dragendoff's reagent were added to the first tube, then 3 drops of Mayer's reagent were added to the second tube, and 3 drops of Wagner's reagent were added to the third tube. If an orange precipitate was formed in the first test tube, in the second test tube a white to yellow precipitate was formed, and in the third test tube a brown precipitate was formed, this indicates a positive alkaloid (Leswara et al., 2024).

2.3.4 Tannin

According to Ikalinus et al., (2015), the presence of a white precipitate was indicated by adding 1% gelatin solution after 2 mL of 70% ethanol *A. vulgaris* extract reacts with 10 mL of 10% NaCl solution.

2.3.5 Saponins

According to Munadi & Arifin, (2022), a positive saponin result is indicated when the foam is occurred in the solution for at least 30 seconds after combining 10 mL of distilled water with 2 mL of 70% ethanol extracted from *A. vulgaris* and shaking for approximately 30 seconds.

2.3.6 Steroids

One gram of extract was weighed, 2 mL of chloroform was added, and the mixture was agitated. The resulting filtrate was mixed with two drops of chloroform and one drop of concentrated H_2SO4. Triterpenoid molecules were indicated by a red or purple color shift, while steroid compounds are indicated by a blue or green color change (Sari et al., 2021).

2.4 Determination of Total Flavonoid Content (TFC)

The total flavonoid extract levels in each treatment were found using the Chang method with UV-Vis spectrophotometry and quercetin as a standard. Subsequently, the results obtained were expressed in g QE/mg (Budiana et al., 2017b; Chia-Chi et al., 2002).

2.4.1 Preparation of quercetin standard solution (1000 ppm)

After 50.0 mg of quercetin was measured, it was dissolved in ethanol p.a to the limit line in a 50.0 mL volumetric flask. The mixture was shaken until homogenous.

2.4.2 Determination of the optimum wavelength of quercetin

The maximum wavelength was determined by using a standard concentration of quercetin of 60 ppm, 1 mL was taken and placed in a 10 mL measuring flask. In the measuring flask, 1.0 mL of 10% $AlCl_3$ and 5% CH_3COOH were added to the limit line. The absorbance at λ 350-500 nm was then read (Nisaa et al., 2023).

2.4.3 Determination of the operating time (OT) of quercetin

A standard solution of 60 ppm was created from the standard 1000 ppm of quercetin. 1 mL of 60 ppm standard solution was placed in a 10 mL measuring flask, and then topped off with 1.0 mL of 10% aluminum chloride ($AlCl_3$) and 5% acetic acid (CH_3COOH). The absorption between 0 and 60 minutes was then measured at the highest wavelength achieved (Nisaa et al., 2023).

2.4.4 Standard curve for quercetin

A six concentration series: 30, 45, 60, 75, 90, and 105 ppm in ethanol p.a was created using a 1000 ppm quercetin standard solution. Each solution was added, 1 mL of 10% $AlCl_3$ and 5% CH_3COOH, to a 10.0 mL volumetric flask and filled. The absorbance at quercetin's optimal λ was measured after incubation with OT.

2.4.5 Determination of total flavonoid content of *A. vulgaris* leaf extract

A stock solution of 1000 ppm extract was prepared for each treatment by weighing 100.0 mg of extract in a 10.0 mL measuring flask and adding ethanol solvent p.a. After that, 1 mL was transferred to a 10-mL measuring flask and the limit line was filled with 1.0 mL of 10% $AlCl_3$ and 5% CH_3COOH. Incubation was done during OT and absorbance was measured at the appropriate

wavelength (Nisaa et al., 2023). Three replications of the extraction were performed, and Total Flavonoid Content (TFC) was calculated using the Chang method formula.

$$TFC = \frac{C \times V \times Fp}{W} \times 100\% \quad (2)$$

3. Results and Discussion

3.1 Sample preparation

A. vulgaris L. leaves were obtained from CV. Merapi Farma Herbal, Sleman, Yogyakarta had previously determined to ensure that the leaves taken were *A. vulgaris* L leaves. The determination was carried out at Merapi Farma Herbal Yogyakarta with the number SK-SMPL/MFH/VII/2024. Based on the results of the determination, it was found that the sample used is Baru Cina leaves with the Latin name *A. vugaris*. Fresh leaves were dried using direct sunlight and covered with black cloth for five days. After that, the water content is checked using Moisture Balance. If the air content exceeds 10%, proceed with drying in an oven set to 40^{o}C to obtain a moisture content of 5.8%. In this study, the water content of *A. vulgaris* leaf simplicia was 5.80%, which meets the Ministry of Health's guideline for a water content of less than 10%. The dry material is ground into powder and sieved through mesh numbers 40 and 80. The goal of powdered simplicia is to increase the contact surface area between the sample and the solvent, speeding up the extraction process by reducing the solute diffusion distance. The sieving technique ensures that the size of the simplicia particles employed in the extraction process is consistent (Asworo & Widwiastuti, 2023). A finer simplicia particle size can improve extraction efficiency, but if the particles are too small, the likelihood of contaminants in the extract increases (Illing et al., 2023). In this study, the maceration method was used for extraction. The maceration procedure creates a pressure difference between the interior and outside of the simplicia cell, causing the cell wall and membrane to rupture. This allows the active chemicals in the cytoplasm to be removed and dissolved in the solvent utilized (Handoyo, 2020).

70% ethanol solvent was used because 70% ethanol has good solubility for many bioactive compounds, including flavonoids. Ethanol is a polar solvent. Therefore, it can dissolve polyphenolic substances like flavonoids, known to dissolve in polar solvents. A 70% ethanol solvent was used to sift the dried leaves through 40 and 80 mesh sieves, with each sieve number macerating for 12 and 36 hours. The yield percentage value was calculated by comparing the thick extract results to the beginning weight of the simplicia, as shown in Table 1.

Table 1. Results of extraction of *A. vulgaris* leaves

Sieve Number	Maceration Time (hours)	%Yield
40	12	1.580
40	36	1.190
80	12	2.975
80	36	1.895

Table 1 shows that the average yield value of *A. vulgaris* leaf extract increases with increasing particle size and maceration time. This is consistent with the hypothesis that the smaller the particle size, the higher the surface area available for interaction with the solvent. The extraction procedure involves transferring active chemicals from the cell to the solvent. greater active chemicals can be recovered from smaller particles because the greater surface area is exposed to the solvent (Gil-Martín et al., 2022). However, the yield fell at a particle size of 80 mesh and a maceration time of 36 hours. This can be produced by over-saturation during extraction. After 36 hours of extraction, the solvent may approach a saturation point (over-saturation) with the active molecule, which means that the solvent can no longer dissolve new compounds. When the solvent reaches saturation, extending the extraction time will not increase the amount of substance removed and may reduce extraction efficiency, lowering yield. Smaller particle size can increase extraction, however excessive extraction time can reduce extraction efficiency.

3.2 Phytochemical screening

The phytochemical screening of a 70% ethanol extract of *A. vulgaris* in each treatment yielded positive results containing flavonoids, phenolics, alkaloids, tannins, saponins, and steroids, as illustrated in Table 2.

Table 2. Phytochemical screening of 70% ethanol extract of *A. vulgaris*

Compound	Reagent	Results	Description
Flavonoids	H_2SO_4 p	Yellow Brown Color	+
Phenolic	$FeCl_3$ 5%	Blackish Green Color	+
Alkaloids	Mayer	Yellowish White Precipitate	+
	Dragendorff	Red Precipitate	+
	Wagner	Brown Precipitate	+
Tannin	NaCl + Gelatin	Yellowish Precipitate	+
Saponin	Aquadest + HCl 1 N	Foam	+
Steroid	Chloroform + H_2SO_4	Red Ring	+

Note: (+) positive containing secondary metabolites

An interaction between concentrated sulfuric acid ($H_2\ SO_4$) and flavonoids is possible due to their hydroxyl group (-OH). Depending on the kind of flavonoid, adding sulfuric acid to flavonoid molecules causes a chromogenic reaction that can result in a yellow, purple, or red color. Sulfuric acid is involved in the dehydration of flavonoids and forms a compound with them, responsible for their color. For example, sulfuric acid can degrade flavonoids by releasing air molecules, which can alter their structure into more stable compounds and give them certain colors.

The reaction between tannin, NaCl, and gelatin produces precipitation resulting from the formation of a complex between tannin and protein in gelatin. The reaction between steroids, sulfuric acid ($H_2\ SO_4$), and chloroform can be used in a qualitative test to detect the presence of steroids in samples. This reaction is known as the Salkowski test. Sulfuric acid can oxidize sterols in the sample to form complex compounds, causing a change in the red ring in the chloroform layer.

3.3 Determination of the maximum wavelength and OT of quercetin

In this study, the maximum wavelength was determined in the range of 0–60 minutes, and a maximum wavelength of 414 nm was obtained. The optimum wavelength is determined when a complex between quercetin – $AlCl_3$ occurs, which provides optimum absorption. Measurement at the maximum wavelength allows for the largest change in absorbance for each unit level so as to minimize measurement errors and increase the accuracy of results when replication or repeated measurements are carried out (Suharyanto & Prima, 2020). Meanwhile, the operating time (OT) of quercetin was determined at a concentration of 60 ppm using the optimum wavelength obtained, namely 414 nm. The measurement data show that the operating time (OT) starts from 42 to 47 minutes, and an absorbance value of 0.397 is obtained. Similar results were also found in research by Pujiastuti & Ma'rifah (2022), where the operating time for quercetin was 40 minutes. Determining the operating time aims to determine the time at which absorbance reaches maximum stability, which indicates that the reaction between compounds has reached an equilibrium point. This is important to minimize errors in measurement (Suharyanto & Prima, 2020).

Several concentration series of 1000 ppm quercetin stock curves created a quercetin standard curve, including 30, 45, 60, 75, 90, and 105 ppm. Next, in each concentration series, the absorbance was measured at a wavelength of 414 nm, and measurements were carried out in the OT range of 42–47 minutes. The absorbance obtained from each concentration series can be seen in Table 3.

Table 3. Standard curve series of quercetin

Concentration (ppm)	Absorbance			Absorbance Average
	1	2	3	
30	0.189	0.182	0.185	0.185
45	0.263	0.265	0.263	0.264
60	0.339	0.338	0.347	0.341
75	0.469	0.452	0.452	0.458
90	0.571	0.538	0.550	0.553
105	0.656	0.670	0.667	0.664

From the results of absorbance data analysis at various concentrations, a linear regression equation y=0.0064x–0.0236 was obtained with a correlation coefficient (r) of 0.9973. An r value that is greater than the r table (0.8783) indicates a linear relationship between concentration and absorbance. The higher the standard concentration of quercetin, the higher the absorption value. The choice of concentration is based on the Lambert-Beer law which states that the absorption requirement is 0.2 to 0.8. The resulting standard curve will be a straight line if the Lambert-Beer law is fulfilled (Grace et al., 2015). This equation is valid and can be used to determine the ethanol 70% total flavonoid content of *A. vulgaris* leaf extract in each treatment. The calibration curve graph is shown in Figure 1.

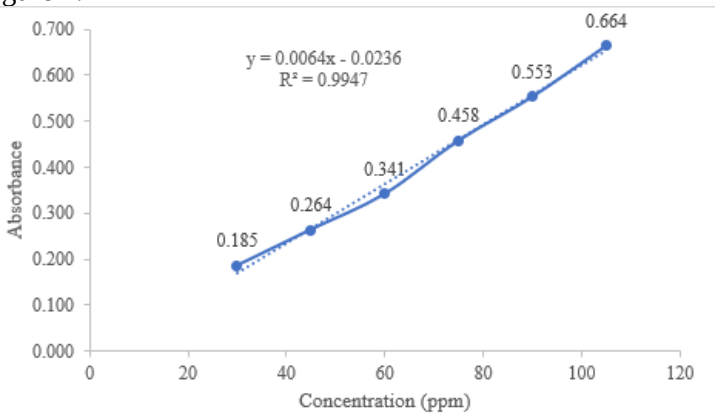

Figure 1. Calibration curve of quercetin

3.4 Determination of flavonoid content of 70% ethanol extract of *A. vulgaris* leaves

The total flavonoid content of a 70% ethanol extract of *A. vulgaris* leaves was quantified using the UV-Vis spectrophotometric technique at a wavelength of 414 nm. The samples were then incubated for 42–47 minutes, depending on the results. The total flavonoid concentrations were determined using the derived calibration curve. The test results indicated that the average total flavonoid content for sieve number 40 with a maceration duration of 12 hours was x ± LE = 72.073±1.126 mgQE/g extract, for sieve number 40 with a maceration duration of 36 hours was x ± LE = 65.645±0.229 mgQE/g extract, for sieve number 80 with a maceration duration of 12 hours was x ± LE = 69.70±0.278 mgQE/g extract, and for sieve number 80 with a maceration duration of 36 hours was x ± LE = 70.169±0.480 mgQE/g extract. The assay results are presented in Table 4.

Table 4. Determination of total flavonoid content

Particle Size (mesh)	Maceration Time (hours)	
	12 (mgQE/g extract)	36 (mgQE/g extract)
40	72.073±1.126	65.645±0.229
80	69.70±0.278	70.169±0.480

The average amount of flavonoids in *A. vulgaris* leaves was highest in the 40 mesh particle size treatment, which was macerated for 12 hours and had a value of 72.073±1.126 mgQE/g extract (Table 4). A larger particle size can provide good permeability for solvents, which allows flavonoid compounds to dissolve more efficiently without the risk of dissolved impurities. Finer particles, specifically those with an 80 mesh particle size, offer a greater surface area. However, they often dissolve impurity compounds alongside active compounds, potentially lowering the quality of the extract. In addition, in very fine particles, apart from flavonoids, other compounds such as salts or proteins can also dissolve, which can reduce the levels of pure flavonoids that will be obtained (Hijaz et al., 2020).

4. Conclusion

The conclusions of the study indicate that secondary metabolites, including flavonoids, phenolics, tannins, alkaloids, saponins, and steroids, are present in 70% ethanol extract of *A. vulgaris* leaves. The most effective method for extracting secondary metabolites from *A. vulgaris* leaves is the treatment that involves a particle size of 40 mesh and a maceration duration of 12 hours with a total flavonoid content of $\bar{x}$ ± LE = 72.073±1.126 mgQE/g extract.

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