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The Effect of Maceration and Soxhletation Extraction Methods on The Flavonoid Concentration of Anting-anting Leaves Extracts (*Acalypha indica* L.) Using Uv-Vis Spectrophotometry

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ABSTRACT

Anting-anting (Acalypha indica L) is an annual plant that reaches 30 to 50 cm in height. This plant is often found on mountain slopes, along roadsides, and meadows. Anting-anting leaves (Acalypha indica L) have anti-inflammatory, antibacterial, and antifungal activities. The high content of flavonoids in the leaves of the earrings is efficacious as an antioxidant. This study aims to determine the effect of maceration and soxhletation extraction methods on the levels of flavonoids in the leaves of the anting-anting. This type of research is an experimental study conducted with two extraction methods using 96% ethanol. Qualitative analysis was carried out by color reaction test while quantitative analysis was carried out using a UV-Vis spectrophotometer with the calorimetry method. The standard solution used was quercetin to measure the levels of flavonoids. The results showed that the average concentration of flavonoids in soxhletation extraction was 5.2266% w/b and in maceration extraction was 5.0084% w/b. The data were tested using the Independent Sample T Test showing a significance value of 0.097 (> 0.05), meaning that there was no significant effect between the maceration and soxhletation extraction methods.

Keywords: Anting-Anting Leaves, Levels Flavonoids, Maceration, Soxhletation.

INTRODUCTION

Around 13,000 plant species in the world have been used as traditional medicines. In written medical science, around 20,000 species of medicinal plants are known to the public. Apart from that, it is estimated that 70,000 types of plants can most likely be processed and developed as medicinal ingredients. Empirically, the anting-anting plant (*Acalypha indica* L.) is widely used for traditional therapy. This plant is believed to be able to treat certain diseases, for example, coughs, nosebleeds, diarrhea, dysentery, external wounds, vomiting blood and bleeding (Dalimartha, 2003).

This plant has high potential for treatment as an anti-inflammatory, antibacterial, anti-diabetic, and anti-cancer (Chekuri *et al*., 2020). In Susanti's (2018) research, it was found that beneficial chemicals found in earrings include flavonoids, saponins, tannins, and alkaloids. The results of the phytochemical screening carried out by Chekuri (2020) showed that the anting-anting leaf extract contained tannin, saponin and essential oil compounds. Apart from that, the anting-anting leaves also found the chemical compounds triacetonamide, aurantiamide, acaindinin, ferulic acid, corilagin and resin. In Susanti's (2018) research, it was found that beneficial chemicals found in earrings include flavonoids, saponins, tannins and alkaloids. Meanwhile, the ethanol extract of Acalypha indica leaves contains flavonoids, steroids, saponins, alkaloids and aleurone (Handayani *et al*., 2018).

The flavonoid content in plants has anti-cancer, antihistamine, antiviral, antibacterial, and antioxidant effects (Alam *et al*., 2017), the effective flavonoid in the magenta purslane gel

preparation against the bacteria (Puradewa&Amelia, 2022) *Propionibacterium acnes*. Antioxidants in flavonoids have a mechanism of action by inhibiting the enzymes glutathione S-transferase, mitochondrial succinoxidase, monooxygenase, and NADH oxidase which are important for forming Reactive Oxygen Species. Antioxidants can absorb ROS and protect antioxidant defenses (Kumar and Pandey, 2013). Flavonoids has function as antioxidants and plays a role in reducing ROS levels so that the inflammation stage occurs quickly (Sari *et al*., 2023). The antioxidant properties of flavonoids play role in wound healing activity (Budiawan *et al*., 2023). Flavonoid also known for its antioxidant activity in sunscreen effect (Cahyani *et al*., 2022).

This research was conducted to compare the flavonoid content in anting-anting leaf extract using the maceration and soxhletation extraction methods. In research conducted by Saadah *et al* (2017), it was stated that the flavonoid content of Dayak onion bulbs in maceration extraction was greater than using soxhletation extraction, namely in maceration the flavonoid content was 1.09%, while in soxhletation it was 0.81%. The results of this study are inversely proportional to the research of Ramayani (2021) which stated that the highest levels of flavonoids from taro leaf extract (*Colocasia esculenta* L) were found in soxhletation extraction. Therefore, it is necessary to optimize the extraction method to determine the highest flavonoid content in anting-anting leaf extract.

METHODS

A. Tools and Material

The tools used in this research were a socket set, maceration container, funnel, analytical balance, beaker, stirrer rod, test tube, dropper pipette, micropipette, filter paper, measuring cup, Erlenmayer, UV-Vis spectrophotometer, rotary evaporator, and cuvette. The materials needed in this research are fresh anting-anting leaves obtained in Madiun, distilled water, 96% ethanol, ethanol p.a, quercetin comparison standard, Mg powder, $AlCl_3$, concentrated HCl, concentrated H_2SO_4, 1% $FeCl_3$, Dragendrof, Bouchardat, and Mayer.

B. Extract Preparation

Fresh green earring leaves are washed thoroughly and then dried in the oven for around 2 days to 4 days at a temperature of 35-40 °C. Imawati *et al* (2023) showed that the drying process affects the content of chemical compounds in plants. After that, the simplicia is powdered by blending until smooth. The maceration method is carried out with a ratio of extract powder and 96% ethanol solvent, namely 1:8. A total of 80 g of powder was soaked in 640 ml of 96% ethanol in a maceration container. The container is stored for approximately 4 days in a place protected from sunlight and shade, tightly closed, and stirred occasionally. To get a perfect filtrate, remaceration is carried out twice until the filtrate is clear. The soxhletation method is carried out by weighing 10 g of Simplicia each, coating it using filter paper, and then placing it in a soxhletation tube and adding 200 ml of 96% ethanol solvent to wet the sample. The ratio of extract and solvent using the soxhletation method is 1:20. The results of each maceration and soxhletation extraction were thickened using a rotary evaporator and the yield of the extract was calculated.

C. Qualitative and Quantitative Test

To determine the chemical content contained in anting-anting leaf extract, a qualitative test was carried out in the form of a flavonoid test, saponin test, tannin test, alkaloid test, and steroid test. Quantitative testing of flavonoids in the extract was carried out by weighing 50 mg each of the extract resulting from maceration and soxhletation, then dissolving it using 15 ml of ethanol in a glass beaker using a sonicator. After that, put it in a 25 ml measuring flask and add ethanol to the mark. Filter the solution with a filter holder.

2 ml of each maceration and soxhletation solution was pipetted and put into a 10 ml volumetric flask, then ethanol was added until the mark. Determining the flavonoid content of anting-anting leaf extract using UV-vis spectrophotometry. 2 ml of each sample solution resulting from maceration and soxhletation was pipetted. Add 0.1 ml AlCl3 and 0.1 ml potassium acetate, 2.8 ml distilled water, and 1.5 ml ethanol p.a. Leave for 30 minutes. The absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 424.6 nm. Replicate the sample solution 3 times (Sa'adah *et al*., 2017). After that, enter the average absorbance (y value) and the equivalent of mg quercetin per 100 mg sample (x value) into the standard curve equation.

D. Data Analysis

This research uses the SPSS application to analyze data. The data obtained is quantitative data which will be analyzed using the Independent Sample T Test method to determine differences in the levels of flavonoids in anting-anting leaves from the two extraction methods. The initial steps taken before the difference test are the normality test and homogeneity test. Data is declared normally distributed if the significance value is >0,05. Homogeneous data also shows a significance value of >0,05.

RESULTS AND DISCUSSION

Anting-anting (*Acalypha indica* L) leaves, which were still fresh green, were collected and weighed 1,433 grams, then dried in an oven and obtained a dry weight of 162 grams. Drying with the help of an oven at 40°C can maintain the active substance content in simplicia (Cholapandian *et al*., 2013). Dried simplicia that has been ground can be extracted. The extraction of anting-anting leaf simplicia (*Acalypha indica* L) was carried out using maceration method. Research by Indriasari (2022) confirmed that the extracted using the soxhletation method to obtain a thick extract of n-hexane, ethyl acetate, and 96% ethanol as much as 0.61g; 1.23g; 2.22 g so that the yield is 2.3%;

4.1%; 7.4%.

Table 1. Yield of Anting-Anting (*Acalypha indica* L) Leave Extract

Simplicia	Powder weight (grams)	Extract weight (grams)	Yield (%)
Maceration	80	9,26	11,575
Soxhletation	40	7,11	17,775

Qualitative analysis was carried out on anting-anting leaf extract (*Acalypha indica* L) to determine several chemical compounds contained in it. Qualitative analysis carried out was the flavonoid test, tannin test, saponin test, steroid test, and alkaloid test. In this analysis, several reagents are used to determine color changes in the sample which are indicators of the chemical compound. Anting-anting leaf extract showed positive results in tannin, saponin, and steroid tests. However, it did not show positive results in the terpenoid and alkaloid tests.

Table 2. Table of Qualitative Test Result on Maceration Anting-Anting (*Acalypha indica L*) Leaves Extract

Compound class	Acalypha Indica Extract
Flavonoid	+
Saponin	+
Alkaloid	-
Tanin	+
Steroid	+
Terpenoid	-

This research aims to determine the effect of maceration and soxhletation extraction methods on the flavonoid content of anting-anting leaf extract (*Acaplypha indica* L) using a UV-Vis spectrophotometer. The principle used is determining flavonoid levels colorimetrically using an aluminum chloride reagent. The principle of the colorimetric method is the reaction between aluminum chloride and the keto and hydroxy groups of Flavonoids to form a complex. Meanwhile, potassium acetate aims as a stabilizer.

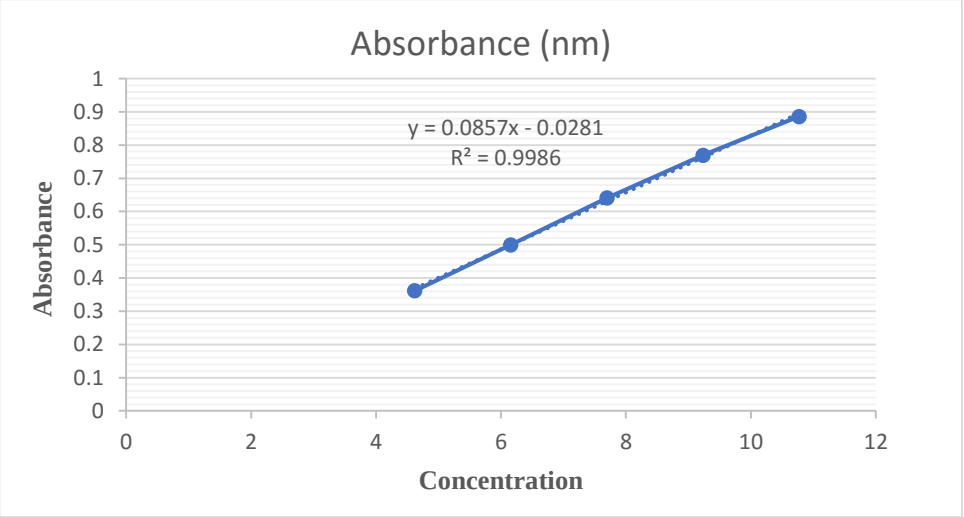

Figure 1. Quercetin Standard Curve Equation

To determine flavonoid levels, it is necessary to first measure absorbance in a standard solution, namely quercetin as a comparison. The standard solution of quercetin has a dark yellow color indicating a greater concentration. The measurement of absorption at the maximum wave is 424.6 nm using a UV-Vis spectrophotometer. Linearity values are used correlation coefficient r in linear regression analysis y = ax+b (Indriasari, 2021). The absorbance obtained is then entered into the standard curve equation y=ax+b. The y value is absorbance and the x value is concentration.

Based on the standard curve graph above, the linear regression equation obtained is y = 0.0.0857x – 0.0281 with an r value (correlation coefficient) of 0.9986. The correlation coefficient value indicates the existence of a strong or weak relationship between the variables in the study. From this graph, we get linear results between concentration and absorbance in the standard quercetin solution. A correlation coefficient (r) value close to 1 indicates a strong level of trust (Winahyu, 2019). Flavonoid levels in anting-anting (*Acalypha indica* L) leaf extract can be calculated using a linear regression equation.

Table 3. Table of Results of Determination of Flavonoid Content of Anting-Anting (*Acalypha indica* L) Leaves Extract

Method	Replication	Absorbance Average	Flavonoid Content (w/w%)	Flavonoid Content Average (w/w%)
Maceration	1	0,5023	5,0284	5,0084
	2	0,4992	4,9990	
	3	0,4991	4,9980	
Soxhletation	1	0,5393	5,3792	5,2266
	2	0,5271	5,2636	
	3	0,5032	5,0370	

The data above is the result of research using a UV-Vis spectrophotometer which shows the largest average absorbance, namely the soxhletation method. Based on calculations, it was found that the average flavonoid content in the maceration extraction method was smaller than in soxhletation extraction. Prasetyo *et al* (2022) showed that the flavonoid total content level from soxhletation was 9,3106% and from maceration was 6,2756%. The flavonoid levels in soxhletation extraction are more optimal, possibly influenced by the temperature during the extraction process. The greater yield in soxhletation also causes more flavonoids to be extracted from maceration. Soxhletation uses indirect heating which causes the solvent to easily extract secondary metabolite compounds that are insoluble at room temperature. With indirect heating, volatile compounds are not easily degraded. Apart from that, the extraction process takes place repeatedly using new solvents so that the extract can be completely extracted. Flavonoid levels in the maceration method also did not differ much although they were greater in soxhletation. This condition occurs because remaceration is used where the maceration is repeated twice (Wahyuni & Guswandi, 2014). The maceration method at room temperature allows compounds that are easily damaged by heat to not be damaged. Regular stirring aims to ensure that the compounds contained in the bottom maceration powder can be penetrated evenly by the solvent (Rosita, 2017).

From the data above, it is necessary to carry out different tests to determine the effect of the two extraction methods. The initial steps taken were the normality test and the homogeneity test. The normality test shows a significance value of 0.55 for maceration and a significance value of 0.646 for soxhletation. From these results, it can be stated that the data is normally distributed (>0.05). The homogeneity test also shows that the data is homogeneous with a significance value of 0.07 (>0.05). Because the data is normally distributed and homogeneous, the difference test that can be carried out is the independent sample t-test. The results of the difference test show a significance value of 0.097 (>0.05), so it is stated that there is no significant influence from the two extraction methods. However, the highest flavonoid levels were found in the soxhletation extraction method. Similar results were shown by Ramayani (2021) in his research that soxhletation extraction of taro leaves (*Colocasia esculenta* L) had the highest levels of flavonoids.

CONCLUSION

From the results of this research, it was concluded that the maceration and soxhletation extraction methods did not have a significant effect on the levels of anting-anting leaf extract (*Acalypha indica* L). The flavonoid concentration in maceration extraction was 5.0084% w/w,

while in soxhletation extraction it was 5.2266% w/w.

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