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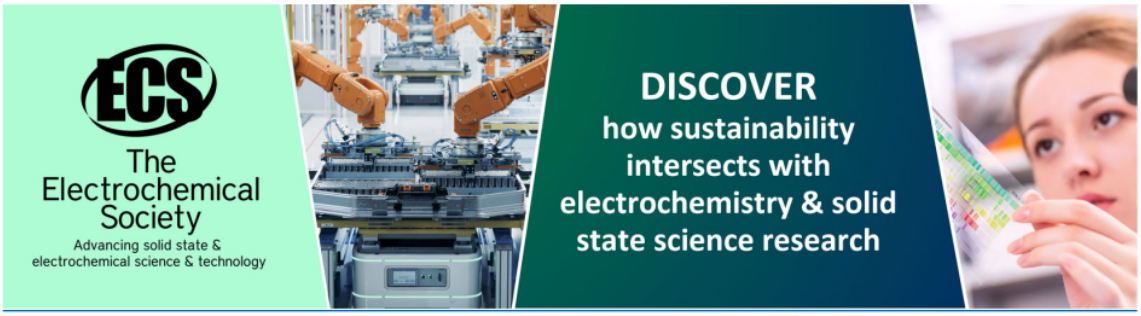

Effect of Boiling Time on Content of The Total Flavonoid of Kitolod (*Isotoma longiflora* (L.) C. Presl.)

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Abstract. Kitolod (*Isotoma longiflora* (L) C. Persl) is a annual herbaceous plant that usually grows on the edge of a moist wall. The leaves of the kitolod plant can be used as medicine for healing wounds, asthma, bronchitis, rheumatism and so on. Kitolod contains chemical compounds namely alkaloids namely lobelamin, isotomine and lobelin. In the leaf part of kitolod contains chemical compounds namely alkaloids, saponins, polyphenols and flavonoids. This study aims to determine the effect of boiling time on the total flavonoid content of water extract of kitolod leaves. Extraction was done by boiling, the determination of the total amount of flavonoids was carried out by the $AlCl_3$ method and then the absorption was measured using Spectrophotometry UV-Vis with a wavelength of 431 nm. The results showed that qualitatively the samples contained flavonoids by showing red. Quantitatively the kitolod leaves are boiled with longer time, the total flavonoid content will decrease. The total flavonoid content of kitolod leaves in boiling 5 minutes obtained an average level of 0.31995% w/w or 31.9 mg/kg, boiling 10 minutes 0.25635% w/w or 25.6 mg/kg, boiling 15 minutes 0.2239% w/w or 22.3 mg/kg, boiling 20 minutes 0.17254% w/w or 17.2 mg/kg.

1. Introduction

Medicinal plants and traditional medicines are an asset that needs to be constantly explored, one of the plants that has the potential to become a medicinal plant. The leaves of the kitolod plant can be used as a remedy for wound healing, asthma, bronchitis, rheumatism and so on[6]. Kitolod plants (*Isotoma longiflora* (L) C. Persl) contain chemical compounds including alkaloid compounds namely lobelamin, isotomin and lobelin. Kitodod plants contain other metabolite compounds such as phenol, terpenoids and steroids [4]. In addition, kitolod plants also contain flavonoid compounds [8].

Regular reductions are made by the community to increase acceptance of traditional food and medicine. Heat processes such as decomposition cause changes in the structural and cellular matrix interactions that have a negative and positive effect on the phytochemical content. The heat treatment of food and traceric drugs usually has a destructive effect on flavonoid and phenolic compounds as they are both unstable components. It is thought that boiling can affect the levels of flavonoids in the leaves of the kitolod [7]

Flavonoids are secondary metabolite compounds that are formed through the ammonia pathway. This compound is manufactured from sinnamoil-CoA units by extension the chain using 3 malonil-CoA. The khalkhon synthase enzyme combines this compound into the khalkon. Khalkon is a precursor of flavonoid derivatives in many plants.

Flavonoid levels can be set by different methods, each analytical method having different levels of advantage. One of the methods used is the UV-Vis Spectrophotometry method. Some of the factors underlying this selection are speed, accuracy, sensitivity, accuracy, selectivity and practicality. The UV-Vis spectrophotometry method is used to determine consentration of the compounds. UV-Vis spectrophotometers are instruments that combine wavelengths and frequencies of absorption intensity (transmission or absorbance) and are expressed in the form of a spectrum or absorption band. The formation of absorption bands is due to the excitation of more than one type of electron in a very complex group of molecules [2].

2. Research Methods

2.1. Tools and Materials

Tools used in this study include a set of glass appliances (Pyrex), UV-Vis Spectrophotometer 1800 (Shimadzu), electric scales (Ohaus), quartz, vortex, incubators, mixing sticks, electric stoves.
Materials used in this study were: Kitolod leaves (*Isotoma longiflora* (L.) C. Presl.) Dried to 200 grams, technical methanol solvents, methanol *pa*, ethanol *pa*, quercetine, aquadest, $AlCl_3$ 10% solution, 1 M potassium acetate, HCl concentrated, Mg.

2.2. Determined plant

Determination is made to determine the identity of the plant material used in the research, namely kitolod leaves obtained from the villagers located in East Talang, Talang Village, Bayat District, Klaten District.

2.3. Powder simplisia making

Fresh kitolod leaves of 4000 g were washed with running water and then rinsed, then dried under sunlight covered with black cloth. Dried kitolod leaves are reduced in [1]

2.4. Extract Making

Kitolod leaves water extract is made by means of boiling with varying degrees of boiling time. 50 grams of simplified powdered kitolod leaves were added to 250 mL boiled boil until boiling time was 5 minutes, 10 minutes, 15 minutes and 20 minutes while stirring occasionally. The infusion results are filtered hot and squeezed [5]
The extracts obtained are calculated by means of:
Randemen : (weight of the extract) / (weight of simplisia) x 100%

2.5. Characterization of organoleptic extract characteristics

The organoleptic parameters of the extract are descriptive, color, taste and smell.

2.6. Flavonoid Qualitative Test

The extracts were extracted 1 ml in a reaction tube and heated on a flame of flame, and then dissolved in 1 ml of 70% ethanol, followed by a small amount of Mg metal and 3–5 drops of concentrated HCl. Positive results when formed in red, orange or yellow solution indicate the presence of flavonoids.

2.7. Flavonoid Quantitative Test

2.7.1. Determination of Maximum Wave Length

10,0 mg quercetin was dissolved in 100 ml of methanol (1000 ppm concentration) as a stock solution. 1 ml of quercetin solution (quartz) was diluted with 2 ml of methanol and then 10% 1 mL aluminum (III) chloride was added. After waiting for 30 minutes, the absorbance of the comparison solution was

measured with a UV-visible spectrophotometer at 380 nm – 780 nm. Created a calibration curve and obtained linear regression [2]

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2.7.2. Operating time

The assay was performed by mixing 1.0 ml of quercetine solution with methanol to a volume of 5.0 ml in a squash, the mixture was shaken and kept for 5 minutes. The solution was measured at a 431.0 nm wavelength with a 45 minute stabilization time [9].

2.7.3. The production of a standard curve of quercetine

A total of 10 mg of quercetin was dissolved in 100 ml of methanol as a stock solution. The solution of the quercetine was made at a concentration of 10, 20, 30, 40, 50 ppm. A total of 1 ml of querecetin solution of each concentration was added with 1 ml of aluminum (III) chloride 10%, 1 ml Potassium acetate 1 M and added aquadest to 10 ml. For 15 minutes, the absorbance was readed using a UV-Vis Spectrophotometer at 431 nm [2]

2.7.4. Total Flavonoid quantitative test

A total of 5,0 grams of the extract sample was dissolved in methanol at a concentration of 500 ppm. 1 ml of the extracts were added with 2 ml of methanol, then 1 ml of 10% aluminum (III) chloride, 1 ml of 1 M sodium acetate, and aquadest to 10 ml in volume. After incubation for 30 minutes, the absorbance of the comparison solution was measured with a spectrophotometer at the measured wavelengths (431,0 nm). It is then calculated using linear regression equations of the quercetine calibration curve which has also been measured previously [2].

$$y = b\,x + a$$

Description:

y = absorbance
a = intersep
b = slope
x = concentration

This assay was used to determine the levels of flavonoids in the kitolod leaves (*Isotoma longiflora* (L) C. Persl).

$$K = (C.V.FP) / BS$$

Results obtained are converted to%
K = Flavonoid level (%)
C = Concentration (ppm)
V = Sample volume (ml)
Fp = Dilution factor
BS = Sample weight (grams)

2.8. Data Analysis

Concentration series data made from standard quercetine are then made by the standard curve equation. The standard curve equation y = bx + a with the description y = absorbance, x = the rate in units of ppm (mg / ml). Absorbance of the obtained leaves extract was included in the standard curve equation to obtain the total flavonoid content of the kitolod leaves.

Statistical analyzes were performed using Anova, to determine dispersal normality and homogeneity were analyzed using Kolmogrov-Smirnov test. The obtained data are normal and uniform when the values are P> 0.05. To find out whether there is a significant difference or not, then LSD analysis is useful to determine the difference between the average difference between treatments.

3. Results and Discussion

3.1. Determined plant

The results of the plant determination indicate that this plant belongs to the family Lobeliaceae, a species (*Isotoma longiflora* (L.) C. Presel)

3.2. Extract Results

The extraction results of kitolod leaves (*Isotoma longiflora* (L.) C. Presel) were performed by varying the 5-minute, 10-minute, 15-minute, and 20-minute boiling method with aquadest solvent. 50 grams of dried kitolod leaves were obtained from 13.9 grams, 12.2 grams, 12.1 grams, 14.3 grams. The extract of kitolod leaves (*Isotoma longiflora* (L.) C. Presel) obtained in dark brown and has an aromatic, bitter taste.

Table 1. Randemen Results

Treatment	Weight sample	Weight Extract (g)	Rendemen (%)
5 minutes	50	13.9	27.8 %
10 minutes	50	12.2	24.4 %
15 minutes	50	12.1	24.2 %
20 minutes	50	14.3	28.6 %

Data source: Primary Data, 2019

3.3. Flavonoid qualitative test results

Qualitative flavonoids identification of kitolod leaves (*Isotoma longiflora* (L.) C. Presel) positively by showing a yellow formed, when extract reacted with powder Mg and HCl concentrated . This is in line with the study conducted by Hapsari Atika [3] where at the time of addition of reagents appeared yellow indicating the presence of flavonoid compounds. This is because flavonoids include phenolic compounds, when the phenol is treated with the base it forms a color due to the conjugation system of the aromatic group.

3.4. Maximum Wave Length and Absorption

Table 2. Maximum Wave Length and Absorption

wavelength (nm)	Absorbance
431	0.7450

Data source: Primary Data, 2019

Setting the maximum wavelength for measurement at the maximum absorption wavelength will result in maximum absorption, at the maximum wavelength having maximum sensitivity due to the greatest absorbance change. The resultant maximum wavelength of the standard solution was 431.0 nm with an absorbance of 0.7450.

3.5. Operating Time (OT) setting

Operating time was performed with the aim of knowing the stable measurement time when the sample reacted perfectly to the color reagent, the stabilization time being 45 minutes.

3.6. *Standard Curve Making*

The results of the concentration and absorbance of the standard curve solution can be seen in table 3:

Table 3. Concentration of Standard Curve Solution

Concentration (ppm)	Absorbance
10	0.259
20	0.330
30	0.403
40	0.485
50	0.562

Data source: 2019 Primary Data

The concentration variation is used to detect differences in absorbance, where the higher the concentration of absorbance values the greater.

The production of a standard curve aims to obtain the standard solution of equilibrium in the determination of the sample rate, the relationship between the quercetin concentration and the absorption, the data obtained a correlation coefficient (r) of 0.9993 approaching 1 shows that the regression equation is linear.

Based on table 3 we obtain y = 0.0076x + 0.1795 with values of r = 0.9993 indicating that these values are linear.

2.8. Determination of Flavonoid Level

Determination of total flavonoid readings with absorbance at a maximum wavelength of 431 nm was obtained with total flavonoid content of kitolod leaves (*Isotoma longiflora* (L.) C. Presel) of 5 min incubation at an average rate of 0.31995% w /w or 31.9 mg / kg, 10 min infusion 0.25635% w / w or 25.6 mg / kg, 15 min 0.2239% w/w or 22.3 mg / kg, 20 min 0.17254% w/w or 17.2 mg / kg . Based on the observational data obtained test statistically using the One-Kolmogorov-Smirnov Test obtained 0.992> 0.05 (H_0 accepted). Then, with the one-way Anova test, homogeniity was found to be 0.504> 0.05 (H_0 accepted). From the Anova test data show that the sign of 0.000 <0.05 then H_0 is rejected, followed by LSD test. From the data between treatments it can be said that there is a significant difference between the treatments that is in each comparison of the time of reduction of flavonoid levels.

4. Conclusion

The longer the boiling time affects the total flavonoid content of the leaves kitolod (*Isotoma longiflora* (L.) C. Presl), the longer the decomposition of the flavonoid content compound decreases.

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