THE EFFECT OF MACERATION METHOD AND SOXHLETATION METHOD ON TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF KIRINYUH (*Chromolaena odorata* (L.) R.M.King & H.Rob) LEAF

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ABSTRACT

This study evaluated kirinyuh leaf extract as a natural antioxidant using Soxhlet extraction and maceration extraction methods. Dried leaves of kirinyuh were extracted with 96% ethanol. The total phenolic content was measured spectrophotometrically according to the Folin Ciocalteu method, calculated as gallic acid equivalent (GAE), while the antioxidant activity was measured using ABTS. The results of the phytochemical test of kirinyuh leaf extract using maceration (EKM) and Soxhletation (EKS) methods revealed alkaloids, flavonoids, saponins, steroids, and tannins. The total phenolic content of EKM was 39.85 ± 0.20 mgGAE/gram and EKS was 46.95 ± 0.15 mgGAE/gram extract. Antioxidant activity in the form of IC_{50} EKM was 46.10 ± 0.09 mg/L and EKS was 45.27 ± 0.07 mg/L, both of them have a very strong category. There were significant differences in the total phenolic content and antioxidant activity of the ethanol extract of the Kirinyuh leaf using maceration and soxhletation methods based on the results of the T-test.

Keywords: Kirinyuh, Eupatorium odoratum, ABTS, phenolics, antioxidant.

INTRODUCTION

Kirinyuh is a plant species with antioxidant potential. Siam or kirinyuh leaves have the potential to reduce blood glucose with alloxan induction in male mice (Amaliah et al., 2019). Research conducted on kirinyuh leaves (Ance et al., 2018) has reported positive results for phenols, flavonoids, and sterols. Phenolic compounds in Kirinyuh leaves can scavenge free radicals by the presence of a hydroxyl group. Choosing the right extraction method can obtain a higher phenolic content, it has more potential as an antioxidant (Alara *et al*., 2021).

The maceration method is a cold *discontinuous* method, whereas the *soxhletation* method is a hot *continuous* method. Soxhletation and maceration are extraction processes that differ in the temperatures used, but both undergo the immersion process. Immersion in soxhletation occurs after the condensation process, it is the change from vapor to liquid (Rosita *et al*., 2017). The solvent is heated and then evaporated into the cooler until the solvent drips to immer the sample, when the sample is immersed in the solvent, the maceration and soxhletation methods would undergo hypertonic. It is a concentration of the solution outside the cell is higher than inside the cell (Saptorini *et al*., 2015). This study evaluated the effects of extraction using soxhletation and maceration on phytochemical screening, total phenolic content, and antioxidant activity in a 96% ethanol extract of Kirinyuh leaf.

RESEARCH METHODS

Equipment and Materials

The tools were used in the research are Soxhlet set, extractor, condenser, water bath, rotary vacuum evaporator (Heidolph Tipe Heizbad Hal-VAP®), spectrophotometer UV-Vis (Shimadzu), quartz cuvette, macerator, oven (Memert UN 30), moisture balance, micropipette (Eppendorf) stirring rod, beaker (Pyrex), dropper pipette, porcelain cup, mesh 60 sieves, analytical balance (Memert UN 30).

The materials were used in this study included kirinyuh leaf, 96% ethanol (Medika), ethanol (Merck), distilled water (Multi Kimia), $FeCl_3$ (Merck), Mg powder (Merck), gallic acid p. a. (Sigma Aldrich), *Folin Ciocalteu* reagent (Sigma Aldrich), ABTS (Sigma Aldrich), HCl (Merck), and H_2SO_4 (Merck).

Methods

1. Sample Preparation

Light green colored and not too old kirinyuh leaf was taken as much as 2 kg. Kirinyuh leaves were collected from Pesu village RT 06 RW 03, Wedi, Klaten. The results of the sample determination on the number KM.04.02/2/2435/2022 showed that the sample is *Chromolaena odorata* (L.) R.M.King & H.Rob. Fresh Kirinyuh leaves were sorted and dried in the oven at 50 °C. Dry kirinyuh leaves were blended and sieved using 60 mesh sieve. A total of 2 g of kirinyuh leaves were dried with *a moisture balance*.

2. Production of Kirinyuh Leaf Extract

a. Maceration Extraction Method

Dry powder of kirinyuh leaf (100.0 g) was placed in the large glass bottle was macerated for 3 days with 750 mL of 96% ethanol at room temperature, protected from light, and stirred occasionally. Then, immers in 250 mL 96% ethanol for 2 days. The liquid extract was then evaporated into a thick extract. The thick extract was weighed to calculate yield (Komala *et al*., 2021).

b. Soxhletation Extraction Method

Dry powder of kirinyuh leaf (100.0 grams) in filter paper was placed into a *soxhlet* device, and 1 liter of 96% ethanol was added, then the temperature was set at 70°C until the droplest cycle was no longer colored (±5 h). The liquid extract was then evaporated into a thick extract. The thick extract was weighed to calculate yield (*Rosita et al*., 2017).

$$\text{Yield of extract (\%)} = \frac{\text{Weight of extract}}{\text{Weight of sample used}} \times 100$$

3. Phytochemical Screening

The ethanol extract of kirinyuh leaf from maceration (EKM) and ethanol extract of kirinyuh leaf from soxhletation (EKS) of as much as 250 mg were diluted in a 25 ml volumetric flask with ethanol solvent.

a. Phenolic Analysis

Each EKM and EKS were pipetted 1 ml and then added $FeCl_3$ 5% 2 drops. The formation of a greenish or blue-black color indicated a positive phenolic extract (Ramees *et al*., 2019).

b. Flavonoid Analysis

Each EKM and EKS were pipetted 1 ml and then added small amount of Mg powder and 2 drops of concentrated HCl and heated in a water bath. The extract is positive for flavonoids if a dark red or violet color is formed (Ramees *et al*., 2019).

c. Alkaloid Analysis

Each EKM and EKS were pipetted 1 ml and then added Mayer reagent 2 drops to tube, contained alkaloids if there was an orange-brown precipitate (Ramees *et al*., 2019).

d. Steroid Analysis

Each EKM and EKS were pipetted 1 ml and then added 2 drops of chloroform, 2 drops of concentrated H_2SO_4, and 2 drops of anhydrous acetic acid. A bluish-green color is formed when the extract is positive for steroids (Ramees *et al*., 2019).

4. Total Phenolic Content Testing

Each standard concentration series of gallic acid (10, 20, 30, 40, and 50 mg/L) was pipetted into 300 µL, and then incubated with 1.5 mL Folin Ciocalteau and Na_2CO_3 1.2 mL for 45 minutes at room temperature. Measurement of samples by using UV-Vis spectrophotometry at λmax 758.5 nm. The total phenolic content of each sample was 25 mg, and EKM and EKS were dissolved in 25 mL of distilled water in a volumetric flask. Each extract (300 µL) was pipetted and then incubated with 1.5 mL Folin Ciocalteau and Na_2CO_3 1.2 mL for 45 minutes at room temperature. The absorbance was measured in three times replications (Utami *et al*., 2023).

5. Antioxidant Activity Test

A total of 5.0 mL of 7 mM ABTS solution was incubated for 12-16 hours with 5.0 mL of 2.45 mM potassium persulfate solution in a closed place to make ABTS a radical solution. Sample solutions of EKM and EKS were prepared at different concentrations (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L). Each concentration was pipetted to 2 ml, reacted with 2 ml ABTS, and then incubated for 33 minutes in the dark. and then measured using UV-Vis spectrophotometry. The test was carried out with 3x replication using standard ascorbic acid. Absorbance was measured at λmax = 732 nm using UV-Vis spectrophotometry in three times replication (Utami *et al*., 2023).

RESULT AND DISCUSSION

1. Kirinyuh Leaf Extraction

According to the FHI, the suitable drying shrinkage for dry powder of kirinyuh leaf is less than 10%. Dry powder of kirinyuh leaf showed a drying shrinkage of 5.67%. Kirinyuh leaf dry powder weighing 100.0 grams was extracted using 96% ethanol solvent using soxhletation and maceration. Evaporation was carried out using a rotary evaporator and water bath at 50°C to evaporate the ethanol solvent. The thick extract color was blackish-green. The yield of the EKM extract was 7.05% and the EKS extract was 31,11%. The yield of the soxhletation results was higher than maceration. It can be caused the compounds dissolve in the solvent until that compounds reach the saturation point, so that the yield in the maceration method no longer increases. Moreover, the extraction time that too long could damage the chemical compounds and cause oxidation, so that it could reduce the yield. The advantage of the soxhletation method is the extraction process that continuous and the solvent always fresh, this factor can be caused the yield of soxhletation is higher than that of maceration extraction.

2. Phytochemical Screening of Kirinyuh Leaf

The content of phytochemical compounds in the ethanol extracts of the maceration and soxhletation methods on Kirinyuh leaves was determined through phytochemical screening for qualitative testing. The results of phytochemical screening of Kirinyuh leaves are shown in **Table I**. Phytochemical screening of EKM and EKS showed that the results could include phenolic, flavonoids, tannins, and saponins.

Table I. Phytochemical Screening of Ethanol Extract of Kirinyuh Leaf

Chemical Content	Results	EKM	EKS
Phenolic	Blue-Black	+	+
Flavonoids	Orange Color	+	+
Alkaloids	White Precipitate	+	+
Steroids	Bluish-Green	+	+

Note:
+ : present
−: absent, based on the color reaction

3. Total Phenolic Content

Folin-Ciocalteu was used to ensure the total phenolic content. Phenolic compounds react with the Folin-Ciocalteu reagent to form a color solution that can be measured as absorbance in the visible wavenumber. Gallic acid was used as a stable standard (Utami & Damayanti, 2023). The purpose for adding Na_2CO_3 is to get an alkaline solution. The blue color is a form of molybdenum-tungsten complex, and increasing the concentration of phenolic ions make the color of the solution more intense blue (Tahir et al., 2017). The total phenolic content of EKM and EKS was analyzed using the linear regression equation obtained from the standard curve of the gallic acid concentration series shown in **Figure 1**.

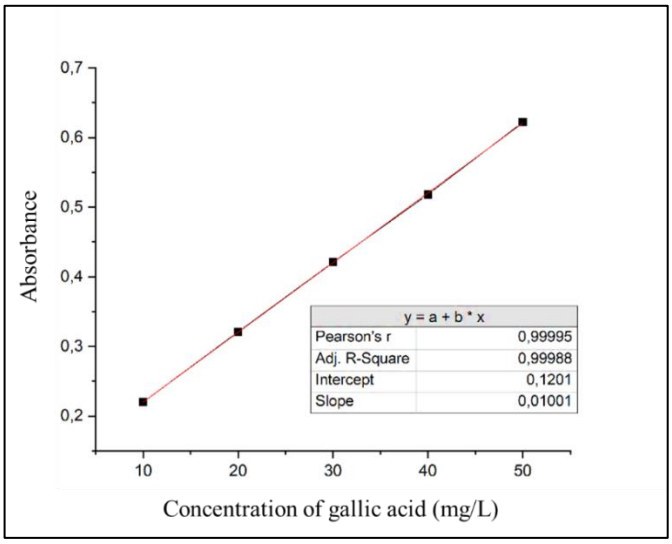

Figure 1. Calibration Curve of Gallic Acid

The absorbance of the sample obtained from UV-Vis spectrophotometric measurements was calculated using the regression equation of gallic acid with the absorbance of EKM and EKS as the Y-ordinate. The total phenolic content of EKM and EKS was shown in **Table II**.

Table II. Determination of Total Phenolic Content of EKM and EKS

Sample	Total Phenolic Content (mgGAE/gram extract)
EKM	39.85±0.20
EKS	46.95±0.15

Table II showed that the soxhletation method of Kirinyuh leaf extraction has a higher total phenolic content than the maceration method. There was a significant difference a value < 0.00 in the independent sample t-test between the two extraction methods. Soxhletation increases the total phenolic content because of the influence of higher temperatures than the maceration method. Higher temperature in soxhletation was controlled, it could be caused the phenolic compounds to be withdrawn and increase when the temperature used in the extraction process is higher (Mokoginta dan Runtuwene, 2013). Higher temperatures could promote solubility and diffusion, but the important aspect is avoid overheating because at too high a temperature during extraction, the solvent would evaporate, and the phenolic compounds would begin to damage *(*Zhang *et al*., 2018). This result was in line with previous research (Puspitasari & Proyogo, 2017), which showed that the soxhletation method was able to extract higher concentrations of total phenolics than the maceration method in ethanol extracts

of kersen leaves. According to a previous study (Harfiani *et al*., 2022), several phenolic substances, including ferulic acid and protocatric acid, are present in the kirinyuh leaves.

The efficiency of maceration method depends on the duration of extraction within a specific time period. Higher extraction efficiency was attained with longer extraction times. However, when the solute reached equilibrium between the inside and outside of the solid material, the longer extraction times had no effect on the extraction. The solvent-to-solid ratio that is too high, an excessive extraction solvent was required, lengthening the extraction time. As a result, the maceration extraction process is insufficiently effective because it uses a large amount of solvent and takes a long time to extract. Soxhletation method requires less time and uses less solvent. Soxhletation method uses solvents that are always fresh because the solvent could be recycled to extract components continuously, but if the extraction takes too long and uncontrolled high temperatures, it can cause thermal damage during the extraction time.

4. Antioxidant Activity

This research method utilizes ABTS as a measure of the antioxidant activity of kirinyuh leaf ethanol extract. The comparator used in this method is vitamin C because vitamin C, a natural antioxidant that neutralizes free radicals (Suharyani *et al*., 2022). ABTS·+ is a radical that has a characteristic blue-green color; when reduced by antioxidants, it changes to colorless. The manufacture of ABTS radicals was very sensitive to light so it takes 12-16 hours of incubation under dark conditions (Setiawan *et al*., 2018). ABTS·+ radical cation is formed from potassium persulfate, which oxidizes ABTS. In this study, ascorbic acid was used as a positive control because it is an antioxidant that inhibits the oxidation of free radical molecules. This positive control was used as a correction factor for the determination of antioxidant activity. The IC_{50} value of ascorbic acid in this study obtained a value of 7.39±0.04 mg/L, the method used in the study was suitable for determining the antioxidant activity of a sample.

The IC_{50} (Inhibitory Concentration) value is a value that states the concentration in units of mg/L that can prevent an oxidation process by 50% or the 50% concentration of the sample that can reduce ABTS free radicals. The ability of the antioxidants to scavenging free radicals is strong, which cause the result of IC_{50} value is lower, while higher IC_{50} value indicates the ability of the antioxidants to scavenging free radicals is weak. The percentage inhibition of ABTS radicals or the percentage of inhibition was obtained from the decrease in absorbance of ABTS samples against the absorbance of the ABTS control. Figure 2 shows the relationship between the concentration of EKM and EKS with the percentage of inhibition ABTS radical. This linear regression equation can be used to calculate the IC_{50} value by replacing the y value with 50 and obtaining the x value as the IC_{50} value (Tristantini *et al*., 2016).

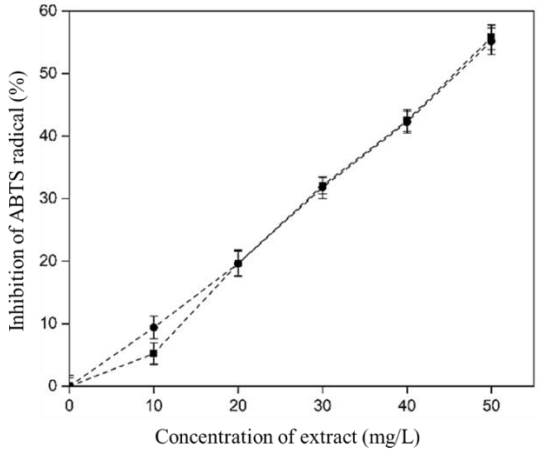

Figure 2. The effect of varying concentration extract of (■) EKS and (●) EKM in the free radical scavenging ABTS assay

Based on the independent sample t-test of EKM and EKS in Figure 3 showed a significant value of 0.000, which is a significant difference in antioxidant activity between the two extraction methods used.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Nilai	Equal variances assumed	3.580	.131	16.270	4	.000	.837	.051	.694	.979
	Equal variances not assumed			16.270	2.755	.001	.837	.051	.664	1.009

Figure 3. The Independent Samples T-Test of EKS and EKM

Table III. Antioxidant Activity of EKM and EKS

Sample	IC_{50} (mg/L)
EKM	46.10 ± 0.09
EKS	45.27 ± 0.03

Continuous extraction and controlled temperature in soxhletation could enhance the ability of the solvent to extract compounds that were insoluble at room temperature, so that the extraction of kirinyuh leaf more effective than macerated extraction. This can be seen as the result of the soxhletation method, which has a higher phenolic content and smaller in vitro ABTS radical scavenging assay with IC_{50}, a smaller IC_{50} indicates the ability of EKS as an antioxidant is stronger.

CONCLUSION

The ethanol extract of kirinyuh leaves by maceration and soxhletation methods showed positive results for phenolics, flavonoids, tannins, alkaloids, and steroids. By maceration, the ethanol extract of kirinyuh leaf had a total phenolic content of 39.85±0.02 mgGAE/gr, whereas soxhletation produced a value of 46.95±0.15 mgGAE/gr. Antioxidant ethanol extract of kirinyuh leaf maceration method has IC_{50} 46.10±0.09 mg/L and soxhletation method has IC_{50} value of 45.27±0.03 mg/L, which was categorized as a very strong antioxidant.

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