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Determination of Total Phenolic Content of Ethanol Extract of Broken Bone Twigs (Euphorbia tirucalli Linn.) by Folin-Ciocalteu Method Spectrophotometrically

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ABSTRAK

Broken bones plant (Euphorbia tirucalli Linn. contain secondary metabolites such as phenolics, (flavonoids and tannins), alkaloids, steroids, triterpenoids, saponins and hydroquinones. This study aims to determine the total phenolic content of ethanol extracts of Broken Bone Twigs (Euphorbia tirucalli Linn.) Spectrophotometrically. Broken bone twigs (Euphorbia tirucalli Linn.) were extracted using maceration method with ethanol solvent. Total phenolic content was determined using Visible Spectrophotometric method with Folincio-Calteu reagent. The principle of this method is the formation of a blue complex compound of Fossosmolibdat-phosphotungstad reduced phenolic compounds in an alkaline atmosphere that can be measured Spectrophotometrically. As a comparator used gallic acid. Examining the total phenolic content contained in broken branches using the Folin-Ciocalteu method by spectrophotometry. From the results of absorbance measurements obtained linear regression equation $\hat{y} = 0.0012x + 0.0316$ with a coefficient of determination (R^2) of 0.9661. Phenolic levels in bone fracture twigs (Euphorbia tirucalli Linn.) amounted to 0.102% w/b calculated as gallic acid.

1. INTRODUCTION

Indonesia has around 30,000 plant species, 940 of which are used as medicinal plants. The use of medicinal plants as traditional medicine is a treatment option that is now increasingly in demand, especially with the awareness to return to nature and also because it is relatively safe and inexpensive, even with current developments there is increasing attention for alternative health services. From various studies, traditional medicine has been recognized by the community, thereby increasing the benefits of plants for health and creating conditions that encourage the development of traditional medicine.[1]. One of the medicinal plants that is often used by people for generations is the broken bone plant. Broken bones plant has been used by the Indonesian people to treat stomach pain, nasal cavity ulcers, rheumatism, bone pain, nerve pain, hemorrhoids, syphilis, skin diseases, leprosy, numb feet and hands and is also used as an anti-cancer drug.[1] [2]. Spectrophotometric methods can be used to identify and measure the concentration of certain compounds in medicinal plant extracts. if any of these compounds have the potential to influence fracture healing, research of this kind could help understand the relationship between medicinal plants and fractures from a chemical and biological perspective.

Broken bones plant (Euphorbia tirucalli Linn.) is a plant originating from tropical Africa, in Indonesia this plant is grown as a hedge, ornamental plant or wild plant. This plant can be found from the lowlands to 600 m above sea level[3]. Fractured twigs contain secondary metabolites such as phenolics, (flavonoids and tannins), alkaloids, steroids, triterpenoids, saponins and hydroquinones. [4][5].

Determination of total phenolic content was carried out using the Folin-Ciocalteu reagent because phenolic compounds can react with Folin to form a colored solution whose absorbance can be measured. Folin-Ciocalteu will reduce phenolic into complex compounds, phenolic compounds that react with Folin-Ciocalteu will dissociate protons into phenolic ions in alkaline conditions[6].

Phenolic compounds are secondary metabolites which are found scattered in almost all parts of the plant and have a wide range of biological activities including anti-bacterial, anti-inflammatory, antithrombotic, anti-cancer.[7]. Phenolic compounds are also antioxidants, this is because phenolic compounds can react with free radicals and eliminate their radical activity so that they are no longer harmful to human body cells.[8]. Determination of the total phenolic compounds content in the sample using a standard solution of gallic acid. Gallic acid is a phenolic compound derived from hydroxybenzoic acid which is classified as a simple phenolic acid. Gallic acid reacts with the yellow Folin-Ciocalteu reagent with the addition of Na2CO3 base which produces a blue color, indicating the presence of phenolic compounds[9].

Based on the background of the problems above, researchers are interested in examining the total phenolic content contained in broken branches using the Folin-Ciocalteu method by spectrophotometry. Successful research could provide new insights into the biological mechanisms behind bone healing and how plants might influence it. However, it's important to remember that scientific research can have mixed results, and it doesn't always achieve the desired results.

2. METHOD

A set of UV-Vis Spectrophotometer (double beam genesis 10 S) a set of distillation apparatus, scissors/knife, analytical balance (Ohaus), measuring flask, spatula, volume pipette, dropper pipette, suction ball, tissue, test tube, funnel, dark bottle , measuring cup, evaporating cup, filter paper. The materials used were broken bone twigs (Euphorbia tirucalli Linn.), 96% ethanol, gallic acid, Folin Ciocalteu reagent,Na2CO3,distilled water.

The sample used is a broken bone branch obtained in the Agam Ladang Laweh area, West Sumatra Province. The sampling technique is Simple Random Sampling. Broken bone twigs cleaned, then chopped and weighed 300 grams as a fresh sample. All of them were dried outside the influence of direct sunlight and then obtained a dry weight of 50 grams. Maceration with ethanol for 3x 3 days at room temperature, shake 1x 24 hours then filter. The maceration results were combined, then the solvent was evaporated using vacuum distillation to obtain a thick extract of 2.615 grams.

Qualitative Test of Phenolic Compounds

Matoa stem bark extract added with a few drops of FeCl3. A positive result is indicated by the appearance of a strong green, red, purple, blue or black color[10].

Reagent Manufacturing

a. Preparation of gallic acid mother liquor (1000µg/ml)
   Weigh 50 mg of gallic acid dissolved in 96% ethanol, then dilute with distilled water to 50 ml
b. Preparation of gallic acid solution (100µg/ml)
   Pipette 1 ml of gallic acid mother liquor (1000µg/ml) then add distilled water to 10 ml
c. 10% Na2CO3 solution
   As much as 10 g of Na2CO3 added with distilled water to 100 ml, let stand for 24 hours then filtered.

Maximum wavelength determination

Pipette 0.2 ml of gallic acid solution with a concentration of 100 µg/ml plus 1 ml of Folin Ciocalteu reagent and then let it stand for 8 minutes. Into the solution was added 3 ml of 10% Na2CO3 and 15.8 ml of distilled water. Then the absorbance is measured at a wavelength of 745 - 785nm.[7].

Determination of Operating Time

Pipette 0.2 ml of gallic acid solution with a concentration of 100 µg/ml plus 1 ml of Folin Ciocalteu reagent and then let it stand for 8 minutes. Into the solution was added 3 ml of 10% Na2CO3 and 15.8 ml of distilled water. Measure the absorbance so that the maximum wavelength constant absorbance is obtained, record the time required.

Preparation of gallic acid standard curve

Gallic acid mother liquor 1000µg/ml pipetted 1.5 ml each; 2ml; 2.5 ml; 3ml; 3.5 ml was then diluted with 10 ml distilled water so that a solution with a concentration of 150, 200, 250, 300, 350 µg/ml was obtained from each concentration in a 0.2 ml pipette plus 1 ml of Folin Ciocalteu reagent, let stand for 8 minutes. After standing, each solution was added 3 ml of 10% Na2CO3 and 15.8 ml of distilled water, let stand for the operating time. All solutions were measured for their absorbance at the maximum wavelength.

Determination of Total Phenolic Content of Ethanol ExtractBroken Twigs (Euphorbia tirucalli Linn.)

1. Weigh 100 mg of broken bones extract, dissolve it with 96% ethanol up to 10 ml and obtain a concentration of 10 mg/ml.
2. From the concentration (10 mg/ml) pipetted 0.2 ml and added 1 ml of Folin Ciocalteu reagent, let stand for 8 minutes, then added 3 ml of 10% Na2CO3 and 15.8 ml of distilled water, shake homogeneously. Leave the solution for the operating time. Measure the absorbance at the maximum wavelength.

Data analysis technique

Using the linear regression formula

The formula for the linear regression equation is

$$\hat{y} = a + bx$$

$$a = \frac{(\sum yi)(\sum_{xi} 2) - (\sum xi)(\sum xi\ yi)}{n(\sum_{xi} 2)(\sum x)^2}$$

$$b = \frac{n(\sum xy) - (\sum x)(\sum y)}{n(\sum_{xi} 2)(\sum x)^2}$$

information :

ŷ = subject in the predicted dependent variable
a = Price y if (x=0)
b = regression coefficient
x = concentration

Correlation Formula

The use of the correlation formula is useful to determine the effect of concentration on absorbance. The formula is as follows:

$$r = \frac{n(\sum xy)\ (\sum x)\ (\sum y)}{\sqrt{\{n(\sum x^2) - (\sum x)^2\}\{n(\sum y^2) - (\sum y)^2\}}}$$

Validation

Standard deviation formula:

$$\text{SD} = \frac{\sqrt{\sum(y - y^1)^2}}{n - 1}$$

Detection limit formula / BD :

$$\text{BD} = \frac{3\ x\ SB}{b}$$

The quantity limit formula/BK:

$$\text{BK} = \frac{10\ x\ SB}{b}$$

Where :

SD = standard deviation
BD = detection limit
BK = quantity limit

3. RESULTS AND DISCUSSION

Results

From the research that has been done, the results are:

1. The regression equation $\hat{y} = 0.0012x + 0.0316$ with the value of the coefficient of determination (R^2) = 0.9661 and the correlation coefficient (r) =0.9828
2. The phenolic content of the ethanol extract of broken bones (Euphorbia tirucalli Linn.) of 0.102% w/w was calculated as gallic acid

Tabel 1. Gallic acid absorbance measurement data

No.	Concentration µg/ml	Absorbance
1.	150	0,215
2.	200	0,262
3.	250	0,343
4.	300	0,424
5.	350	0,439

The results of the graphical visualization of the calibration curve can be seen in Figure 1 below.

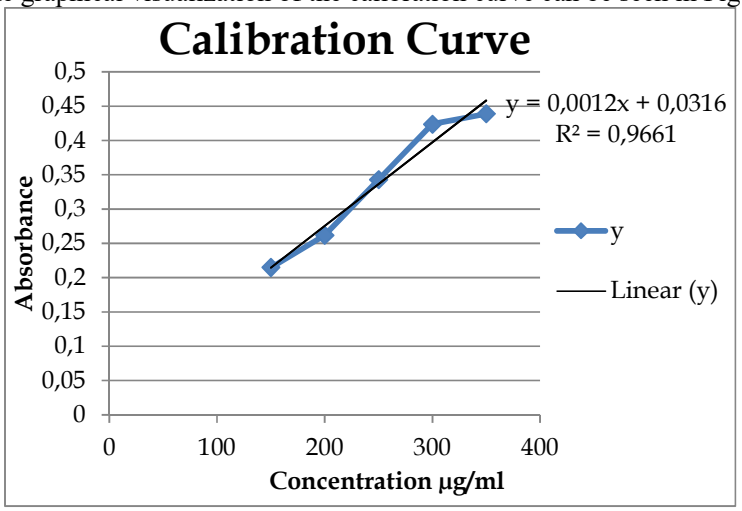

Figure 1. Calibration Curve

Discussion

In this study, the sample used was broken bone branches obtained from the Agam Ladang Laweh Region, West Sumatra Province. Sample processing starts with cleaning the sample to reduce dirt adhering to the sample, then drying it with the aim of reducing the water content in the sample and chopping it.300 grams as a fresh sample was dried outside the influence of direct sunlight and then obtained a dry weight of 50 grams.This study used the extraction method, namely maceration for 3 x 3 days using 96% ethanol as a solvent.Ethanol is a polar solvent that easily evaporates so that the solvent will be separated more easily from the extract.Polar solvents such as ethanol can dissolve polar compounds such as phenol groups. In the sample maceration process, shaking is carried out once every 24 hours to ensure the homogeneity of the solutes, after the maceration process, the solvent is separated from the dregs by filtering[11][12].

Thickening of the filtrate was carried out using vacuum distillation. During vacuum distillation, boiling stones are used to prevent large explosions during boiling and to distribute heat and absorb heat. After distillation, 2.615 grams of broken bone twig ethanol extract was obtained.

Furthermore, the determination of the maximum wavelength is carried out which aims to determine the maximum absorbance value at the time of measurement. Maximum absorption at a wavelength of 745 – 785 nm, obtained at a wavelength of 775 nm[13]. Furthermore, the determination of the operating time is carried out which aims to obtain the measurement time when the reaction has

run optimally which is characterized by a stable absorbance. From the experimental results, the operating time of gallic acid is stable from 110 to 130 minutes. So all absorbance measurements must be carried out at that time.[13].

This study was conducted to determine the phenolic content contained in broken bone twig extract by UV-Vis spectrophotometry using the Folin Ciocalteu method with gallic acid as a comparator. The hydroxyl group in the phenolic compound will reduce the Folin Ciocalteu reagent to become a blue complex compound with the addition of Na2CO3. the blue color formed will be more concentrated in proportion to the concentration of phenolic ions formed, meaning that the greater the concentration of phenolic compounds, the darker the resulting color. Determination of the total phenolic compound content in the sample using a standard solution of gallic acid, gallic acid is a hydrobenzoic derivative which is a simple phenolic acid that is pure and stable.[14][12].

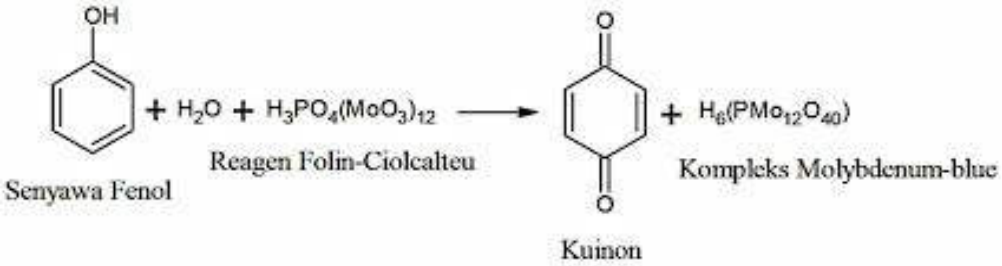

Figure 2. Reaction of Phenol Compounds with the Folin-Ciocalteu Reaction[15].

Making a calibration curve aims to determine the total phenolic content through the linear regression equation of the calibration curve. From making the curve, we get the regression equation $\hat{y}$ = 0.0012x + 0.0316 with the value of the coefficient of determination (R^2) = 0.9661 and the correlation coefficient (r) = 0.9828. This means that 98.2 of the absorbance is affected by concentration, while the remaining 1.8 is influenced by other factors such as temperature, light, chemicals and so on. From the data above it shows that the value of r is close to 1 proving that the regression equation is linear[16].

Furthermore, the data obtained from concentrations with different absorbances were processed to determine the detection limit (BD) and quantification limit (BK), the detection limit is the lowest analyte concentration that can still be detected. The test results on the ethanol extract of broken bones showed a detection limit value of 118 µg/ml while the quantification limit obtained was 393.3 µg/ml[13]

In determining the phenolic content used extracts that have been reacted with reagents. From the calculations performed, it was obtained that the total phenolic content of the ethanol extract of broken bones was 0.102% w/w which was calculated as gallic acid.

4. CONCLUSION

From the research that has been done, it is concluded that the phenolic content of the ethanol extract of broken bones is 0.102% w/w which is calculated as gallic acid.

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