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Antioxidant Activity of Binahong (Anredera cordifolia (Tenore) Steen) Simplicia Leaves

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Abstract

Indonesia is a country rich in natural resources from flora, fauna, and minerals. Many plants have medicinal properties including Binahong leaves. Binahong consists of secondary metabolites such as saponins, quinones, flavonoids, steroids, monoterpenoids, while the rhizome contains flavonoids, polyphenols, tannins, and steroids. The plant has an anti-bacterial, anti-cholesterol, healing of cuts and hepatoprotective wounds activity. The purpose of this study is to identify the comparative content of polyphenols, flavonoids, and the antioxidant activity of binahong simplicia leaves using the oven drying method and room temperature. Observed parameters consisted of yield, water content, polyphenol content, total flavonoid levels, and antioxidant activity. Results showed that the best treatment based on the highest levels of polyphenols (1.782% vs 1.561%), flavonoids (0.702% vs 0.354%) and antioxidant activity (782.79 vs 887.31). Conclusion, the treatment of binahong leaves by oven drying produces the highest content of polyphenols and flavonoids in binahong leaves. They have antioxidant activity better than those which are produced by room temperature drying treatment

Keywords:

Introduction

Traditional medicine with medicinal plants is expected to be utilized in the development of public health. Treatment using raw materials from nature has been known for a long time and was used by our forefathers. The law rule no.36 of 2014 concerning Health Workers issued article 11 stating that traditional health workers are included in the group of health workers. The Government also issued rule no. 103 of 2014 concerning Traditional Health Services article 10 and Permenkes No. 61 of 2016 concerning Empirical Traditional Health Services. The rules are the basis and reference in producing traditional medicines as drugs. Traditional medicines are native medicines from Indonesia, namely finished drugs or packaged medicines derived from plants, animals, minerals and gelenic preparations or a mixture of materials that do not have clinical data and are used as medicines based on experience. (15-17)

One form of traditional medicine preparations in the form of dry leaves or simplicia. The way that can be done to control the quality of simplicia is to standardize simplicia. Standardization of simplicia has the understanding that the simplicia used for drugs as raw material must meet certain requirements. Simplicia quality parameters include water content, total ash content, acid insoluble ash content, ethanol soluble extract, water soluble extract, and truth test (1, 2). Other studies also found natural product, both from animals and plants, have a potential benefit for many diseases such as hypoalbuminemia and tuberculosis. (3-6)

One of the plants that has health properties is Binahong (*Anredera cordifolia* (Ten.) Steenis). Binahong is a plant native to South America, creepers with a plant length can reach five meters and its age can reach dozens of years. This plant can grow both in tropical and subtropical climates. Binahong leaves contain

secondary metabolites such as saponins, quinones, flavonoids, steroids, monoterpenoids, while the rhizome contains flavonoids, polyphenols, tannins, and steroids (7).

Previous research reported that the binahong plant has the potential to be an anti-bacterial (8), anti-cholesterol (9), healing of the wound (10) and hepatoprotective (11). To meet the needs of the community in the use of binahong leaves, it is deemed necessary to make simplicia safer and better. But the best treatment is not yet known either on the leaves of Binahong in order to produce simplicia according to standardization will benefit public health. Therefore, the researchers conducted research on the production techniques of binahong leaf simplicia (*Anredera cordifolia* (Ten.) Steenis) through two methods, namely drying at room temperature and drying using an oven.

Materials and Methods

Materials

The plant material used in this study is the leaves of binahong (*Anredera cordifolia* (Ten.) Steenis) taken from Makassar City. The other ingredients are aquadest, water, standard solution, DPPH, methanol, hexane solvent, acetone, folin, aluminum foil, and label paper. Tools used include cabinet dryers, basins, buckets, trays, digital scales, analytical scales, blenders, test tube racks, laboratory glassware, hotplates, sonicators, UV-Vis spectrophotometers, incubators, autoclaves, centrifuges, evaporators, and moisture analyzer.

Preparation of Plant Materials

The material used is binahong leaves which are still fresh. Intake of binahong leaves is done purposively without comparing with the same plant from other regions. Material is taken from Makassar City.

Selection and Drying of Binahong Leaves

Sorting is performed on the leaves of Binahong to separate the quality of fresh leaves and those which are damaged or blackish colored, then washed clean so that the dirt attached to the leaves is lost, then the withering process. The sample withering process is carried out by aerated without direct sun exposure. This aims to reduce the water content of the sample without damaging the active compounds contained in the sample. This withering process will be carried out for 24 hours at room temperature (33^{o}C) and followed by drying with two different methods, namely by using a cabinet dryer at 40^{o}C for 5 hours and drying at room temperature (33^{o}C) for ± 3 weeks.

Preparation of Simplisia Binahong Leaves

Dried leaves which are marked from the resulting texture become brittle and obtained dry weight, then mashed using a blender and then sieved using a 16 mesh sieve. Simplicia powder is weighed and then packed in a tightly closed plastic container.

Binahong Leaves Yield

Yield is the amount of dried binahong leaf powder produced from a number of fresh leaf raw materials in a clean condition through the drying process. Yields can be obtained using the formula:

$$Yield = \frac{DryWeightOfBinahong}{WeightofFreshBinahong} \text{ x } 100\%$$

Moisture Moisture Analyzer Method

The Moisture Analyzer used must be calibrated first. Calibration is carried out by an external body and is carried out at least once a year. The Moisture Analyzer is turned on, then press the mode button. The lid on the device is opened, so the display status will change. The aluminum pan is cleaned while the position of pan is corrected. The lid is closed again and the the tool is tared automatically. The sample is weighted as

much as 0.5 g, flatten the sample on the pan then the instrument is closed again. The instrument will heat the sample until it shows the value of the sample water content that is read constantly (± 3-5 minutes).

Polyphenol Levels

Analysis of polyphenol levels was carried out by the Folin-Calciteau (12) method. A total of 25 grams of fat sample was freed by the addition of 100 ml of hexane solvent and stored for 24 hours at room temperature, then centrifuged at 2500 rpm for 10 minutes. This process was repeated 3 times. A total of 1 g of fat-free sample was extracted with 50 ml of acetone: water (70: 30) using a sonicator for 25 minutes. Then centrifuged at 3500 rpm for 15 minutes and the solution is taken. The solution is then evaporated to separate the solvent and extract. Pipette as much as 1 mL of extract and then diluted with aquadest as much as 7 mL. After that, pipette as much as 1 mL extract into 50 mL measuring flask and then added 2 mL of 10% follin, 20 mL of Na2CO3 7%, and crushed with aquadest to the limit mark. Leave for 60 minutes. The sample absorption was measured by a spectrophotometer with a wavelength of 740 nm. The experiment was repeated using gallic acid as a standard with concentrations of 100, 200, 300, 400, and 500 ppm. The polyphenol content was calculated using a standard curve and expressed in mg / g of the sample.

Determination of Total Flavonoid Levels

Binahong leaves powder samples were dissolved with ethanol pro analysis (2% -5%), added 0.10 ml of aluminum chloride 10%, 0.10 ml of sodium acetate 1 M and 2.80 ml of aquadest. The mixture is shaken homogeneously then left for 30 minutes. Then the absorption is measured using an ultraviolet-visible (UV-VIS) spectrophotometer at maximum wavelength.

Antioxidant Activity DPPH Method

To make a 0.4 mM DPPH solution, 0.0157 g of DPPH is dissolved in 100 mL absolute ethanol in a measured flask. Furthermore, as much as 100 mL of sample solution of various concentrations of each 1.0 mL DPPH 0.4 mM was added and the volume was sufficient to 5.0 mL with the addition of absolute ethanol. The mixture is then vortexed and left for 30 minutes at room temperature. Absorption is measured at a wavelength of 518 nm. % Inhibition is measured by the formula:

% Inhibition $\frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance} x100$

Data analysis

Data analysis results in this study were processed using Microsoft Excel.

Results and Discussion

The research sample consisted of two treatments namely A1 binahong leaves which were dried using an oven took about 5 hours to obtain the powder with the desired water content. The way to find out the sample is dry and ready to be crushed is to squeeze it. If the binahong leaves when crushed are destroyed, the leaves are ready to be crushed. For A2 treatment, the leaves are dried by placing them at room temperature of about ± 3 weeks. The measurement method used to determine whether the sample is ready to be mashed or not is the same as the sample method of drying with an oven.

Yield Results

$YieldA2 = \frac{113gram}{2000}$ x 100% = 5.65%

$YieldA1 = \frac{146{,}6gram}{2000}$ x 100% = 7.33%

Water content

Water content greatly affects the durability of simplicia. High water content will make it easy for bacteria, fungi and other microbes to multiply so that it will affect the quality of the simplicia. The result of measurement of water content of binahong leaves produced for both treatments was 9%.

This value is still in the range of standards for the quality requirements of drugs in traditional special products that are served by brewing hot water. This is in accordance with the Regulation of the Head of the Republic of Indonesia Drug and Food Supervisory Agency Number 12 of 2014 concerning Quality Requirements for Traditional Medicines for Medicines In that the standard water content value for traditional medicines brewed with hot water is ≤ 10%.

Flavonoid levels

Flavonoid content analysis is intended to determine the amount of flavonoid levels that are still contained in the binahong leaf powder produced. The amount of flavonoids affects the antioxidant activity of the resulting binahong leaf powder. The results of the analysis of the levels of polyphenols produced in different drying methods namely the best flavonoid levels were obtained in the oven drying treatment (A1), which is 0.7020%. While the level of flavonoids in room temperature drying treatment (A2) is 0.3540%. Different levels of flavonoids produced on the powder with different drying methods can be seen in Figure 1.

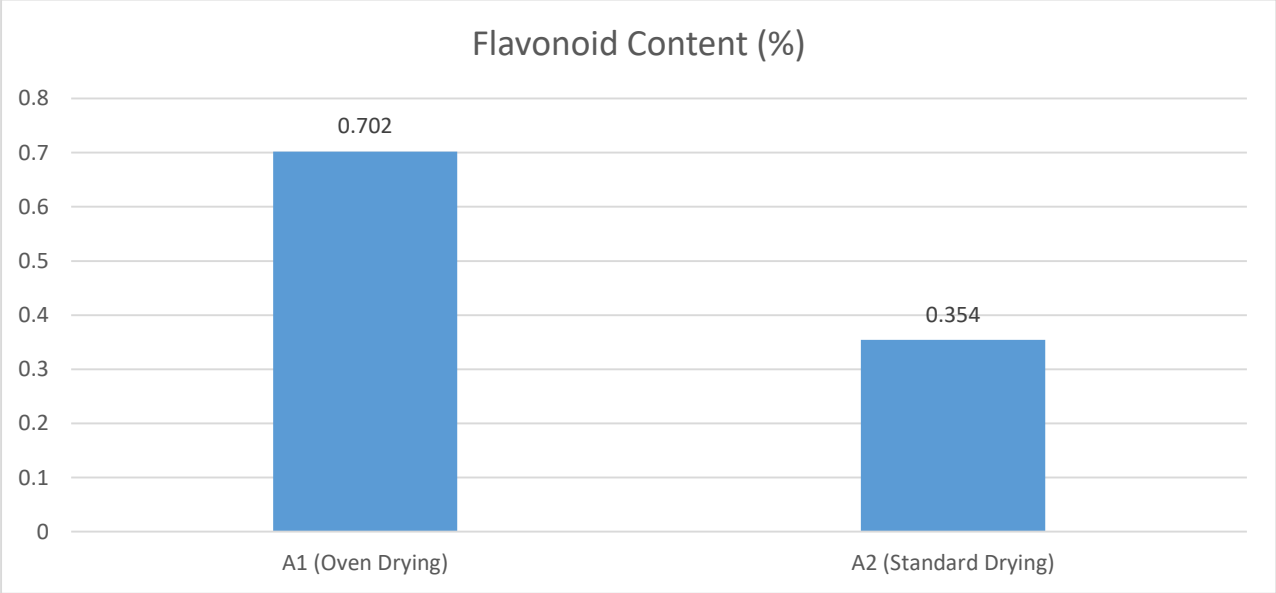

Figure 1. Levels of Flavonoids in Binahong Leaves

Based on the graph in Figure 1, it can be seen that the drying method significantly influences the flavonoid levels in the binahong leaves produced. From the graph drying by the oven method produces higher flavonoid levels compared to the room temperature drying method. The difference in value obtained is probably caused by exposure to sunlight during the drying process at room temperature which causes damage to flavonoid levels. This is in accordance with Winangsih's statement (13), that direct sun drying is the easiest drying process, but in terms of the quality of artificial dryers (ovens) will provide better products. Ultraviolet rays from the sun also cause damage to the chemical content of the dried material.

Polyphenol Levels

In this study fresh binahong leaves were processed into dry powder using two drying methods, namely oven drying and room temperature drying. Then the levels of Polyphenols were tested using the Folin-Calciteau method. The results of the analysis of the best levels of polyphenols were obtained in the drying treatment with an oven (Figure 2).

The results of testing the polyphenol content (Figure 2) showed that the highest levels were obtained in treatment A1 (oven drying) with a value of 1.782%, while the polyphenol content in the A2 treatment (room temperature drying) was 1.561%. The graph shows that the best drying method is obtained on the material by oven drying treatment method. The polyphenol levels obtained are directly proportional to the levels of favonoids in the sample, this is due to the flavonoid compound is one type of antioxidant component of polyphenols, so that treatments containing higher levels of flavonoids will also produce high levels of polyphenols. Polyphenols are one of the antioxidant substances found in food. The antioxidant compounds that include polyphenol components are Flavonoids, Flavones, Flavonols, Heterosides flavonoat.

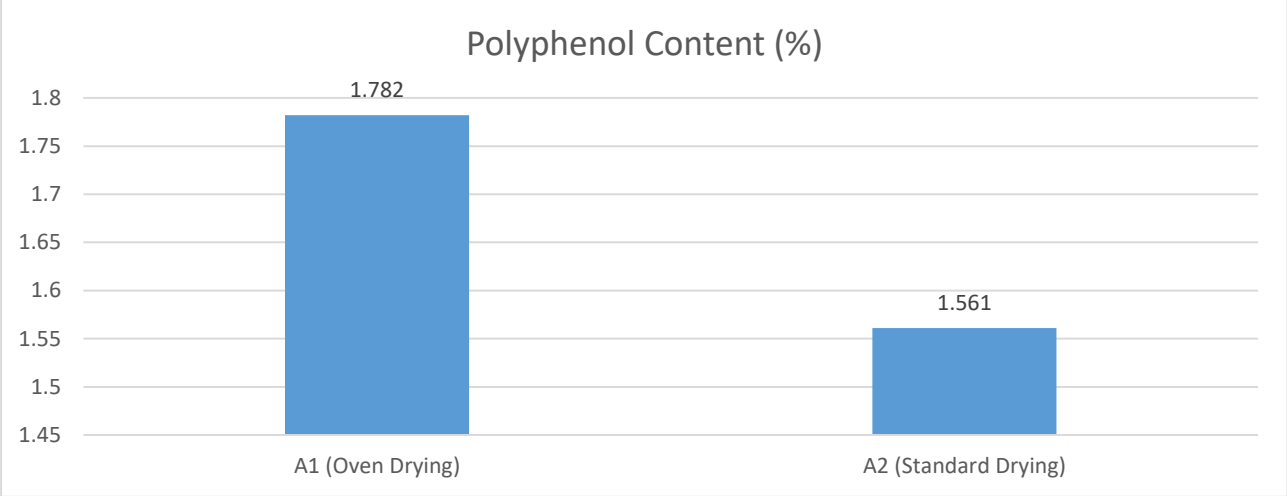

Figure 2. Polyphenol Content in Binahong Leaves

Antioxidant Activity

In this study an antioxidant activity test was carried out to determine the effect of the drying method on antioxidant activity on binahong leaves. The results of the analysis of antioxidant activity in different drying samples is the best value in the oven drying treatment (A1), which is 782.7970 ppm, while the value of antioxidant activity in the treatment of room temperature drying (A2) is 887,315 ppm. The graph of differences in the value of antioxidant activity between oven drying and room temperature drying on bonahong leaves can be seen in Figure 3.

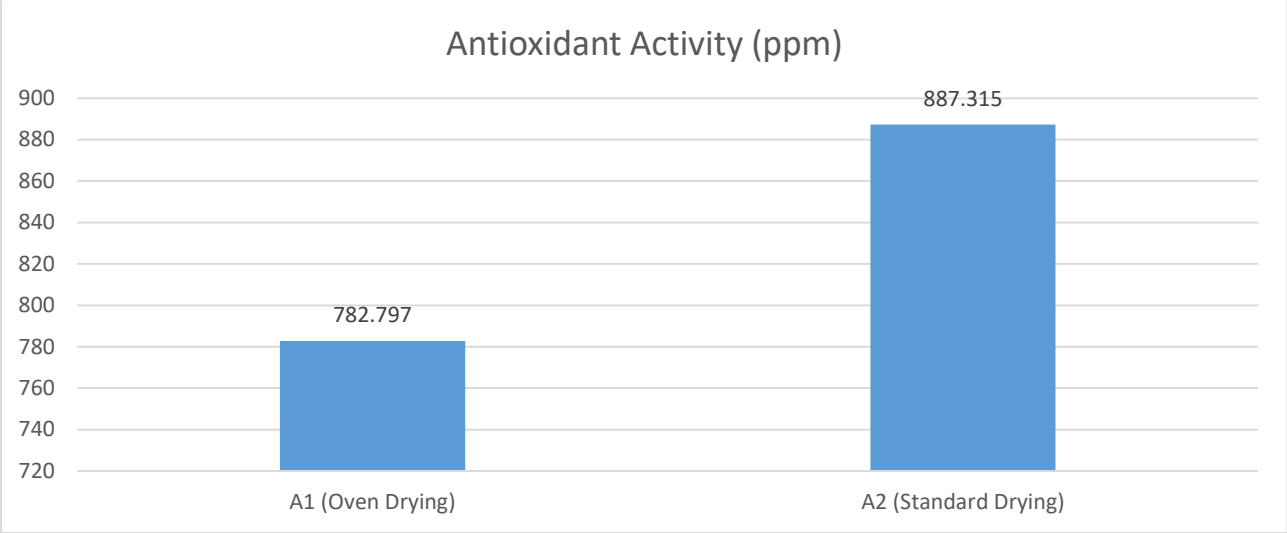

Figure 3. Value of Antioxidant Activity in Binahong Leaves

Graphic data shows that the drying method factor has no significant effect on the level of antioxidant activity of Binahong leaf simplicia. Figure 3 shows that the level of antioxidant activity obtained in the oven method treatment obtained higher values compared to room temperature drying, because the IC_{50} value in the oven method treatment produced is lower. However, the difference in value produced did not show a significant difference to the antioxidant activity of the sample. The difference in the value of antioxidant activity is due to several factors including the method of drying in the form of temperature use, material handling, and also the light conditions and room temperature during processing. other than that drying time also influences the antioxidant activity of binahong leaves. The higher temperature and duration of drying used causes antioxidant activity also decreases. This is consistent with Winarno's statement that temperature and drying time significantly affect antioxidant activity because these conditions cause damage to the active substances contained in an ingredient. (14)

Conclusion

The treatment of binahong leaves by oven drying produces the highest content of polyphenols and flavonoids in binahong leaves. They have antioxidant activity better than those which are produced by room temperature drying treatment.

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