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Effect of Drying Method Treatment on Total Phenolic, Flavonoids and Antioxidant Activity of Kersen (Muntingia calabura l.) Leaves

Nurpudji Astuti Taslim[1*], Muhammad Asfar[2], Indra Yuliana[3], Dewi Lestari[4], Rabiah Waris[5]

[1] Department of Nutrition School of Medicine, Hasanuddin University, Indonesia
[2] Department of Food Science and Technology, Hasanuddin University, Indonesia
[1,3,5] Center for Research and Development of Health Sciences, Institute for Research and Community Service, Hasanuddin University, Indonesia
[4]School of Economic and Business, Hasanuddin University, Makassar, Indonesia
[1]*Correspondent Email: pudji_taslim@yahoo.com

ABSTRACT

The manufacture of kersen leaf simplicia generally goes through a drying process, but it is not yet known which method of drying is best to obtain the best content. This study consisted of two treatments: the drying method of the blower oven and the vacuum oven. The results showed that the type of drying treatment on the simplicia of kersen leaves had a significant effect on the total levels of flavonoids but did not significantly affect the antioxidant activity and total polyphenol levels. The total level of flavonoids in the vacuum oven drying treatment was 4.68%, while in the blower oven drying treatment was 3.61%. The total polyphenol content in the blower oven drying treatment was 14.85%; whereas by drying the vacuum oven it was 16.21%. The antioxidant activity of the vacuum oven drying treatment was 17.77% while the blower drying treatment was 17.26%. The vacuum oven drying treatment should be used for getting higher flavonoid content in Kersen leaves compared to blower oven drying treatment. The analysis showed that the intensity of antioxidant activity for both treatments were very strong. Thus, it is very potential to be used as an antidiabetic.

Keywords:
Functional food; drying method treatment; total phenolic; flavonoids; and antioxidant activity

1.Introduction

Extraction of certain component in both animal and plant products have been proven for its efficiency. For example, *Channa striata* (fish) protein extract can increase serum albumin levels and protein intake in hypoalbuminemic patients.(1) Another example, black rice bioactive compound extract as a local product in South Sulawesi plays a role in gut microbiota modulation which related to many metabolic disorders.(2)

Kersen plant (*Muntingia calabura L.*) is included in the *Elaeocarpaceae* family and is a tropical plant that is commonly found in Indonesia. Nowadays, Kersen leaves are part of the herbal plant which is widely consumed by the public because it has many health benefits. One of the diseases that can be treated with kersen leaf extract is diabetes mellitus. Arum *et al*. (2012) stated that kersen leaves contain flavonoid compounds that can act as antioxidants, antibacterial, and anti-inflammatory.(3) According to Sridhar *et al*. (2011) stated that the methanol extract of kersen leaves has an antidiabetic effect in normal and alloxan-induced mice.(4)

Flavonoid has been proven of its antidiabetic properties by increasing glucose transport, increasing plasma insulin and decreasing programmed cell death.(5) Moreover, high antioxidant diets has a potential to be antidiabetic agent by alleviating insulin resistance.(6)

According to the Ministry of Home Affairs (2014), herbs are Indonesia's biodiversity for the purposes of supporting conventional medicine performed by doctors, standardized herbal medicines, and phytopharmacology. One of the basic ingredients used in the production of herbal medicine is simplicia. The manufacture of simplicia is generally carried out by the drying method, but it is not yet known which method of drying the best that can be applied in the

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manufacture of simplicia leaves with productive results, which can minimize the loss of bioactive content which has the potential to become an alternative anti-diabetic treatment. Therefore in this study to analyze the effect of the drying method on the bioactive components of the simplicia of cherry leaves which act as an alternative anti-diabetic drug.(7)

Diabetes mellitus is the most common endocrine disease. This disease is characterized by increased blood sugar levels (hyperglycemia) and high levels of sugar in the urine (glycosuria).(8) Based on the results of Riskesdas (2018) the prevalence of diabetes mellitus in Indonesians aged ≥15 years has increased by 10.9% from the previous year in 2013 which was 6.9%.(9) Along with the increasing number of diabetics in Indonesia, it is necessary to use horticultural commodities as raw material for herbal products as an alternative treatment for diabetics.

2. Material And Methods

Material. The equipment used in this research is a blower oven, vacuum oven, digital scale, analytical scale, grinder, sieve, test tube rack, laboratory glassware, hotplate, sonicator, UV-Vis spectrophotometer, incubator, autoclave, centrifuge, evaporator, and Moisture Analyzer.

The main ingredient used in this study is kersen leaves *(Muntingia calabura L.).* The other ingredients are aquadest, water, standard solution, DPPH, methanol, ethanol, folin-ciocalteu, aluminum chloride, sodium acetate, distilled water, NaOH, aluminum foil, and label paper. The material used is fresh kersen leaves obtained from Makassar City. Kersen leaf collection was carried out purposively without comparing it with similar plants from other regions.

Methods.

Selecting and Drying Kersen Leaves

Kersen leaves are sorted to separate the quality of fresh leaves from damaged or blackish colored leaves, then washed clean so that the dirt adhering to the leaves is lost, then the wilting process. The withering process of the sample is carried out by aerating it without direct exposure to sunlight then followed by drying using two different methods, namely by using an oven blower and a vacuum dryer at the same temperature, namely 40^{o}C for ± 5 hours.

The dry leaves are characterized by the resulting texture to become brittle (when crushed into pieces) and dry weight is obtained, then mashed using a blender and then sieved using a 16 mesh sieve. The simplicia powder is weighed and then packed in a tightly closed plastic container.

The Yield.

The yield of simplicia is the ratio between the weight of the simplicia produced and the weight of the initial sample before drying. The percentage yield of simplicia is calculated by the following formula:

$$\text{The Yield (\%)} = \frac{\text{dry weight of kersen leaves}}{\text{the weight of fresh kersen leaves}} \times 100$$

Moisture Content.

Moisture content is made based on AOAC (Association of Official Analytical Chemists), 2005.(10)

Antioxidant Activity DPPH Method

a) Sample preparation

The sample was weighed 5 mg, dissolved in methanol p.a until a volume of 5 ml (1000 µg / ml) was obtained. From a stock solution of 1000µg / ml, dilution of the sample was made to obtain a concentration suitable for calculating IC50.

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b) Preparation of DPPH solution

The DPPH solution used as reagent was prepared at a concentration of 40 mM. Then DPPH is carefully weighed as much as 8 mg, dissolved with methanol to obtain a volume of 50 ml, then 1 ml of DPPH solution is taken and the volume is sufficient to 5 ml in a measuring flask and then the maximum wavelength is measured.

c) Measurement of free radical binding activity

Into a 5 ml measuring flask was put in 100 µl of the sample, 1 ml of DPPH solution was added and the volume was sufficient to 5 ml, then left for 30 minutes at room temperature. Furthermore, the absorbance of the sample was measured at the maximum wavelength of the DPPH solution that had been previously measured. Testing of each sample concentration was carried out with three replications.

Total Flavonoid

a) Test solution for simplicia

Weigh carefully approximately 1 g of simplicia powder, put it in an Erlenmeyer flask, add 25 mL of ethanol P, extract for 1 hour with a magnetic stirrer. Filter into a 25 mL flask, add ethanol P through the filter until marked.

b) Test solution for extracts

Weigh carefully approximately 0.2 g of the extract, put it in an Erlenmeyer flask, add 25 mL of ethanol P, stir for 30 minutes with a magnetic stirrer. Filter into a 25 mL flask, add ethanol P through the filter until marked.

c) Reference solution

Weigh carefully about 10 mg of comparison, put in a 25 mL shaped flask, dissolve and add ethanol P to mark. Make quantitative dilutions if necessary gradually to levels of 25, 50, 75, 100µg / mL.

d) Measurement

Pipette separately 0.5 mL of the test solution and the dilution of the comparison solution, add to each 1.5 mL of ethanol P, 0.1 mL of 10% aluminum chloride, 0.1 mL of sodium acetate IM and 2.8 mL of distilled water. Shake and let stand for 30 minutes at room temperature. Measure the absorption at the maximum absorption wavelength. Take blank measurements in the same way, without adding aluminum chloride. Create a calibration curve and calculate the levels of the test solution.

Total Polyphenol

a) Test solution for simplicia

Weigh carefully approximately 1 g of simplicia powder, put it in an Erlenmeyer flask, add 25 mL of methanol P, extract for 1 hour with a magnetic stirrer. Filter into a 25 mL flask, add methanol P through the filter until marked.

b) Test solution for extracts

Weigh carefully approximately 0.2 g of the extract, put it in an Erlenmeyer flask, add 25 mL of methanol P, stir for 30 minutes with a magnetic stirrer. Filter into a 25 mL flask, add methanol P through the filter until marked.

c) Reference solution

Weigh carefully about 10 mg of comparison, put it in a 25 mL shaped flask, dissolve it with methanol P, add methanol P to the mark. Make quantitative dilutions if necessary gradually to levels of 5, 15, 30, 50, 70 and 100 100µg / mL.

d) Measurement

To each 1 mL of the test solution and the dilution of the comparison solution in the test tube, add 5 mL of the dilute folin-ciocalteu LP (7.5% in water). Let stand for 8 minutes, add 4 mL of 1%

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NaOH, incubate for 1 hour. Measure the absorption of each solution at a maximum absorption wavelength of approximately 730 nm. Take blank measurements in the same way, without adding aluminum chloride. Create a calibration curve and calculate the levels of the test solution.

3. Results And Discussion

Yield and Water Content of Kersen Leaf Simplicia

The manufacture of cherry leaves simplicia has been carried out in this study and the results obtained that the drying time of cherry leaves using a blower oven and a vacuum oven at a temperature of 40^{o}C to reach the moisture content according to the specified standard takes 6 hours.

The yield value of simplicia obtained for both treatments was 29.20% for drying the blower oven, while for drying the vacuum oven was 31.54%. In addition, based on the results of the water content test, it was found that the water content in the simplicia was in accordance with the

Table 1. The results of the simplicia analysis of cherry leaves (Muntingia calabura L.)

Yield after washing	The Yield (%)		Water Content
(%)	Blower drying	Vacuum drying	(%)
96	29,20	31.54	6-7

quality requirements, which is ≤ 10%.(11) The simplicia of cherry leaves has a moisture content of 6-7% as seen in Table 1.

Antioxitant Activity

Antioxidants are chemical compounds that can donate one or more electrons to free radicals, so that these free radicals can be reduced. The method of testing antioxidant activity used in this study is the DPPH (1,1 Diphenyl-2-picrylhidrazyl) method. The results of measurements using the DPPH method show the antioxidant ability of the sample in general, not based on the type of radicals that are inhibited.(12)

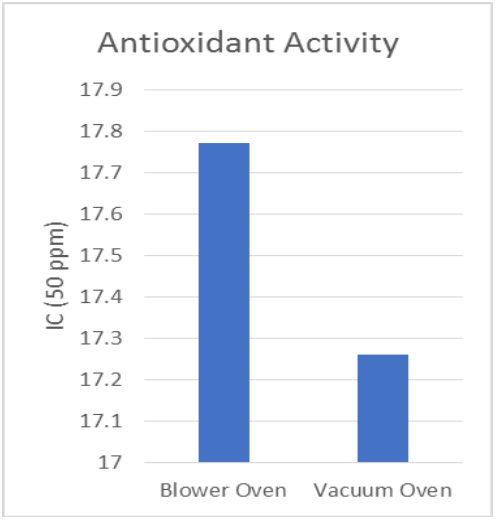

Figure 1. The Simplicia Antioxidant Activity Value of Kersen Leaves Based on the Type of Drying

The principle of measuring the free radical binding activity test is that the antioxidants react or

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reduce the radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and turn it into yellow measured by spectrophotometer as the activity of free radical binding.(13) The results of the antioxidant activity test for the simplicia of cherry leaves can be seen in Figure 1.

Based on the results of the T test, it was found that the type of drying used in this study had no significant effect on the antioxidant activity of the simplicia of kersen leaves. IC50 is a parameter indicating antioxidant activity, IC50 is determined from each extract in order to obtain the amount of extract dosage which can reduce DPPH free radical scavenging 50% in which is calculated linearly. The smaller the IC50 value, the higher the antioxidant activity will be.(14)

The results of the antioxidant activity test for the simplicia of kersen leaves showed that the antioxidant activity in the vacuum oven drying treatment was better than the blower oven. In the vacuum oven drying treatment, the IC50 value was 17.77% and the blower drying treatment was 17.26%. The analysis showed that the intensity of antioxidant activity for both treatments was very strong.

Compounds are considered to have “strong” antioxidant activity if the IC50 value is less than 50 ppm, “active” if the IC50 value is between 50-100ppm, “moderate” if the IC50 value is between 101-250 ppm, “weak” if the IC50 value is between 250-500 ppm and “inactive” if the IC50 value is more from 500 ppm. Referring to this limitation, it can be stated that the two types of drying treatments of kersen leaves can be said to have strong antioxidant activity.

Oxidative stress is known to be an etiology for diabetic complication such as diabetic nephropathy. Hence antioxidant treatment become a potential therapeutic for these complications.(15) A high antioxidant diet was also shown to reduce risk of type 2 diabetes mellitus type 2 in some studies.(6,16) One example of antioxidant that acts as a plasma antioxidant is vitamin E, which can reduce Malonyaldehyde (MDA) and thiobarbituric acid reactive substances (TBARS) in type 2 Diabetes Mellitus Patients in a meta-analysis.(17) Kersen Leaves was known for its strong antioxidant effect.(18)

Total Flavonoids

Analysis of flavonoid levels is aimed to determine the amount of flavonoid levels that are still contained in the resulting binahong leaf powder. The amount of flavonoid levels affects the antioxidant activity of the resulting binahong leaf powder. The test results for the total flavonoids simplicia of kersen leaves can be seen in Figure 2.

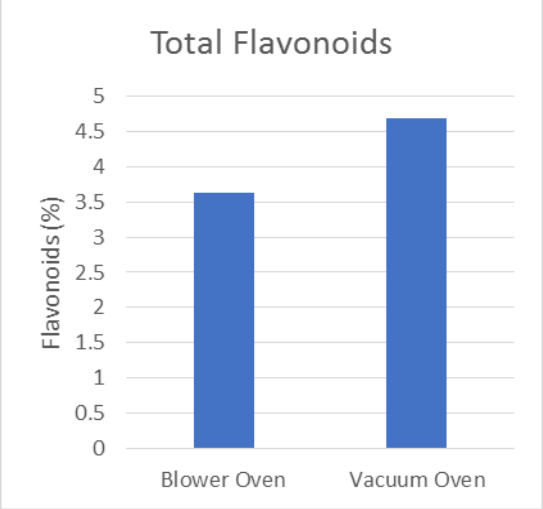

Figure 2. Total Flavonoid Levels Simplicia Leaf Kersen Based on Drying Type

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The T test results showed that the type of drying treatment on the simplicia of kersen leaves had a significant effect on the total levels of flavonoids. The highest levels of total flavonoids were produced in samples with drying treatment using a vacuum oven, namely 4.686%; for treatment with drying oven blower that is 3.615%. Based on the test results of total flavonoid levels, which can be seen in Figure 2, it shows that there was a decrease in the total levels of flavonoids simplicia leaves in cherry blower drying treatment.
Kersen Leaves might have antidiabetic effect through glucose homeostasis due to its flavonoid content. Some possible underlying mechanisms are increasing insulin secretion and inhibition in alpha-glucosidase. These mechanisms can maintain glucose level to be normoglycemia.(19) Another possible explanation, flavonoid in Kersen may act as a scavenger for free radical produced in the body.(20)

Total Polyphenols

The total polyphenol level was tested by using the spectrophotometer method. The results of testing for total polyphenol levels in the simplicia of cherry leaves with two types of treatment can be seen in Figure 3.
The T test results showed that the type of drying treatment had no significant effect on the total polyphenol content. Based on the results of the total polyphenol content comparison, which can be seen in Figure 3, it shows that the total polyphenol content in the vacuum oven drying treatment was higher than the treatment with blower oven drying. The total polyphenol content produced in this test was a sample with a blower oven drying treatment, which is 14.85%; and samples with vacuum oven drying that is 16.21%.

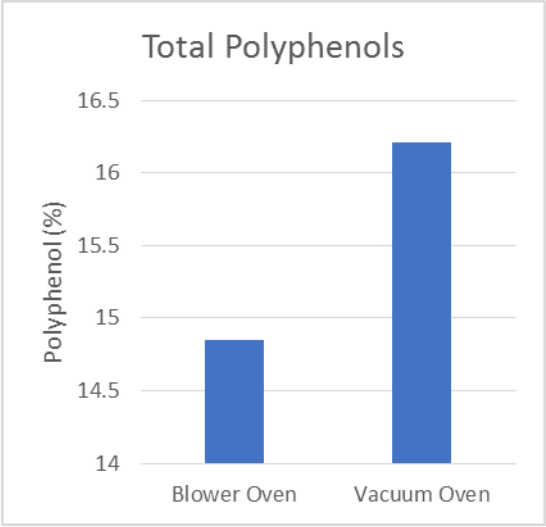

Figure 3. Total levels of symptomatic polyphenols in cherry leaves based on type of drying

The total polyphenol level obtained is directly proportional to the flavonoid level in the sample, this is because flavonoid compounds are one type of polyphenol component antioxidant, hence that treatment containing higher levels of total flavonoids will also produce high polyphenol levels. This findings was in accordance with the results of the Proceedings of the Seminar (1996), which states that polyphenols are one of the antioxidant substances found in food ingredients.(21) The antioxidant compounds that are included in the components of polyphenols are flavonoids, flavones, flavonols, heterosides flavonoates, kalkon auron, biflavonoids.

Intake of polyphenols are known to decrease type 2 Diabetes Mellitus risk and be beneficial for the patients and can improve respiratory health.(25,26) Three possible mechanisms for its antidiabetic properties are anti-inflammation, inhibition of glucose absorption and alleviating insulin resistance.(22) Its benefit has been proven in both human studies and *in vitro* studies. Bioactive polyphenols are found in fruit and vegetables.(23) Kersen Leaves also contain metabolites polyphenols and had been proven to take a role as an α-gluvosidase enzyme.(24)

4. Conclusions

The type of drying treatment on the simplicia of kersen leaves had a significant effect on the total levels of flavonoids but did not significantly affect the antioxidant activity and total polyphenol levels. Moreover, the analysis showed that the intensity of antioxidant activity for both treatments was very strong. Thus, kersen leaves have the potential to be used as an alternative anti-diabetic treatment. Vacuum Oven is a better drying method to extract Kersen Leaves in terms of flavonoid and polyphenol contents, while both treatments did not show any significant difference in terms of antioxidant content.

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