

Effect of Drying Method on Antioxidant Activity and Total Flavonoid Content of Java Tea Crude Drug (*Orthosiphon aristatus*)

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ABSTRACT

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Java tea (*Orthosiphon aristatus*) is a medicinal plant with antioxidant properties due to its polyphenols, flavonoids, phenolic acids, terpenoids, and sinensetin content. Numerous diseases, including hepatitis, diabetes, hypertension, and others, are commonly treated with Java tea. These bioactive compounds are unstable and readily degraded by oxygen, heat, and light. Inappropriate dehydration techniques may compromise the quality of the bioactive compounds in herbal Java tea crude drugs by damaging the bioactive compounds. Consequently, this study aims to determine the effect of various drying methods on the antioxidant activity and Total Flavonoid Content (TPC) of the crude drug of Java tea. In addition, the physical properties, including water content and hygroscopicity, are investigated. The method employs cabinet drying at 30 and 70 degrees Celsius, infrared drying, and sun drying. The results demonstrated that the crude drug dried in a cabinet at 30°C had the maximum flavonoid content ($61.03 \pm 5.35 \text{ mg QE/g}$) and moisture content ($15.12 \pm 0.27\%$). The antioxidant activity of DPPH free radical scavenging and Ferri Reduction Antioxidant Power (FRAP) of Java tea from infrared drying was lower than other drying methods. All samples were non-hygroscopic powders. Consequently, the dehydration method significantly affects the antioxidant activity, TFC levels, and physical properties of the crude drug of Java tea.

Keywords: DPPH, FRAP, cabinet drying, infrared drying, sun drying

1. INTRODUCTION

Java tea (*Orthosiphon aristatus*) is a medicinal plant belonging to the *Lamiaceae* species [1]. This type of plant is known as *Misai Kucing* in Malaysia and *Java Tea* in Europe and is one of the most popular medicinal plants growing wild in tropical countries [2]. Java tea contains various substances, namely potassium, polyphenols, flavonoids, phenolic acids, terpenoids, sterols, and sinensetin, which contain antioxidants [3][4]. Some medicinal plants contain flavonoids believed to have antioxidant, anti-bacterial, anti-viral, anti-inflammatory, anti-allergic, and anticancer activities. According to Hossain in Surahmaida [5], Java tea is widely used to treat diseases such as edema, hepatitis, jaundice, hypertension, diabetes, rheumatism, influenza, and other conditions.

Flavonoid compounds found in Java tea leaves are unstable and easily degraded. Degradation occurs due to temperature, oxygen content, and light [6]. Flavonoid degradation occurs

due to the breaking of molecular chains and the occurrence of oxidation reactions which cause the oxidation of hydroxyl groups and will quickly form other volatile compounds [6].

How does the drying process of herbs affect the content of chemical compounds in plants, especially those responsible for antioxidants? The stability of the drying method can affect the total phenolic and flavonoid content in a plant with stable antioxidant activity[7], especially phenolic and flavonoid compounds.

Improper drying methods will cause losses, including loss of shape, appearance, and quality characteristics. To avoid damage to bioactive compounds, especially flavonoids, a drying method is needed to protect thermolabile bioactive compounds [8]. Therefore, it is necessary to study the effect of the drying method to determine the antioxidant activity and total flavonoid content of

Java tea crude drug, as well as the moisture content and hygroscopicity of samples.

2. MATERIALS AND METHODS

2.1 Materials

In this experimental study, we use java tea leaves, DPPH, ethanol, Trolox, TPTZ, CH₃COONa, FeCl₃, HCl, Folin Cocalteu, NaOH, gallic acid, AlCl₃, quercetin, Cabinet Dryer, IR Dryer, Incubator shaker, spectrophotometer UV-Vis, analytical balance and glassware.

2.2 Preparation of the drying

The Java tea leaves are washed thoroughly with running tap water and separated from the stems, then dried using the sun for 3-5 days (8 hours drying every day), IR drying at 30°C for 5-8 hours, Cabinet Dryer at 30°C for 12-24 hours, and at 70°C for 5 hours. The dried java tea leaves are then blended into a powder. The powder is ready to be further analysis.

2.3 Moisture content analysis.

Moisture content was analyzed according to the AOAC method. Approximately 1 gram of dry crude drug was put into an empty pan, weighed, then put into the oven and heated at 105°C for 4 hours. The sample is considered with the pan and continued drying until the weight is constant.

2.4 Hygroscopicity analysis

Hygroscopicity was measured using a modified version of Cai and Corke's method. One gram of powder was deposited on a crucible and placed in a hermetic desiccator containing a saturated NaCl solution (75% RH) for one week at 25±5°Celsius. After a week, the powder was weighed, and the water absorbed was expressed in grams per 100 grams of dry solids. The weight difference was calculated to determine the hygroscopicity of the java tea crude drug.

2.5 Antioxidant activity analysis

Approximately 0.4 grams of dry crude drug was put into the maceration container (small bottle) and added with 10 ml of ethanol, then extracted using an incubator shaker for 1 hour at 150 rpm. The extract was filtered, and the supernatant was used for further antioxidant activity and TFC analysis.

DPPH free radical scavenging was measured by a mixture of 500 µl of the supernatant and 5000 µl of 25 µg/ml DPPH solution in an amber bottle. The mixture was shaken and incubated for 30

minutes at room temperature and protected from light. Absorbance was measured using a UV-vis spectrophotometer at a wavelength of 517 nm.

FRAP method was analyzed by adding a supernatant of 210 µl into 4000 µl of FRAP reagent, and then the mixture was shaken and left for 30 minutes at room temperature. Absorbance was measured using a UV-vis spectrophotometer at a wavelength of 594 nm.

The DPPH free radical scavenging and FRAP method's antioxidant activity was measured by the Trolox solution standard curve at concentrations of 0-400 µM. It was reported as mM Trolox equivalent (TE)/ gram crude drug.

2.6 Total Flavonoid Content (TFC) analysis

In the TFC method, 0.5 milliliters of extract or standard quercetin solution was combined with 1.5 milliliters of ethanol, 0.1 milliliters of 10% aluminum chloride, 0.1 milliliters of 1M sodium acetate, and 2.8 milliliters of water. After 30 minutes at ambient temperature, the absorbance of the mixture was measured at 425 nm. The standard curve equation was derived from 0-250 ppm Quercetin solutions, and the TFC was expressed as mg quercetin equivalent (QE)/g dry crude drug.

2.7 Data Analysis

The one-way ANOVA and Duncan's post-hoc test were used to evaluate and compare the effects of drying methods on Java tea crude drug characteristics, including moisture content, hygroscopicity, TFC, and antioxidant activity. IBM SPSS Statistics version 26.0 (IBM, United States) established p <0.05 as the significance level

3. RESULTS AND DISCUSSION

3.1 Physical properties of Java tea crude drug from different drying methods

In this study, java tea leaves were used as a sample. The drying process is a very important activity because it can affect the quality of the product produced. The main purpose of drying is to reduce the moisture content of raw materials so that the growth of microorganisms can be inhibited [9]. The dried powdered java tea was measured for its water content using the AOAC method. Based on SNI: (01-7085-2005) for powder, the maximum moisture content of the crude drug is 10% [10].

Table 1. Physical characteristics of Java tea crude drug

Drying methods	Physical characteristics			
	Moisture content (%)		Hygroscopicity (g/100 g)	
Sun drying	9.90	$\pm 0.32^{\text{bc}}$	6.40	$\pm 0.30^{\text{a}}$
Infrared drying	11.00	$\pm 1.55^{\text{b}}$	6.26	$\pm 0.34^{\text{a}}$
Cabinet drying at 30 C	15.12	$\pm 0.27^{\text{a}}$	6.30	$\pm 0.10^{\text{a}}$
Cabinet drying at 70 C	9.31	$\pm 0.15^{\text{c}}$	6.62	$\pm 0.22^{\text{a}}$

Different alphabets on each column represented statistically different values, with $p \leq 0.05$.

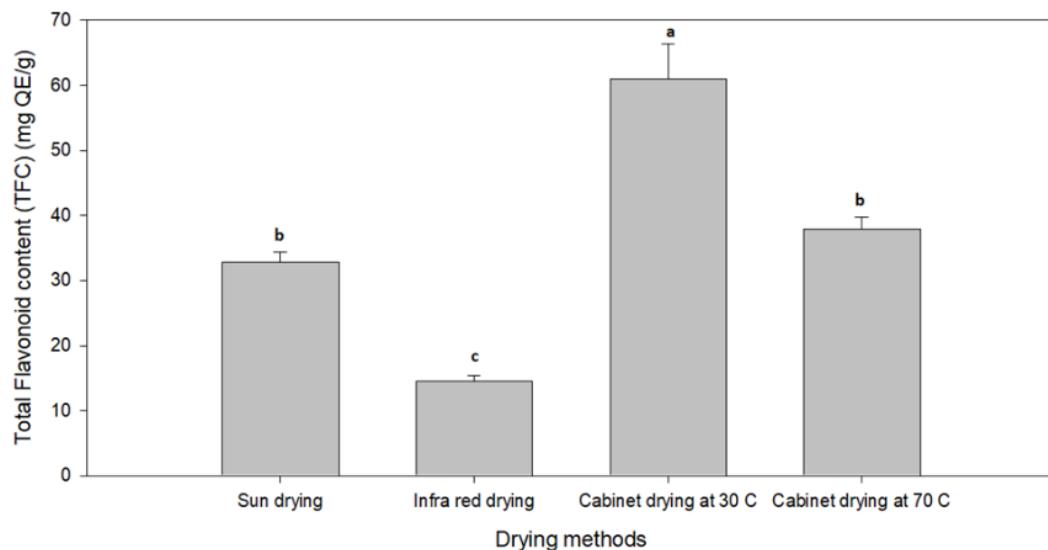


Figure 1. Total Flavonoid Content of Java tea crude drug in different drying methods. Different alphabets on each bar represented statistically different values, with $p \leq 0.05$.

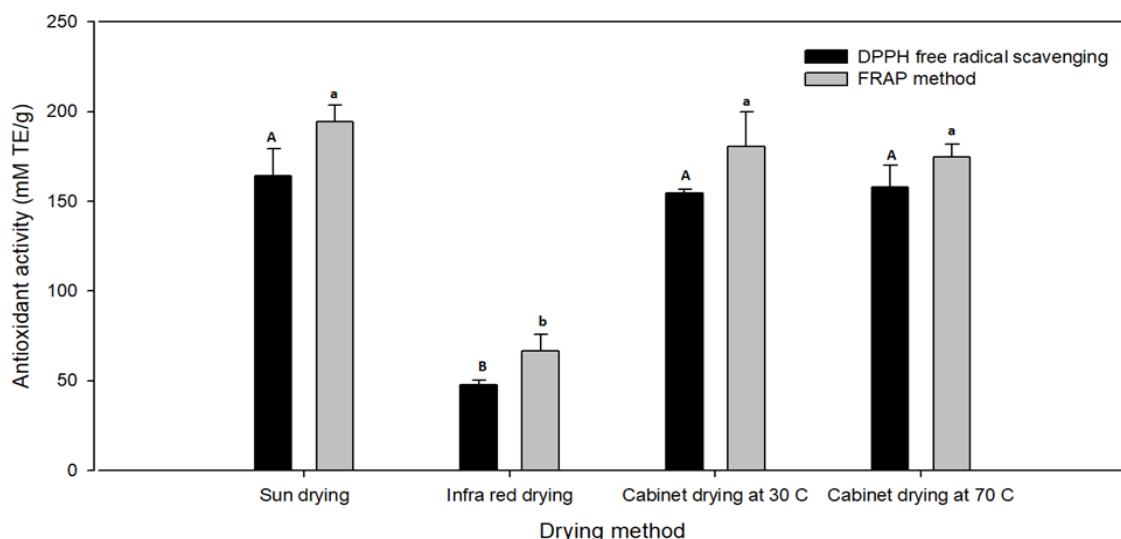


Figure 2. Antioxidant activity using DPPH free radicals scavenging and FRAP method. Different alphabets on each bar represented statistically different values, with $p \leq 0.05$

Statistical test results showed that variations in drying methods significantly affected the moisture content of the Java tea crude drug. Based on the data in **Table 1**, it is known that the water content in the samples using the drying method using cabinet drying at 70°C has the lowest water content, namely 9.31%, a significant effect when compared to 30°C, which has the highest moisture content, namely 15.12 %. It shows that the higher the temperature in the drying process, the greater the heat energy carried by air, so the greater the volume of liquid that evaporates from the material's surface during the drying process.

3.2 The Total Flavonoid Content of Java tea crude drug from different drying methods

The total flavonoid content of the java tea crude drug can be seen in Figure 1. The results showed that the difference in the drying method significantly affected the flavonoid content of the java tea. The highest average value of total flavonoids was obtained in the cabinet drying treatment at 30°C, namely 60 mg QE/g. The lowest average value is found in the infrared drying treatment. In the drying treatment with the cabinet drying method, there was a tendency for the temperature to be too high to produce a lower total flavonoid. This phenomenon also confirmed the previous research conducted by Kusuma and Martini [2][10] that high drying temperatures lead to lower flavonoid content because exposure to heat can damage some of the flavonoid components in the dry materials.

According to Sutjipto (2009) in Astuti [8], in the physicochemical study of Java tea leaves, it was stated that the highest flavonoid content was obtained from the drying treatment in the air. It is possible because flavonoid compounds are active compounds sensitive to temperature (thermolabile) [8]. The drying temperature of the cat's kumis leaves can affect the total flavonoid content contained in the cat's kumis leaves. The drying temperature of 30°C is optimal for obtaining crude drugs with a high content of flavonoids [3].

3.3 Antioxidant activity of Java tea powder from different drying methods

Figure 2 shows the ability to scavenge DPPH free radicals from java tea crude drug with various drying methods. The ability of Java tea powder to capture DPPH free radicals is greater than the FRAP method. The lowest antioxidant activity value based on the DPPH free radical scavenging and the FRAP methods was found in the drying treatment with the infrared drying method, namely

50 Mm TE/g and 60 Mm TE/g. In contrast, the highest average values were found in the drying treatment with the sun drying process, namely 160- and 180-mm TE/g, which were not significantly different from the drying treatment with the cabinet drying method at 30 or 70°C.

Rusnayanti's publication (2018) shows that antioxidant activity decreases if the drying temperature is too high. The drying temperature is too high, destroying secondary metabolites that act as antioxidants [11]. The low value of antioxidant activity in the infrared drying method corresponds to the low value of the total flavonoids of Java tea. According to Pramono (2006) in Martini [10], ingredients with a high enough water content are susceptible to damage. In addition, the relatively high water content in the raw material encourages certain enzymes to exert their action to change the chemical composition of the material into other products [10]

4. CONCLUSION

It can be concluded that the differences in the drying method of Java tea (*Orthosiphon aristatus*) leaves have a significant effect on the flavonoid content and antioxidant activity both using DPPH free radical scavenging and FRAP method. Even though cabinet drying at 30°C gives the better-preserved flavonoid content, the moisture content resulted in a higher than 10%. Only using IR drying has shown lower antioxidant activity compared to the other methods.

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