Total phenolic and flavonoids content, and antioxidant activity of kratom (Mitragyna speciosa Korth.) leaf ethanol extract

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Abstract: This study aims to determine and compare antioxidant activity, total phenolics, flavonoids, and the relationship of phenolic and total flavonoids with the antioxidant activity of the three kratom variants. The ethanol extract of the third variant of kratom leaves was obtained by maceration. Through the antioxidant activity test using DPPH method, all three extracts showed strong antioxidant activity with IC50 values of 26.39, 30.25, and 30.59 μg/mL. The relationship of total phenolics and flavonoids with antioxidant activity was determined by the Pearson correlation test. Examination of total phenolic content using the Folin-Ciocaltelcu method with successive results for green, red, and white kratom are 6.11, 8.67, and 9.09 mg GAE/g extract. Examination of total flavonoid content using the colorimetric method for green, red, and white kratom were 0.86, 0.68, and 1.13 mg QE/g extract, respectively. The total phenolic content and antioxidant activity showed a correlation coefficient value of -0.32 (P>0.05), and the total flavonoid content to antioxidant activity showed a correlation coefficient value of 0.81 (P<0.05). Thus, all three variants of kratom have potency as natural antioxidants, but their total phenolic and flavonoid content does not influence their antioxidant activity.

Keywords: Mitragyna speciosa Korth, phenolic content, flavonoid content, antioxidant activity

INTRODUCTION

Antioxidants are compounds that can reduce the impact of ROS or reactive oxygen species and free radicals and have the ability to act as anti-cancer, anti-aging and prevent heart disease [1]. The formation of free radicals can be inhibited by antioxidants by stabilizing these free radical compounds [2]. Free radicals are defined as atoms or molecules that are unstable, short-lived and reactive, attracting electrons from other molecules in the body to reach a stable state that has the potential to damage the integrity of lipids, proteins and DNA [3]. Decreased antioxidant capacity and increased free radical production can cause oxidative stress. Oxidative stress plays a major role in causing several diseases such as hypertension, diabetes, coronary heart disease, arteriosclerosis, metabolic syndrome, kidney dysfunction, pulmonary insufficiency, rheumatoid arthritis, inflammatory bowel disease, and neurodegenerative diseases such as Parkinson's and Alzheimer [4]. One source of exogenous antioxidants that can play a role in trapping free radicals is plants. One plant that is proven to have antioxidant activity is kratom (Mitragyna Speciosa Korth.) [2]. Kratom is an endemic plant from Putussibau, West Kalimantan. Kratom can be classified based on the color of the veins or veins of the leaf bones, namely green, red, and white kratom [5]. Part of the kratom plant that is often used is the leaves. Kratom leaves are commonly consumed as tea, chewed directly, or made as cigarettes. Kratom is usually sold in capsule form, dried leaves, and flour (powder) [2]. The results of phytochemical screening of green kratom ethanol extract showed the presence of alkaloid compounds, flavonoids, phenolics, triterpenoids or steroids, saponins, and tannins [6]. Meanwhile, mitragynine and 7-hydroxy mitragynine are known to be the alkaloid compounds with the largest percentage in green kratom leaves [7]. The results of the antioxidant activity test on the same extract showed that green kratom ethanol extract has antioxidant activity using the DPPH or 1,1-diphenyl-2-picrylhydrazyl method with a strong category indicated by an IC50 value of 38.56 g/mL [6]. In this research, the DPPH method will also be
used to analyze antioxidant activity because of the efficiency and sensitivity of this method in evaluating antioxidant activity. A literature review revealed no research has ever been conducted on antioxidant activity and total phenolics in 2 other kratom variants. As part of the study of secondary metabolites and bioactivity of kratom plants, this study will be conducted related to the total phenolic and flavonoid content and their correlation with the antioxidant activity of three kratom variants, namely green, red, and white.

**METHODOLOGY**

The simplicia of the three kratom leaf variants used in this research were obtained from PT. Kreasi Alam Borneo. The simplicia comes from local farmers in the Putussibau and surrounding areas whose quality has been processed and tested in the laboratory. Simplicia of the three kratom leaf variants has been declared free from fungal or mold contamination.

**Extraction of Kratom Samples**

The 200 g of kratom leaf powder (green, red, and white powder) (Figure 1) was macerated using a solvent of 96% ethanol at room temperature for 72 hours, and stirring was carried out every 1x24 hours. The extract obtained was then concentrated by evaporating the solvent through a water bath at 50 °C until a thicker extract was obtained.

**Determination of Antioxidant Activity**

Testing antioxidant activity in this study referred to the Masriani & Fadly method (2020) [8]. Ethanol extract of green, red, and white kratom leaves (1 mL) with a concentration range of 15.625-500 μg/mL mixed with 0.1 mM (3 mL) DPPH solution. The negative control was a mixture of DPPH solution (3 mL) and 96% ethanol (1 mL), while the positive control was ascorbic acid. Next, the mixture was incubated for 30 minutes in a dark place. Its absorbance was measured at a wavelength of 517 nm. The ability to ward off free radicals was calculated using the following formula

\[
% \text{ inhibition} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100\%
\]

The linear regression equation would be obtained by plotting between percent inhibition and concentration. The linear regression equation obtained is used in calculating the value of IC\textsubscript{50}, where the value of y is replaced by 50, and the value of x will be found as the value of IC\textsubscript{50} [9].

**Determination of Total Phenolic Content**

Testing of total phenolic content was carried out using the Folin-Ciocalteu method with reference to Zuraida et al. (2017)[10] with modifications. A total of 10 mg of kratom leaf ethanol extract was dissolved in each 25 ml of 96% ethanol. Then Follin-Ciocateu reagents (0.5 mL) and aquades (5 mL) were added to each sample. The mixture is left for 5 minutes. Next, 7% Na2CO3 (1 mL) is added and homogenized using a vortex. The mixture is incubated in a dark place for 60 minutes. The wavelength used to measure absorbance is 725 nm. The standard solution's concentration variation is 15.62 ppm - 500 ppm. The phenolic compound content of each sample is expressed in units of mg GAE/g extract and calculated based on the standard gallic acid curve (Figure 1). The calibration curve obtained from the measurement of a standard solution of gallic acid yields the regression equation \[y = 0.0049x + 0.0036\].
Determination of total flavonoid content

Determination of total flavonoid content was carried out using colorimetric methods referring to Ahmad et al. (2015)[11] with modifications. A total of 100 mg of each sample was dissolved into aquades (100 mL). Then, each sample was taken (0.1 mL) and added 10% AlCl$_3$ (0.2 mL), potassium acetate 1 M (0.2 mL), and aquades up to a volume of 10 mL. The wavelength used to measure its absorbance is 435 nm. The flavonoid compound content of each sample is calculated based on the quercetin standard curve, with concentration variations of 1 ppm – 30 ppm. The total flavonoid content was expressed in mg QE/g units of extract. The calibration curve obtained from the measurement of a standard solution of quercetine yields the regression equation $y = 0.0527x - 0.0344$ (Figure 2).

Determination of the relationship of total phenol content to antioxidant activity

The correlation between total phenolic and flavonoid content and antioxidant activity was tested using a correlation test. The Pearson Product Moment correlation test was used to determine whether there was a significant correlation between them.

RESULTS AND DISCUSSION

Extraction

The study tested antioxidant activity, determination of total phenolic and flavonoid levels, and correlation of total phenolic and flavonoid with antioxidant activity in three kratom variants. The part of the kratom plant used was the leaf part that has been in powder. Before testing, the three kratom leaf powders were extracted first. Extraction was done to separate the active compound content of a plant [12]. The extraction of three kratom variants was carried out using maceration extraction method. The use of maceration extraction techniques in this study is because this method can extract simplisia active substances optimally and is relatively fast and simple. The maceration method can also prevent the loss of the active substance you want to separate because this method is not heated [13].

The extraction process of three variants of kratom leaf powder was carried out using 96% ethanol solvent. The use of 96% ethanol was due to the nature of this solvent, which can capture most substances ranging from polar, nonpolar, and semipolar [14].

Antioxidant Activity of Ethanol Extracts of Three Variants of Kratom

The antioxidant activity test aims to determine how much potential ethanol extract of the three kratom leaf variants as anti-free radicals. The antioxidant activity test was carried out by the 1,1-diphenyl-2-picrylhydrazil or DPPH method. The DPPH method was used in this study because of its advantages which require few samples to be analyzed, fast, and simple [15]. DPPH will react to compounds that function to donate oxidant compounds to electrons that do not have pairs. When electrons have pairs, the absorbance value will decrease according to the number of electrons they take [16] Electrons donated by DPPH after pairing with free electrons from the sample will result in a decrease in absorbance characterized by a
The smallest phenolic content owned by the green kratom variants. Next followed by red kratom and finally the largest total phenolic content among the other two kratom variants.

Data on the total phenolic content of each extract can be considered in Table 1 which shows white kratom has the largest total phenolic content among the other two kratom variants. Next followed by red kratom and finally the smallest phenolic content owned by the green kratom variant.

The parameter for measuring antioxidant activity in this study was the IC₅₀ value. The value of the regression equation obtained was used in calculating the antioxidant activity of all three kratom leaf ethanol extracts and their comparison with ascorbic acid. The results of antioxidant activity testing on ethanol extracts of three variants of kratom leaves and ascorbic acid can be seen in Table 1.

All three kratom variants show strong antioxidant activity, so the three kratom leaf variants have the potential as antioxidants that can function as an alternative to treat various diseases caused by the impact of free radicals, such as aging, cancer, cardiovascular disease, and neurodegenerative diseases and inflammation [3]. The highest antioxidant activity is the green kratom variant because the green kratom variant is known to show the smallest IC₅₀ value among the other two variants.

### Total Phenolic Ethanol Extract Three Variants of Kratom

The purpose of testing the total phenolic content in the ethanol extract of three kratom leaf variants is to find the total phenolic contained in each extract. The method used in the total phenolic test is the Follin-Ciocalteu method, and then analyzed using a microplate reader. The principle of the method is the formation of blue complexes as a result of the reaction between Folin-Ciocalteu reagents and phenolic compounds, where this reaction is known to occur only in an alkaline atmosphere. The results of measuring the total phenolic content of ethanol extracts of three kratom leaf variants are presented in Table 1. The measurement results on these are obtained from the calculation of the linear regression equation of the standard curve of gallic acid.

Data on the total phenolic content of each extract can be considered in Table 1 which shows white kratom has the largest total phenolic content among the other two kratom variants. Next followed by red kratom and finally the smallest phenolic content owned by the green kratom variant.

### Total Flavonoid Ethanol Extract Three Variants of Kratom

Testing the total flavonoid content is intended to determine the total flavonoid content contained in the ethanol extract of three variants of kratom leaves. A colorimetric method using AlCl₃ reagents was used in this study. The principle involved in the colorimetric method with aluminum chloride (AlCl₃) reagent is that AlCl₃ forms a stable acid complex with a C-4 keto group and a C-3 or C-5 hydroxyl group from flavone and flavonol compounds which are flavonoid groups which then form compounds with stable yellow properties [18]. In addition, AlCl₃ also forms labile acid complexes with ortho-dihydroxy groups on the A ring or B ring of flavonoid compounds. Quercetin solution was used as a standard solution because quercetin is a flavonoid of the flavonol group, which has a keto group on carbon atom number 4 and also a hydroxyl group on carbon atom number 3 and carbon atom number 5 that are adjacent so that when reacted it can form a color complex with AlCl₃. During testing, Mg and HCl were added, which have a function to reduce the core of benzopyrone so that it can form a color complex [19].

The results of measuring the total flavonoid content of the three extracts based on the calculation results were for green kratom of 0.86 mg GAE / g extract, red kratom of 0.68 mg GAE / g extract, and white kratom of 1.13 mg GAE / g extract.

### Correlation of Phenolic and Flavonoid Total Ethanol Extract of Three Kratom Variants

After obtaining the total phenolic and flavonoid content, the correlation or relationship between total phenolic levels to antioxidant activity and total flavonoids to antioxidants was analyzed. This correlation test was intended to determine the relationship between total phenolic and flavonoid levels with antioxidant activity in ethanol extracts of the three kratom leaf variants. The correlation test results obtained showed the Pearson correlation value between total phenolics and antioxidant activity, which was -0.315 (P>0.05). This shows that the antioxidant activity of the three variants of kratom leaves is not influenced by phenolic compounds. The correlation results show a significant value of 0.796, meaning greater than 0.05, and the value shows that the correlation between total phenolics and antioxidant activity is not significant. It can be seen in Table 1 that the total phenolic content is not inversely proportional to its antioxidant activity. The correlation between total flavonoids and antioxidant activity of the three kratom leaf variants is not significant. It can be seen in Table 1 that the total flavonoid content of the three variants is not significantly different.

### Table 1. Antioxidant activity of three variants of kratom leaf ethanol extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rendemen (%)</th>
<th>TPC mg GAE/g extract</th>
<th>TFC mg GAE/g extract</th>
<th>AA μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Kratom</td>
<td>12.9</td>
<td>8.67 ± 0.0</td>
<td>0.86 ± 0.0</td>
<td>26.39 ± 0.09</td>
</tr>
<tr>
<td>Red Kratom</td>
<td>17.0</td>
<td>6.11 ± 0.0</td>
<td>0.68 ± 0.0</td>
<td>30.25 ± 0.74</td>
</tr>
<tr>
<td>White Kratom</td>
<td>18.7</td>
<td>9.09 ± 0.0</td>
<td>1.13 ± 0.0</td>
<td>30.59 ± 0.59</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.08 ± 0.07</td>
</tr>
</tbody>
</table>

Note: ± Deviation standard, n=2, TPC = Total Phenolic Content, TFC = Total Flavonoid Content, AA = Antioxidant Activity

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variants obtained a Pearson correlation value of 0.808 (P<0.05). The positive correlation value means that the greater the total flavonoid content, the greater the IC\textsubscript{50} value, and the greater the IC\textsubscript{50} value indicates the smaller the antioxidant activity.

A study [20] states that the contribution of phenolic compounds affects antioxidant activity. However, in this study, total phenolics and antioxidant activity do not correlate significantly. Related research on kratom leaves states that the main chemical content of kratom leaves is alkaloid compounds, where the main content of kratom leaves is mitragynine as much as 66.2% and 7-hydroxy mitragynine 2.0% [21]. Both compounds are indole alkaloid group compounds. Alkaloid compounds have pharmacological effects as antioxidants because alkaloids can reduce the impact of free radicals. Alkaloid compounds also fight microbial infections and spur the nervous system [22]. Mitragynine has high antioxidant activity. Although monoterpenoid alkaloids do not contain phenolic structures so they do not assist in strong hydrogen donation in the antioxidant pathway, monoterpenoid alkaloids have an antioxidant mechanism through hydroxyl groups on the indole (7-hydroxy mitragynine), methoxy groups on the aromatic ring, other alkyl groups on aliphatic esters/ethers, or more dominant H-donation from N-H on the indole ring [23]. In line with this, the strong antioxidant activity in the three kratom variants is thought to be influenced by the content of alkaloid compounds as the largest secondary metabolite content of kratom leaves.

This study examines the the antioxidant activity of the three kratom variants using the DPPH method and correlation between phenolic content and total flavonoids on antioxidant activity in the three kratom variants with results as described. In testing antioxidant activity, the use of the DPPH method itself has several drawbacks, one of which is that in complex samples, it is difficult to show which compound is responsible for the antioxidant effect. This is because the DPPH method relies on the absorption of DPPH free radicals at a wavelength of 517 nm. However, after reacting with antioxidants, there was a decrease in absorption due to the formation of 2,2-diphenyl-1-picrylhydrozfine. In addition, the possibility of the presence of compounds that absorb in the same wavelength range as DPPH could be a significant source of bias [24]. Further research on the correlation of the content of other secondary metabolites with the antioxidant activity of kratom leaves, especially alkaloid compounds, which are the largest secondary metabolite content of kratom leaves, can be a recommendation for future research.

CONCLUSION

The ethanol extract of the three kratom variants, namely green, red, and white, have antioxidant activity with strong categories with IC\textsubscript{50} values of 26.39 ± 0.06, 30.25 ± 0.74, and 30.59 ± 0.59 μg/mL. The green kratom has the highest antioxidant activity among the three kratom variants. Then, there was no significant correlation or relationship between total phenolic and total flavonoid content on antioxidant activity in ethanol extracts of three kratom variants.

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