Inhibition of α-Glucosidase Enzyme by Ethanol Extract of Kratom Leaf Variant (Mitragyna speciosa Korth.)

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Abstract
Diabetes mellitus characterized by acute hyperglycemia has become a worldwide epidemic. One plant in Indonesia that has empirical potential as an antidiabetic is kratom (Mitragyna speciosa Korth.). There are 3 types of kratom variants that are widely used by the public, namely green, red, and white kratom variants. This variant is distinguished by the presence of veins on kratom leaves. However, antidiabetic research with the mechanism of inhibition of the enzyme α-glucosidase from kratom leaves red, green, and white variants has not been widely studied. This study aims to determine the inhibitory activity of α-glucosidase enzyme by Kratom Leaf Ethanol Extract (Mitragyna speciosa Korth.) Various variants. Green, red and white variant kratom leaf powder macerated with 96% ethanol for 72 hours, and inhibition of α-glucosidase enzyme activity using ethanol extract and akarbose as positive control. In vitro testing was carried out using a microplate reader with a wavelength of 405 nm. Ethanol extract of kratom leaf red, green, and white variants of 96% at a concentration of 500 μg/ml can inhibit the activity of α-glucosidase enzymes by 10.57%, 13.06%, and 5.33%, respectively. This shows that the three kratom variants have activity as inhibitors of α-glucosidase enzymes but are classified as very weak, with IC50>200 μg/ml. The inhibitory activity of all three extracts differed markedly (p<0.05). This difference can be caused by the content of secondary metabolites in each variant of Kratom.


INTRODUCTION
Diabetes mellitus characterized by acute hyperglycemia has become a worldwide epidemic. Indonesia ranks 5th after India, China, America, and Brazil from the number of people with diabetes mellitus (IDF Atlas., 2022). The International Diabetes Federation (IDF) reports that around 537 million people worldwide suffer from diabetes with a mortality rate of 6.7 million each year (WHO, 2022). This figure is expected to increase to 783 million by 2045. Diabetes poses an economic burden of 966 billion USD or about 17% of the total health budget in the world (IDF Atlas., 2022). The total cost incurred by JKN (National Health Insurance) amounted to 6.1 trillion for the treatment of diabetes and its complications. The cost needed by patients without complications is 5.4 million/person/year and for the treatment of complications is 14 million/person/year (Patty, Mufarrihah, and Nita 2021). This data shows the urgent need to implement strategies to overcome and reduce mortality from diabetes mellitus.

The main and effective strategy to improve the condition of diabetes is to control post-prandial hyperglycemia (Nguelefack et al., 2020). α-glucosidase inhibitors as one of the antidiabetes
mellitus ingredients that work by inhibiting the enzyme α-glucosidase, the main enzyme responsible for the hydrolysis process of oligosaccharides and disaccharides into monosaccharides (Hamid et al., 2015). Inhibition of α-glucosidase in diabetics lowers post-prandial glucose levels by delaying glucose absorption (Mataputun, Rorong, and Pontoh 2013). α-glucosidase inhibitors such as akarbose, voglibose, miglitol, and nojirimicin have been widely used as antidiabetics and have been shown to be effective in reducing hyperglycemia, but have side effects and increase diabetes complications. Given the side effects caused by oral drugs, natural α-glucosidase inhibitors from plants can be used as an alternative to the pharmacological therapy of diabetes mellitus. Some natural plants have shown activity as α-glucosidase inhibitors such as cat's whiskers (Orthosiphon aristatus) (Yuliana et al., 2016), bungur (Lagerstroemia speciosa (L.) Pers) (Riyanti, MA, and E 2021), okra fruit (Abelmoschus esculentus (L) Moench), Dutch teak leaves (Guazuma ulmifolia), black sticky rice (Oryza sativa Var glutinosa) (Budiman, 2011), and Simpur (Dillenia suffruticosa) (Elmaniar and Muhtadi 2017; Mahargyani 2019; Masriani, Fadly, and Bohari 2020; Mohamed et al. 2012). One plant that has potential as an inhibitor of α-glucosidase is kratom.

Kratom (Mitragyna speciosa Korth.) nicknamed the leaf of paradise from Borneo, is a plant from the Rubiaceae family. This plant is widely found in West Kalimantan, especially in Kapuas Hulu Regency. Based on the color of the leaf veins, kratom plants are divided into three variants, namely red kratom, green kratom, and white kratom (Figure 1). The three variants have different activities. The red vein variant tends to be a strong pain reliever, the white vein variant tends to increase endurance, and the green variant tends to increase excitement (Warner, Kaufman, and Grundmann 2015).

Traditionally kratom has been used by the people of Indonesia, Malaysia and Thailand to increase stamina, treat diarrhea, stomach pain, insomnia, cholesterol, gout and diabetes (Prozialeck et al., 2020; Purintrapiban et al., 2011). Several empirical properties have been tested and proven that kratom leaves have pharmacological effects including analgesics (Nugraha, Robiyanto, and Luliana, 2018), antinociceptives (Luliana &; Islamy, 2018), antidiarrheal (Suhaimi Dian, 2020), antibacterial (Suhaimi, Puspasari, and Apriani, 2019), antioxidants (Setyawati, 2020), and have cytotoxic effects on breast cancer cells (Ikhwan, Harlia, and Widiyantoro, 2018).

Kratom leaves are known to have 57 compounds dominated by alkaloids and other compounds including flavonoids, phenolics, triterpenoids, and saponins (Meireles et al., 2019). The content of alkaloids, phenolics and flavonoids is known to have the ability to deal with diabetes problems. Alkaloids work as antidiabetics through various mechanisms including signaling pathways, inhibition, stimulation of various systems such as PTP-1B blockade, improving insulin sensitivity, modulating oxidative stress and inhibition of α-glucosidase enzymes (Ajebli, Khan, and Eddouks 2020). In the study of Yin et al (2014) mentioned that alkaloids and phenolics from several types of extractive substances showed inhibitory activity of α-
glucosidase. Examples are alkaloid compounds isolated from Adhatoda vasica Nees leaves, deoxynojirimycin isolated from adenophores, phagomine alkaloids and cytidine from Orus atropurpurea leaves which show stronger inhibitory potential than akarbose (Zhenhua Yin, Wei Zhang, Fajin Feng, Yong Zhang 2014)

Research on the biological activity of kratom leaves has been widely conducted, including as analgesics, antinociceptives, anti diarrheals, antibacterials, and antioxidants (Meireles et al., 2019; Ramanathan et al., 2021). However, research related to activity on three types of kratom leaves, namely red, green and white kratom as inhibitors of the enzyme α-glucosidase has not existed. Not yet known with certainty the value of inhibition in ethanol extract of kratom leaf powder (M. speciosa Korth.) Green, red, and white variants and which extracts have the best potential in inhibiting the enzyme α-glucosidase. Therefore, research was conducted on the inhibitory activity of the three kratom variants against the enzyme α-glycosidase.

METHOD

Tools and Materials

The tools used consist of drying cabinets, incubators, analytical balances, vortexes, pH meters, centrifuges, microplate readers, cuvettes, microtubes, micropipettes, freezers, and laboratory glassware. The plant material used in this study was green, red, and white kratom leaves (Mitragyna speciosa korth.). The chemicals used are 96% ethanol, Na₂CO₃, dimethyl sulfoxide (DMSO), α-glucosidase enzymes, p-Nitrophenyl-α-D’glucopyranoside (p-NPG), akarbase tablets, and aquades.

Extraction

A total of 200 grams of kratom leaf powder each (green, red and white powder) was macerated with 96% ethanol solvent at room temperature for 3 days and stirring was done every 1x24 hours then the filtrate was separated from the pulp. Repeated extraction process for 2 times in the same way. The result is an extract which is then concentrated using a waterbath at a temperature of 50 °C until a concentrated extract is formed. The extract was then used to test the inhibitory activity of the enzyme α-glucosidase (Masriani et al., 2023).

α-Glucosidase Enzyme Activity Test

Testing the activity of α-glucosidase enzyme refers to research (Masriani et al. 2020). A mixture of 150 L samples with a concentration range of 15.625-500 μg/mL; and 100 μL of 0.1 M sodium phosphate buffer (pH = 6.8) containing the enzyme α-glucosidase (0.1 μg/mL) were incubated for 10 min at 37 °C. After pre-incubation, 200 μL of 1 mM solution pNPG was added in 0.1 M sodium phosphate buffer (pH = 6.8). Next, incubated for 30 minutes at 37°C. After pre-incubation, 200 μL of 1 mM solution pNPG was added in 0.1 M sodium phosphate buffer (pH = 6.8). Next, incubated for 30 minutes at 37°C. Then, 1.0 mL of 0.1 M Na₂CO₃ solution was added. The inhibition activity of the enzyme α-glucosidase was determined by measuring the yellow color of p-nitrophenol released by pNPG at a wavelength of 405 nm (Masriani et al., 2020). Akarbose is used as a positive control. The percentage of inhibition activity of the enzyme α-glucosidase is calculated by the following equation:

\[
%\text{Inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100
\]

The IC₅₀ value of each sample is expressed in a linear regression equation with a y value equal to 50 and the x value to be determined is the value of IC₅₀.
Data Analysis

The data obtained in this study was expressed by averaging ± standard deviations from three tests. Next, it was analyzed using the SPSS program version 21.0 with the ANOVA One Way method and Tukey to determine the average difference among the samples. If a sig value of p <0.05 is obtained, it is stated that there is a significant difference in the sample (Nasution, 2018).

RESULTS AND DISCUSSION

Sample Extraction

The extraction of three dry kratom variants powder was carried out by maceration method. This method uses the principle of solubility, namely polar compounds will dissolve in polar solvents, vice versa nonpolar compounds will dissolve in non-polar solvents (Ministry of Health RI, 2005). The solvent used is 96% ethanol because this solvent is able to dissolve polar compounds, volatile, non-toxic, and the work is safer for all secondary metabolites (Mardhiani et al., 2018). The hydroxyl group in ethanol plays a role in dissolving polar ions and molecules, while the alkyl group is responsible for binding non-polar compounds. This characteristic causes ethanol to dissolve polar and nonpolar compounds well (Bayani, 2016).

The extraction results on the three types of kratom leaves produce different yields, where in green kratom the yield obtained is dark brown with a stickier texture resembling caramel, while in red and white kratom the yield obtained is deep brown and has a texture that is not too sticky and less dense (Figure 2.)

![Figure 2. Kratom leaf extract (a) green kratom, (b) red kratom, (c) white kratom](image_url)

α-Glucosidase Enzyme Activity Test

Diabetes mellitus is an endocrine disease characterized by hyperglycemia. There are 2 types of diabetes that are most commonly suffered, namely type 1 and type 2. As many as 9% of deaths are contributed by type 2 diabetes. This indicates that there is an urgent need to find potential therapeutic agent treatment alternatives (Syed Ahsan Elahi Bukhari, Syed Mubashar Sabir, Shabaz Ali, Ali Turaiib 2022). Inhibition of α-glucosidase enzyme aims to delay glucose absorption in the intestine and reduce postprandial hyperglycemia (Rachmatiah, Nurvita, and D 2018). Akarbose is an enzyme inhibitor compound α-glucosidase which is widely used as an oral antidiabetic and clinically proven. However, this drug has side effects such as triggering hypoglycemia and stomach pain so other alternatives are sought such as α-glucosidase inhibitors from natural ingredients, one of which is kratom leaves which are traditionally used as antidiabetics (Heri et al., 2020; Ningrum et al., 2021a).

The antidiabetic activity of a plant can be carried out by testing the inhibitory ability of α-glucosidase. In this study, the inhibitory activity of ethanol extract was tested for three kratom variants, namely the red, green, and white variants against the enzyme α-glucosidase. The principle of this test is that a substance that acts as an inhibitor will bind to the enzyme α-glucosidase so that the hydrolysis of pNPG substrates (p-nitrophenyl-α-D-glucopyranosida)
into p-nitrophenol will be inhibited. The amount of p-nitrophenol formed will be measured for absorbance using a microplate reader at a wavelength of 405 nm. The smaller the absorbance of p-nitrophenol formed, the greater the inhibitory activity (Tran et al., 2021).

The results of testing the inhibitory activity of ethanol extracts of three kratom variants against the α-glucosidase enzyme showed that the three kratom variants were able to inhibit the activity of the α-glucosidase enzyme. The higher the concentration of the extract, the percentage of inhibition increases. Green kratom ethanol extract showed the highest inhibitory activity against the enzyme α-glucosidase (Figure 3). Sequentially, the percentage of inhibition of kratom leaf ethanol extract against the enzyme α-glucosidase is green kratom>red kratom>white kratom. The inhibitory effect of α-glucosidase enzymes from the same compound can differ depending on substrate level, temperature conditions, pH, incubation time, and enzyme concentration at the time of the enzymatic reaction (Masriani et al., 2020). The enzyme form is maintained at a certain temperature and pH. Changes in temperature and pH can break the intramolecular bonds of the enzyme, allowing it to change its shape. Optimal enzyme activity usually occurs at temperatures of 30 to 40 °C. In the human body, enzymes have optimal activity at pH 6.5 – 7.4 (Sismindari et al., 2016).

The difference in the ability of the three types of kratom variants to inhibit the α-glucosidase enzyme may also be caused by differences in the type and / or concentration of secondary metabolites contained in kratom leaves. According to Sinulungga (2020), the inhibitory activity of α-glucosidase can differ depending on the content of secondary metabolites possessed by the extract. Secondary metabolites with a structure that has an affinity resembling a substrate, will easily occupy the active side of the enzyme α-glucosidase and inhibit the work of the enzyme. Thus, the more similar the structure of secondary metabolites to the substrate, the higher the inhibition obtained (Sinulingga, Subandrate, and Safyudin 2020).

![Figure 3. % Inhibition of Ethanol Extract of Three Variants of Kratom Leaves against α-glucosidase Enzyme of Kratom putih (white veins Kratom), Kratom merah (red veins Kratom), Kratom hijau (green veins Kratom).](image)

The inhibitory activity of enzymes shown by kratom leaves is inseparable from the content of secondary metabolites contained in it. All three types of kratom leaves are known to contain alkaloid compounds, flavonoids, tannins, saponins, phenolics, and triterpenoids (Masriani et al., 2023).

Alkaloids are known to lower blood sugar levels by various mechanisms. Alkaloids can inhibit glucose absorption in the intestine, improve glucose transport in the blood, inhibit blood glucose absorption through inhibition of α-glucosidase, glucose-6-phosphatase, fructose-1,6-
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Inhibition of α-glucosidase enzyme... (Fatmawati, Susilawati, Liniyanti D Os wary, Fadiya 2021). Looking at the weak inhibitory activity, it is suspected that the empirically proven antidiabetic activity of the three kratom variants is not through the mechanism of inhibition of the α-glucosidase enzyme but from various other mechanisms (Sari sasi gendro, 2022).

In addition to alkaloids, the content of secondary metabolites such as tannins, saponins, triterpenoids, flavonoids and phenolics also plays a role in the inhibitory activity shown. Tannins are predators of free radicals and antidiabetic agents with a mechanism of lowering glucose levels in the blood of insulin mediator action (Kumari & Jain, 2012). Flavonoids and phenolics are compounds rich in hydroxyl groups (Wibawa, 2021). The antidiabetic mechanism by this compound occurs through hydroxylation and substitution bonds in β-pancreatic installments (Pratiwi et al., 2021). Oxygen bound to the hydroxyl group can bind to hydrogen from the active side of the enzyme α-glucosidase so that there is an inhibition of glucose production in the blood (Herdien et al., 2020).

When referring to IC50 values (Table 1), all kratom variants show very weak inhibitory activity of α-glucosidase. According to Riyanti et al (2019) the inhibitory activity of a sample is categorized into 5, namely >200 μg/ml (very weak), 150-200 μg/ml (weak), 100-150 μg/ml (medium), 50-100 μg/ml (strong) and <50 μg/ml is very strong. Based on these criteria, the inhibitory activity of red, green, and white kratom leaf ethanol extract against α-glucosidase enzymes is very weak because of the IC50>200 μg/mL value (Riyanti et al., 2019). Akarbose as a comparison showed very strong inhibitory activity and much higher than sample extracts (Table 1). In this study, IC50 akarbose amounted to 0.2058 μg/ml.

The inhibitory activity of the enzyme α-glucosidase kratom leaf extract is much lower than that of akarbose because akarbose is a pure compound, while kratom extract is still a crude extract where the compound content in this extract is still mixed with compounds that may work antagonistically in inhibiting α-glucosidase enzymes (Nguelefack et al., 2020). The low activity of an extract can also be caused by several things such as modifications in testing and the concentration of active compounds contained in the sample (Suprihatin et al., 2020).

Table 1. IC50 Value of Inhibition Activity of α-Glucosidase Enzyme by Kratom Leaf Ethanol Extract (M.Speciosa Korth)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Line Equation</th>
<th>IC50 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Kratom</td>
<td>y = 2.7064ln(x) - 6.2778</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>R² = 0.9949</td>
<td></td>
</tr>
<tr>
<td>Green Kratom</td>
<td>y = 3.2841ln(x) - 8.4683</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>R² = 0.9126</td>
<td></td>
</tr>
<tr>
<td>White Kratom</td>
<td>y = 2.6438ln(x) - 9.4031</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td>R² = 0.964</td>
<td></td>
</tr>
<tr>
<td>Akarbose</td>
<td>y = 13.05ln(x) + 70.627</td>
<td>0.20587</td>
</tr>
<tr>
<td></td>
<td>R² = 0.9982</td>
<td></td>
</tr>
</tbody>
</table>

Kratom has 57 compounds that are dominated by achaloid compounds. The main compounds are indole alkaloids mitragynine (66%) and 7-hydroxy-mitragynine (2%) (Kamble et al., 2021). The results of the study (Purnama Melania, Masriani 2023), the total phenolic levels of green, red, and white kratom were 6.11 mg GAE/g, 8.67 mg GAE/g, and 9.09 mg GAE/g, while flavonoid levels were 0.086 mg GAE/g, 0.68 mg GAE/g, and 1.13 mg GAE/g, respectively. It can be seen that compounds that are thought to play an important role in the inhibitory activity of α-glucosidase, namely flavonoids and phenolics are minor compounds in kratom leaves and are suspected to be the cause of low inhibitory activity against α-glucosidase. In addition, it is
suspected that the mechanism of antidiabetic activity possessed by kratom leaf extract is not through inhibition of the enzyme α-glucosidase, but through other mechanisms. Although empirically kratom leaves are beneficial for the treatment of diabetes, based on these findings, it is necessary to test other antidiabetic mechanisms of kratom leaves (Ningrum et al., 2021b).

CONCLUSION
From the results of the study it was concluded that red, green and white kratom leaves have activity as inhibitors of α-glucosidase enzymes. Of the three kratoms, green kratom has the strongest inhibitory activity compared to red and white kratom, but its inhibitory properties are still very weak, because of the IC50>200 μg / ml value. The results of this study add new information about the inhibitory activity of the α-glucosidase enzyme owned by the three variants of kratom leaves. Based on these findings, it is hoped that it can be a reference for future research to explore the potential of kratom leaves as an antidiabetic.

RECOMMENDATIONS
Based on the results of this study, it is recommended that future studies can test other antidiabetic mechanisms of kratom leaves both in vivo and in vitro.

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