Phytochemical Screening of Ethanol Extracts from Three Variants of Kratom Leaves (*Mitragyna speciosa* Korth.)

Masriani*, Rini Muharini, Dinda Kartika Wijayanti, Purnama Melania, Margareta Lita Widian Sari

1 Chemistry Education Study Program, Faculty of Teacher Training and Education, Tanjungpura University, Pontianak, Indonesia.

* Corresponding Author e-mail: masriani@fkip.untan.ac.id

**Article History**
Received: 31-01-2023
Revised: 05-03-2023
Published: 28-04-2023

**Keywords:** *Mitragyna speciosa* Korth., secondary metabolite, ethanol extract

**Abstract**
Kratom (*Mitragyna speciosa* Korth.) has been traditionally used in the community for medicinal purposes. Kratom leaves have been reported to possess various biological activities such as antioxidant, antibacterial, anti-inflammatory, and antinociceptive effects. The biological activity of kratom leaves is believed to be attributed to the presence of secondary metabolites. The aim of this study was to investigate the content of secondary metabolite compounds in three types of kratom leaves, namely green, red, and white kratom. Each 200 g of kratom leaf powder was subjected to 72 hours of maceration with 1 L of 96% ethanol solvent. The resulting extract was filtered and concentrated using a water bath and an oven at a temperature of 50°C to obtain a concentrated extract. The extract was evaluated for its yield and secondary metabolite content using specific reagents for each test. The results revealed the presence of secondary metabolites such as alkaloids, flavonoids, triterpenoids, phenolics, tannins, and saponins in the red, green, and white kratom leaves. These findings provide a valuable reference for future bioactivity research and identification of the bioactive compounds present in the three variants of kratom leaves.

**INTRODUCTION**

Kratom (*Mitragyna speciosa* Korth.), also known as Purik leaves in West Kalimantan (Adang Firmansyah, Melvia Sundalian, 2021), is a plant widely distributed in Southeast Asian regions including Indonesia, Malaysia, the Philippines, and Thailand. The spread of kratom plants in Indonesia is centered on Sumatra, Sulawesi, Kalimantan, and Papua regions, where it is also referred to as the leaves of paradise (Wahyono et al., 2019). Previous pharmacological studies on kratom leaves have reported various effects, including analgesic, antipyretic, sedative, stimulant, anti-inflammatory, antioxidant, and antimicrobial properties (Dania Cheaha et al., 2015; Griffin & Webb, 2018; Nadya Sodia, Yuniarti, 2022; Norsita Tohar et al., 2016; Reanmongkol et al., 2007; Suhaimi Dian, 2020). Kratom is often used in low doses to enhance concentration, energy, and alertness, and in Europe and America, it is widely used for pain management, emotional and mental conditions, and treatment of opioid withdrawal symptoms (Raini, 2017). The pharmacological effects of kratom are attributed to the presence of secondary metabolites in its leaves.

Kratom leaves are categorized into three types based on their vein color: green, red, and white. Previous phytochemical screening studies of kratom leaves have primarily focused on...
individual variants, such as those conducted by Yuniarti et al. (2022) and Setyawati (2020) (Nadya Sodia, Yuniarti, 2022; Setyawati, 2020). However, no studies have been found that investigate the phytochemical screening of all three kratom variants.

Therefore, the present study aims to conduct a phytochemical screening analysis of the secondary metabolite compounds present in green, red, and white kratom leaves, including alkaloids, flavonoids, tannins, terpenoids, steroids, phenols, and saponins. This study is expected to provide valuable references for future researchers in assessing the bioactivity of secondary metabolite compounds and identifying the types of compounds present in the three kratom variants.

METHOD
This type of research is laboratory experimental research. Phytochemical screening of green, red, and white kratom ethanol extracts. The place of research is the Chemistry laboratory of the Faculty of Teacher Training and Education at Tanjungpura University. This research uses glassware that is often used in chemical laboratories. Mayer's reagent, Wagner, iron(III) chloride, concentrated sulfuric acid, concentrated HCl, chloroform, Liebermann-Burchard reagent, 96% ethanol, and aquades were the materials used in this study.

Sample Preparation
Samples in the form of three types of kratom leaf powder, namely green, red, and white kratom obtained from PT. Kreasi Alam Borneo, Pontianak, West Kalimantan.

Extraction
Each 200 g of kratom leaf powder was macerated with 1 L of 96% ethanol solvent. After maceration for 72 hours, the extraction was filtered so that liquid ethanol extract was obtained (Masriani and Fadly, 2020). The extract was concentrated in a water bath and oven at 50°C until a thick extract was produced (Kusuma et al, 2020). The viscous extract is further used for phytochemical screening. The yield percentage is calculated by the formula: (Nasution, 2018)

\[
\text{%Yield} = \frac{\text{Weight of extract}}{\text{Weight powder}} \times 100
\]

Phytochemical Screening
Phytochemical screening is carried out by referring to several modifications (Riwanti &; Izazih, 2019). Phytochemical screening of extracts is carried out to determine the presence of secondary alkaloid metabolites, flavonoids, tannins, terpenoids, steroids, phenols, and saponins in red, green and white kratom leaves.

Alkaloid Screening
The alkaloid test was carried out by means of a thick extract of kratom leaves dissolved with 1 mL of 96% ethanol then added HCl 2N. The solution is divided into two, tested with Mayer's reagent and Wagner's reagent, respectively. Positive samples contain alkaloids if Mayer's reagent shows a yellow color change accompanied by a white precipitate and with Wagner's reagent to an orange-red color accompanied by the formation of an orange to brownish-yellow precipitate (Lantah et al., 2017).

Flavonoid Screening
Each 10 mg of condensed ethanol extract of kratom leaves was dissolved with 1 mL of 96% ethanol. The solution is then added magnesium powder and two drops of HCl 2 N. Kratom
leaves are declared to contain flavonoids when an orange solution is formed (Anna Hidayati, 2013).

**Triterpenoid Screening**

A total of 1 mL of kratom leaf ethanol extract was added with 2 mL of chloroform and three drops of \( \text{H}_2\text{SO}_4 \) concentrated. Kratom leaves are stated to contain triterpenoid compounds when a brownish-red color is formed between surfaces (Mardhiani et al., 2018).

**Phenolic Screening**

A total of 1 mL of ethanol extract of kratom leaves was reacted with \( \text{FeCl}_3 \) 1%. Samples containing phenol are characterized by the formation of blackish-green to solid black (Riwanti & Izazih, 2019).

**Saponin Screening**

Kratom leaf extract is added in a test tube added 1 mL of aquades and shaken vigorously for 10 minutes until foam forms. Kratom leaves are said to contain positive saponins formed if foam with a height of 1-10 cm that lasts for 15 minutes (Riwanti &; Izazih, 2019).

**Tannin Screening**

Kratom leaf extract is dissolved in aquades then heated and then added \( \text{FeCl}_3 \) 1% solution. The sample tested positive for tannin compounds if a brownish-green or bluish-green color was formed (Riwanti &; Izazih, 2019).

**RESULTS AND DISCUSSION**

**Sample Extraction**

The extraction of three variants of kratom leaves was carried out by maceration using 96% ethanol. This method uses the principle of solubility of the compound from the sample in the solvent used. 96% ethanol is used as a solvent because ethanol is able to dissolve both polar and nonpolar compounds. The hydroxyl group of ethanol will bind to polar molecules and ions, while the alkyl group, \( \text{C}_3\text{H}_2^- \) can bind to nonpolar compounds. Thus, ethanol can dissolve both polar and nonpolar compounds. Another flagship of ethanol is non-toxic, safe for all secondary metabolites, and volatile (Mardhiani et al., 2018).

Table 1. Percent Yield of Kratom Leaf Extract

<table>
<thead>
<tr>
<th>Kratom Variants</th>
<th>Heavy (g)</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dried</td>
<td>Extract</td>
</tr>
<tr>
<td>Red</td>
<td>200</td>
<td>34.0744</td>
</tr>
<tr>
<td>Green</td>
<td>200</td>
<td>25.8401</td>
</tr>
<tr>
<td>White</td>
<td>200</td>
<td>37.4868</td>
</tr>
</tbody>
</table>

The calculation of the proportion of ethanol extract yield of three kratom leaf variants shows that white kratom has the highest yield (Table 1). The difference in yield of the three kratom variants may be caused by differences in the polarity properties of each kratom compound (K.H Row and Jin Y, 2005). Based on yield proportions, it is estimated that white kratom has the most soluble compounds in ethanol.

Ethanol extract testing of three kratom variants showed that red, green, and white kratom leaf extracts contained alkaloids, flavonoids, tannins, saponins, phenolics, and triterpenoids (Table 2). These results are as per previous research showing that kratom leaves contain alkaloids, flavonoids, tannins, phenolics, and triterpenoids (Gogineni et al., 2014).
Table 2. Phytochemical Screening Results of Red, Green, and White Kratom Ethanol Extract

<table>
<thead>
<tr>
<th>Testing</th>
<th>Reagents</th>
<th>Kratom Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Red</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>Mayer</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Shinoda</td>
<td>++</td>
</tr>
<tr>
<td>Steroid</td>
<td>Lieberman-Buchard</td>
<td>-</td>
</tr>
<tr>
<td>Tanin</td>
<td>Heating and FeCl₃</td>
<td>+++</td>
</tr>
<tr>
<td>Saponin</td>
<td>Air + Shaking</td>
<td>+++</td>
</tr>
<tr>
<td>Fenolik</td>
<td>FeCl₃</td>
<td>+++</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>Salkowski</td>
<td>+</td>
</tr>
</tbody>
</table>

Alkaloid Screening

Alkaloid testing is based on precipitation reactions between alkaloid compounds and heavy metal ions. Alkaloids are organic compounds that contain nitrogen and are found in many plants (Barnawi Hasan, 2017). The nitrogen atoms in alkaloids form alkaline properties that have the ability to interact with metal ions. The negative charge of the lone pair of electrons on the nitrogen atom is reactive to metal ions (Azizi et al., 2013).

The results of kratom leaf alkaloid tests with Mayer's reagent demonstrate that all three variants of kratom leaves contain alkaloids characterized by the formation of white deposits. Based on the amount of white precipitate formed differently, it indicates that the three variants of kratom leaves have different levels. Red kratom contains more alkaloid compounds compared to other kratom variants because it has most white deposits.

White precipitate reduction in kratom leaf extract test with Mayer reagent is a potassium-alkaloid complex that occurs from the reaction between nitrogen in alkaloids with potassium ions from potassium tetraiodomercurate (II) (Riwanti & Izazih, 2019) (Figure 1).

\[
HgCl_{2(aq)} + 2KI_{(aq)} \rightarrow HgI_{2(aq)} + 2KCl_{(aq)}
\]

\[
HgI_2(aq) + 2KI_{(aq)} \rightarrow K_2[HgI_4](aq)
\]

Figure 1. Reaction of alkaloids with Mayer Reagent

Alkaloid testing using Wagner reagent (Iodine) showed that all three variants of kratom leaves contain alkaloids characterized by the formation of brown deposits. The nitrogen atoms in the alkaloids will form a precipitate through interaction with iodine ions. Iodine in comparison with ions I⁻ from potassium iodide gives ions I₃⁻ which are brown. In the Wagner test, K⁺ metal ions will form coordinate covalent bonds with nitrogen in alkaloids to form potassium-alkaloid complexes that precipitate (Fajrin & Susila, 2019) (Figure 2).

\[
I_2(aq) +KI_{(aq)} \rightarrow KI_3(aq)
\]
Based on the amount of brown deposits formed, the green kratom leaf variant has the most brown deposits compared to the red and white kratom variants. This indicates that green kratom has the highest levels of alkaloids compared to red and white kratom. This is in line with the previous findings of Boffa et al., (2018) who used HPLC-DAD, HPLC-MS, HPLC-MS/MS and GC-MS for identification and quantification of the main components of various kratom leaf variants and found that green veins contain mitragynine and total alkaloids that are highest compared to other variants (Boffa et al., 2018).

**Flavonoid Screening**

Screening of flavonoid compounds on three variants of kratom leaves was carried out with the Shinoda test and concentrated sulfuric acid. The test results showed that all three variants of kratom leaves contain flavonoids characterized by the formation of orange (Figure 3) (Anna Hidayati, 2013). Based on the intensity of the resulting color, white kratom leaves show the strongest orange color compared to green and red kratom. This indicates that the flavonoid content in white kratom is higher than the other two kratom variants. Differences in flavonoid levels in kratom leaves will likely have an impact also on differences in biological activity in the third variant of kratom.

Flavonoids are part of phenolics (Wibawa, 2021). The formation of orange in the flavonoid test occurs due to the reduction of polyhydroxy compounds from flavonons contained in the sample by magnesium metal in hydrochloric acid, which forms benzopyrylium salts (Manongko et al., 2020).

$$\text{MgCl}_2(\text{s}) + 2\text{HCl}(\text{aq}) \rightarrow \text{MgCl}_2(\text{aq}) + \text{H}_2(\text{g})$$

**Steroid Screening**

Steroids are compounds composed of 17 carbon atoms and 4 rings. The principle requires steroids to be done based on the ability of compounds to form color when reacted with concentrated sulfuric acid. Concentrated acid will reduce the acetyl group in steroid compounds. The green, red variant of kratom leaves does not show color changes when reacting with these reagents. The results of this study are different from Yuniarti & Nadia's
research, (2020) where kratom leaves show the presence of steroid compounds characterized by the formation of greenish color ((Riwanti & Izazih, 2019).

Differences in phytochemical screening results of a plant with the same species can be caused by factors from outside or from within. External factors can be environmental conditions where plants grow, while internal factors are the genetic properties of the plant (Sholekah, 2017).

**Tannin Screening**

The identification of tannin compounds is carried out by reacting tannin compounds with FeCl$_3$ reagents (Setyawati, 2020). The results of testing on three kratom variants gave positive results characterized by the formation of a blackish-green color. The change indicates that there has been an intercondensation reaction in the hydroxyl group of the tannin compound. The presence of tannins in kratom leaves is thought to act as an antioxidant (Noer et al., 2018).

Based on the color formed, all three kratom variants show the same color strength. This shows that the three variants of kratom leaves have almost the same tannin levels by dissolving the extract into water then followed by whisking. Saponins have polar properties so they are easily soluble in water. These compounds include glycoside forms on sapogenins. A positive test result for saponins is indicated by the presence of foam that arises after shaking. Foam formation after shaking is caused by hydrophilic groups of saponins interacting with air, while hydrophobic groups interacting with air. The polar group in saponins faces outward, while the nonpolar group faces the inside of the micelle structure (Ravelliani et al., 2021).

Stable foam after 15 minutes of letting stand indicates saponins in water hydrolyzed into glycosides and other compounds, which is characterized by the formation of foam (Abu Hayullah, Mohammad Basyuni, 2012). Red kratom shows more froth formation, while white and green kratom show just as much froth. Thus, it is suspected that the presence of saponins in red kratom leaves is more than the other two variants.

**Triterpenoid Screening**

Triterpenoids are carbon compounds of six isoprene units which are biosynthesized from squaenea C$_{36}$. The compounds of these compounds in kratom leaves are identified with the Liebermann-Burchard reagent. This reagent is a mixture of chloroform and concentrated sulfuric acid. Figure 4 shows the mechanism of the Liebermann-Burchard Reaction (Dhurhania & Novianto, 2019).

![Figure 4. Mechanism Liebermann-Burchard reaction](image)

The third variant of kratom leaves showed positive results against the triterpenoid test which was characterized by the formation of two layers, a clear brown top layer and a brownish-red
bottom layer. This result is in accordance with Setyawati's research, (2020), namely kratom leaves are positive on the Triterpenoid test.

**Phenolic Screening**

Based on qualitative testing, the three variants of kratom leaves show a change in concentrated green color, which is blackish green quickly after adding FeCl₃ solution (Figure 5), so it is suspected that the third variant of kratom leaves has good antioxidant activity (Bayani, 2016). Muharini & Lestari (2021) found that there is a correlation between the content of phenolic compounds and the antioxidant activity of a plant. Setyawati (2020) showed that kratom leaves have antioxidant activity. It is thought that phenol compounds contained in kratom leaves are the compounds responsible for these activities.

![Figure 5. Reaction of Phenol with FeCl₃](image)

**CONCLUSION**

In conclusion, this study has successfully identified the presence of various secondary metabolite compounds in the three variants of Kratom leaves, including alkaloids, flavonoids, phenolics, tannins, saponins, and triterpenoids. The findings of this research add new information to the existing body of knowledge on the phytochemical composition of Kratom leaves, particularly regarding the content of secondary metabolites in red and white Kratom, which have received less attention in previous studies compared to green Kratom. The results of this study also support and reinforce previous research on the phytochemical screening of Kratom leaves. These findings are expected to serve as a valuable reference for future research in exploring the bioactivity and therapeutic potential of the secondary metabolites present in Kratom leaves.

**RECOMMENDATIONS**

Based on the results of this study, it is recommended that future research should conduct quantitative testing of the secondary metabolite compounds in the three variants of Kratom leaves. This would provide data on the differences in levels of each secondary metabolite present in each variant. Such data would be valuable in understanding the bioactivity of the compounds and their potential therapeutic benefits. Moreover, further identification of these compounds may provide additional insights into their potential applications in medicine. The results of this study can serve as a reference for researchers interested in conducting bioactivity research and identifying compounds in the three Kratom variants.

**ACKNOWLEDGMENTS**

The author would like to thank the Faculty of Teacher Training and Education Tanjungpura University (UNTAN), for providing PNBP FKIP DIPA funding assistance in 2022 Number:
The author would also like to thank PT. Kreasi Alam Borneo West Kalimantan which has provided support for this research by providing samples in the form of 3 types of kratom variants used as samples in this study.

BIBLIOGRAPHY


