Characterization, Phytochemical Screenings and Antioxidant Activity Test of Kratom Leaf Ethanol Extract (Mitragyna speciosa Korth) Using DPPH Method

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Abstract. Antioxidants are compounds that have an important role in health because they can be used as anti-toxic molecules in the body which are the cause of various diseases. One of the plants that have antioxidant content is kratom (Mitragyna speciosa Korth). The purpose of this study was to determine the antioxidant activity of kratom leaf ethanol extract by using the DPPH trapping method. Exploration of kratom leaf samples was carried out by maceration using ethanol 96%, macerate was evaporated with a rotary evaporator, phytochemical screening of kratom leaf ethanol extract and antioxidant testing of DPPH as Free radical. Result of Simplisila Characterization of kratom leaves containing water, air soluble extract contents, ethanol-soluble extract levels, total ash content, and acid insoluble ash content sequentially as follows: 6.65; 18.01; 9.45; 7.14; and 1.06%. Phytochemical Screening results containing kratom leaf ethanol extract containing chemical composition: alkaloids, flavonoids, triterpenoids/steroids, saponins, and tannins. The results of antioxidant activity testing showed that ethanol extract had an IC50 value of 38.56 μg / ml. The results showed that the ethanol extract of kratom leaves had antioxidant activity in a very strong category.

1. Introduction
Antioxidants are defined as compounds that inhibit oxidation by way of reacting with reactive free radicals that form reactive free radicals are unstable. In the chemical sense antioxidants are electron-giving compounds, but in a broader biological sense, that is, all compounds that can reduce the negative effects of oxidants, including enzymes and protein-binding proteins. Antioxidants are compounds that can inhibit reactive oxygen species and also free radicals so that antioxidants can prevent diseases that are associated with free radicals such as carcinogenesis, cardiovascular, and aging [1].
Antioxidants can be obtained in synthetic and natural forms. Natural antioxidants come from the extraction of natural ingredients that have the potential to capture free radicals, while synthetic antioxidants are obtained from synthetic chemicals. However, concerns about the side effects of using synthetic antioxidants have led to many studies on the potential of natural antioxidants derived from
plants [2] Some plants are known to have high antioxidant content such as kratom, carrots, cabbage, tomato, rosella, basil, mangosteen, and others.

Kratom (Mitragynaspeciosa Korth.) can be used to treat various diseases. Kratom leaves contain alkaloids, glycosides, terpenoids, flavonoids, and saponins. Alkaloids are the main group of compounds found in kratom leaves [3]. Several studies on the pharmacological effects of kratom leaf have also been investigated such as analgesic, stimulant, antidepressant, anti-inflammatory, antinociceptive, antioxidant, and antibacterial activities [4].

Antioxidant activity can be tested by several methods such as the method of capturing radical attenuation 2, 2-diphenyl-1-picrylhydrazyl (DPPH), reducing power method, oxygen radical absorption capacity test (ORAC) method, FRAP method and CUPRAC method [5]. Of all the methods available DPPH method is the method most often used for testing antioxidant activity because the DPPH method is a method that is simple, fast, easy, requires little sample and is sensitive to evaluate the antioxidant activity of natural compounds [6].

Based on the background described above, the authors are interested in examining the antioxidant activity of Kratom leaves. The kratom leaf antioxidant activity test was carried out using the DPPH method.

2. Methodology

2.1 Material
Plant material used is kratom leaf (Mitragyna speciosa Korth) chemicals used, namely: ethanol 96%, toluene, 2 N hydrochloric acid, iron (III) chloride, chloroform, aquadest, Dragendorff reagent, Lieberman-Burchard reagent, magnesium powder, 1% ammonia solution, 1% gelatin solution, distilled water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), vitamin C, methanol and ash-free filter paper.

2.2 Tools
The equipment used consists of glassware (beaker cups, funnels, measuring cups, water flasks, stirring rods, test tubes). Suction pipettes, vaporizer cups, water baths (water bath), rough balance, electric balance, a set of the rotary evaporator (Stuart) and UV-Visible Spectrophotometer (Shimadzu UV-1700).

2.3 Research procedure
2.3.1 Making Simplisia
The kratom leaves that have been collected are washed under running water until they are clean, drained, then weighed as wet weight, then dried in a drying cupboard to dry. Kratom leaves are said to be dry if easily destroyed, then weighed as dry weight. Dry simplisia is then made into powder using a blender, extracted by maceration method.

2.3.2 Simplisia Characterization Examination
Simplisia characterization examination conducted by determination of water content, levels of water soluble extract, content of ethanol soluble extract, total ash, and acid insoluble ash content.

2.3.3 Ethanol Extraction of Kratom Leaves
Samples of kratom leaves that have been pollinated weighed as much as 500 g put into maceration container, then added with 75 parts of ethanol solvent (3750 ml). The maceration container is closed and kept for 5 days in a place protected from direct sunlight while stirring occasionally. Then filtered, separated between pulp and filtrate 1. The pulp is extracted again with the rest of ethanol (1250 ml) and allowed to stand for 2 days (filtrate 2). Filtrate 1 and filtrate 2 were mixed and evaporated using a rotary evaporator with a temperature of 50 °C until a thick ethanol extract of kratom leaves was obtained.

2.3.4 Phytochemical Screening of Kratom Leaf Ethanol Extract
Phytochemical screening of kratom leaf ethanol extract was carried out by examination of alkaloids, examination of flavonoids, examination of triterpenoids and steroids, examination of saponin, and examination of tannins.
2.4 Antioxidant Activity Testing
2.4.1 DPPH free radical trapping principle
The ability of the test sample to dampen the DPPH oxidation process (2,2'-diphenyl-1-picrylhydrazyl) as a free radical in a methanol solution (resulting in a reduction in DPPH purple colour) with IC$_{50}$ (the concentration of the test sample capable of reducing free radicals by 50%) was used as a parameter determining the activity of the test sample [7].

2.4.2 Determination of Operating Time
The standard mother kratom leaf solution is pipetted as much as 5 ml, put in a 25 ml flask and then the volume is sufficient with methanol up to the mark line (concentration of 200 g/ml). Then pipette as much as 2 ml, put into a 10 ml flask, added 1 ml DPPH solution (concentration of 200 g/ml) and sufficient volume with methanol to the mark line (concentration of 40 g/ml), measured its absorption to determine the operating time of the test solution until the 60th minute at the maximum absorption wavelength that has been obtained. Then determine the operating time with vitamin C samples (comparison).

2.4.3 Kratom Leaf and Vitamin C Antioxidant Activity Test Procedure
The standard kratom leaf solution is pipetted as much as 5 ml, put in a 25 ml flask and then the volume is sufficient with methanol up to the mark line (concentration of 200 g/ml). Then pipette as much as 1, 2, 3, 4, and 5 ml into a 10 ml flask to get the concentration of the test solution 20, 40, 60, 80, and 100 g/ml. Each test solution was added 1 ml of DPPH solution (concentration of 200 g/ml) and then the volume was sufficient with methanol to mark the line, and then allowed to stand for 10 minutes, then the absorption was measured using a UV-Visible spectrophotometer. Furthermore, the same way is done for vitamin C.

2.4.4 IC$_{50}$ Determination
The calculation used in determining radical capture activity is IC$_{50}$ (Inhibitory Concentration 50%). This value indicates the concentration of the test compound that can reduce the DPPH oxidation process by 50%. A value of 0% means it does not have antioxidant activity, while a value of 100% means total reduction and testing needs to be continued with dilution of the test solution to see the concentration limit of its activity. The calculation results are entered into the regression equation with the extract concentration (g/ml) as abscissa (x axis) and the value of% damping (antioxidants) as the ordinate (y axis). The formula determines the IC50 value:

\[ Y = ax + b \]

Note: y = dependent variable (IC 50)
\[ x = \text{free variable (kratom leaf)} \]
\[ a = \text{slope} \]
\[ b = \text{intercept} \]

Specifically, a compound is said to be a very strong antioxidant if the IC$_{50}$ value is less than 50 g/ml, strong for IC 50 isworth 50-100 g/ml, while if IC50 isworth 100-150 g/ml and weak if IC 50 isworth 151-200 g/ml [8].

3. Result And Discussion
3.1 Simpilisia Characterization Results
The results of the simpilisia characterization of kratom leaves can be seen in Table 1.

| No. | Examination                              | Earnings Rate (%) |
|-----|-----------------------------------------|-------------------|
| 1   | Water content                           | 6.65              |
| 2   | The essence of water soluble essence    | 18.01             |
| 3   | Soluble essence in ethanol              | 9.45              |
| 4   | Total ash content                       | 7.14              |
| 5   | Acid insoluble ash content              | 1.06              |
Based on data from Table 1 shows that the water content obtained 6.65% has met the general requirements, which is no more than 10%. Water content checks are carried out to provide maximum limits or ranges of the amount of water content in simplisia. The maximum allowable value is related to purity and contamination. If the water content contained in simplisia is more than 10%, the simplisia will be easily overgrown with fungi at the time of storage so that the quality of the simplisia will decrease. Water soluble extract concentrations obtained by 18.01% and soluble extract concentrations in ethanol obtained by 9.45%. Water soluble extract and ethanol soluble extract are indicators of the amount of efficacy substances that can be found by both water and ethanol solvents. Total ash content was 7.14% and acid insoluble ash was 1.06%. Determination of total ash content and acid insoluble ash content was carried out aimed at providing an overview of internal and external mineral content from the initial process until the formation of the extract and to evaluate the extract against contamination of silica-containing materials, such as soil and sand.

### 3.2 Phytochemical Screening Results

Phytochemical screening tests were carried out to determine the class of chemical compounds contained in kratom leaf extract. The results of screening of Simplisia extract of kratom leaves can be seen in Table 2.

#### Table 2. Phytochemical screening results of kratom leaf extract

| No. | Examination          | Results |
|-----|----------------------|---------|
| 1   | Alkaloids            | +       |
|     | Dragendorff reagents | +       |
|     | Wagner reagent       | +       |
| 2   | Flavonoids           | +       |
| 3   | Triterpenoids / Steroids | + |   |
| 4   | Saponin              | +       |
| 5   | Tannin               | +       |

Description: (+) positive: Contains the compound being examined  
(-) negative: Does not contain the compound being inspected

Based on the results of phytochemical screening tests of ethanol extract of kratom leaves containing Alkaloids, Flavonoids, Triterpenoids / Steroids, S. aponin and Tannin extracts. The screening results in Table 2 show that the kratom leaf simplisia extract contains a class of chemical compounds that have potential as antioxidants, namely flavonoid compounds.

Alkaloid testing with Dragendorff and Wagner reagents obtained positive results with the formation of orange deposits and brown deposits. In reactions using Dragendorff and Wagner reagents, the K⁺ metal ion forms a covalent bond in coordination with the alkaloid nitrogen atom to form a precipitating potassium-alkaloid complex.

### 3.3 Results antioxidant activity

The results of antioxidant activity tests of ethanol extract of kratom leaves and Vitamin C with concentrations of 20, 40, 60, 80 and 100 μg/ml compared with DPPH solution can be seen in Table 3 and Table 4.

#### Table 3. The results of the antioxidant activity test of kratom leaf ethanol extract

| Sample      | Absorbance measurement to |
|-------------|----------------------------|
|             | I  | II | III |
| DPPH (blank)| 0.979 | 0.979 | 0.979 |
| 20 ppm      | 0.549 | 0.547 | 0.549 |
| 40 ppm      | 0.310 | 0.309 | 0.313 |
| 60 ppm      | 0.228 | 0.234 | 0.240 |
| 80 ppm      | 0.207 | 0.201 | 0.203 |
| 100 ppm     | 0.142 | 0.143 | 0.142 |
From Table 3 and Table 4, it can be seen that the absorbance value of the test sample decreases with increasing concentration. This can occur because of the reduction of DPPH radicals by antioxidants, where the higher the concentration of the test sample, the more antioxidant compounds contained will increase the greater antioxidant activity and cause its absorbance to decrease. In principle, the absorbance measured is the absorbance of DPPH solution that does not react with the test material or DPPH that is still left in the solution, so that with increasing concentration of free radicals in the solution, the absorbance of the solution will decrease. This means that the activity of capturing free radicals is increasing. So from these results it can be said that the test sample has antioxidant activity. In this test, a comparison solution is used namely Vitamin C (ascorbic acid) because Vitamin C is a very powerful antioxidant and is most often used as a comparison.

3.4 Results of Free Radical Damping Percentage (DPPH) by Samples and Vitamin C

The relationship between concentration and percent reduction of DPPH free radicals by kratom leaf ethanol extract and Vitamin C can be seen in Figure 1 and Figure 2.
Both Figure 1 and Figure 2 show that the increase in concentration is directly proportional to the increase in the reduction of DPPH free radical scavenging. This shows the antioxidant activity of kratom leaf ethanol extracts and vitamin C (as a comparison). The interaction of antioxidants with DPPH either electron transfer or hydrogen radicals to DPPH, will neutralize DPPH free radicals. Free radical scavenging causes electrons to be paired which then causes a colour loss that is proportional to the number of electrons taken. The reduction in colour intensity of the DPPH solution is produced by the reaction of the DPPH radical molecule with one hydrogen atom released by the components of the test sample to form a yellow compound of 2,2 diphenyl-1-picrylhydrazine.

3.5 Results of Inhibitory Concentration Value 50% (IC\textsubscript{50})

The antioxidant activity of the DPPH method is expressed by IC\textsubscript{50} (Inhibitory Concentration) where IC\textsubscript{50} is a number that shows a sample concentration (ppm) that can reduce free radicals by 50%. The smaller the IC\textsubscript{50} value indicates the higher the free radical reduction activity. Conversely, if the IC\textsubscript{50} value is greater the lower the free radical mitigation activity. Antioxidant activity can be divided into very strong, strong, medium, weak and very weak categories. Antioxidants are said to be very strong if they have an IC\textsubscript{50} value of less than 50 \textmu g / ml, antioxidants are categorized as strong if they have an IC\textsubscript{50} value of 50-100 \textmu g / ml, antioxidants are categorized as moderate if they have an IC\textsubscript{50} value of 100-150 \textmu g / ml, antioxidants are categorized as weak if has an IC\textsubscript{50} value of 150-200 \textmu g / ml and an IC\textsubscript{50} value of more than 200 \textmu g / ml is an extremely weak category of antioxidant. IC\textsubscript{50} values were obtained from the linear regression equation which states the relationship between the concentration of the test sample with DPPH reduction as a parameter of antioxidant activity, wherein the concentration of the test solution (ppm) as abscissa (x axis) and% damping value as ordinate (y axis). The results of the analysis of the IC\textsubscript{50} value of the antioxidant activity test of ethanol extract of kratom leaves and vitamin C can be seen in Table 5.

| No | Test Solution       | Regression equation     | IC\textsubscript{50}(\textmu g / ml) | Category       |
|----|---------------------|-------------------------|-------------------------------------|----------------|
| 1  | Kratom leaf extract | Y = 0.7725x + 20.210    | 38.56                               | Very strong    |
| 2  | Vitamin C           | Y = 0.6945x + 36.919    | 18.84                               | Very strong    |

Based on Table 5, showed that the antioxidant activity of kratom leaf extract had a very strong category with IC\textsubscript{50} values obtained at 38.56 \textmu g / ml. The measurement of vitamin C as a comparison has a very strong category of antioxidant activity with IC\textsubscript{50} values obtained at 18.84 \textmu g / ml. From these results it can be concluded that vitamin C has a stronger antioxidant activity than kratom leaf extract.

4. Conclusion

Based on the data obtained, it can be concluded as follows:

1. Phytochemical screening results of kratom leaf (\textit{Mitragynaspeciosa}Korth) ethanol extract showed that kratom leaves contained Alkaloids, Flavonoids, Polyphenols, Triterpenoids / Steroids, Saponins and Tannins.
2. The results of antioxidant activity testing using UV-Visible spectrophotometer at a wavelength of 515.5 nm with DPPH method obtained IC\textsubscript{50} values of kratom leaf ethanol extract at 38.56 \textmu g / ml and IC\textsubscript{50} values of vitamin C as a comparison of 18.84 \textmu g / ml. From these results it was concluded that the test sample and vitamin C as a comparison have very strong antioxidant activity.
References

[1] Suhaling, S. 2010. *Uji Aktivitas Antioksidan Ekstrak Metanol Kacang Merah (Phaseolus vulgaris L.) Dengan Metode DPPH*. [Skripsi]. Fakultas Ilmu Kesehatan. Universitas Islam Negeri (UIN) Alauddin Makassar. Makassar.

[2] Sadeli, R. A. 2016. *Uji Aktivitas Antioksidan Dengan Metode DPPH (1,1-Difenil-2-Pikrilhidrazil) Ekstrak Bromelain Buah Nanas (Ananas Comosus (L.) Merr.*). [Skripsi]. Fakultas Farmasi. Universitas Sanata Dharma. Yogyakarta.

[3] Hidayati, Anna. 2013. *Uji Efek Sedatif Ekstrak n-heksan Dari Daun Kratom (Mitragyna speciosa Korth.) Pada Mencit Jantan Galur BALB/c*. [Naskah Publikasi Skripsi]. Program Studi Farmasi. Universitas Tanjungpura. Pontianak.

[4] Luliana, S., Robiyanto., Islamy, M., R. 2018. *Aktivitas Antinosiseptif Fraksi Diklorometana Daun Kratom (Mitragyna speciosa Korth) Rute Oral Pada Mencit Jantan Swiss*. Pharmaceutical Sciences and Research (PSR), 5(2), 2018, 58-64.

[5] Ikhlas, N. 2013. *Uji Aktivitas Antioksidan Ekstrak Herba Kemangi (Ocimum americanum Linn) Dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil)*. [Skripsi]. Program Studi Farmasi. Universitas Islam Negeri Syarif Hidayatullah. Jakarta.

[6] Cahyani, A. I. 2017. *Uji Aktivitas Antioksidan Dari Ekstrak Kulit Batang Kayu Jawa (Lannea coromandelica) Dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil)*. [Skripsi]. Program Studi Farmasi. Universitas Islam Negeri Syarif Hidayatullah. Jakarta.

[7] Molyneux, P. 2004. *The Use of The Stable free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity*. Songklanakarin. J. Sci. Technol. 26(2): 212, 214, 216.

[8] Mardawati, E., Achyar, C.S., dan Marta, H. 2008. *Kajian Aktivitas Antioksidan Ekstrak Kulit Manggis (Garcinia Mangostana L) dalam Rangka Pemanfaatan Limbah Kulit Manggis Di Kecamatan Pusaphiang Kabupaten Tasikmalaya*. Laporan Akhir Penelitian Peneliti Muda (LITMUD). Semarang: Universitas Padjajaran.