Comparison of phenolic, flavonoid, and tannin contents from ethanol extract of Kratom stem (*Mitragyna speciosa* Korth.) and senggani flower (*Melastoma malabathrium* L.)

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Abstract. Indonesia is a country known for its useful natural resources, especially for a health maintenance and treatment of diseases. Some of the plants that can be used as traditional medicine are Kratom (*Mitragyna speciosa* Korth) and Senggani (*Melastoma malabathrium* L.).

The purpose of this research was to determine the total phenolic content, total flavonoids content, and total tannins content in the ethanol extract of Kratom stems and flowers using the UV-Vis spectrophotometric method. The comparator used in determining the phenolic content was gallic acid, for the flavonoid content was quercetin, and for the tannin content was tannic acid. The results of this study showed that the total phenolic content in the Kratom stem extract was 23.59 mg/gram of extract, while in the senggani flower was 43.97 mg/gram of extract. The total flavonoid content in the Kratom stem extract was 10.65 mg/gram of extract, while the senggani flower was 11.26 mg/gram of extract. Lastly, the total tannin content in the Kratom stem extract was 17.99 mg/gram of extract, while in the senggani flower it was 13.74 mg/g extract.

1. Introduction

The use of traditional medicine in Indonesia has been going on since ancient times and traditional medicine has been used for generations. Generally traditional medicine is used to maintain health, prevent disease, treat disease, and restore health [1]. Some of the plants that can be used as traditional medicine are Kratom (*Mitragyna speciosa* Korth) and Senggani (*Melastoma malabathrium* L.).

Kratom, *Mitragyna speciosa* Korth. (Rubiaceae), is a plant endemic to Southeast Asia, especially in Thailand, Malaysia, and Indonesia [2]. Kratom plant (*Mitragyna speciosa* Korth) has been used as a medicinal plant since ancient times. Kratom plant have some benefits such as stimulant, analgesic, relaxing, anti-diarrheal, antipyretic, and anti-diabetic [3]. *Melastoma malabathrium* L plant possess biological functions such as antioxidant and anti-cancer, antiviral, anti-inflammatory, antinociceptive and anti-pyretic, and anti-ulcerogenic [4].

Kratom and senggani plants were native to West Kalimantan Province. This plant contains many secondary metabolites, such as flavonoids, and tannins. Phenolic compounds are probably the most explored natural compounds due to their potential health benefits as demonstrated in a number of studies [5]. The phenol group consists of several compounds, including flavonoids and tannins. Its ability as a biologically active compound provides a big role for human. One of them is for the treatment of degenerative diseases [6]. Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure are found in fruits,
vegetables, grains, bark, roots, stems, flowers, tea and wine. They have miscellaneous favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer’s disease (AD), atherosclerosis, etc. [7]. Flavonoid compounds are found in almost all parts of the plant, including fruit, roots, leaves, and stems. Tannins are potential antioxidants. They have been considered to be cardio-protective, anti-inflammatory, anti-carcinogenic, antidiabetic and anti-mutagenic [8].

Plant secondary metabolites play an important role in determining of biological activities of medicinal plants used in Traditional medicine. Therefore, analysis of content the secondary metabolites are important to standardize and to increase quality of the traditional medicine [9]. Literature reviews indicated that no studies about total phenolic, flavonoid and tannin content of kratom stem and senggani flowers. This prompted researchers to analyze the total phenolic content, total flavonoid content, and total tannin content from kratom stem extract and senggani flower so that they could be used as herbal medicine. The research used a UV-Vis spectrophotometric instrument to measure the contents. The method used to analyze the content in the plant was to compare it with standard solutions. The standard solution for flavonoid was quercetin, for phenolic was gallic acid, and for tannin was tannic acid.

2. Methods

2.1. Plant determination
Plant determination was carried out at the Faculty of Agriculture, Laboratory of Land Quality and Health, Tanjungpura University (UNTAN), Pontianak, West Kalimantan.

2.2. Determination water content
The water content in simplicia was determined by using Moisture Balance. A total of 2 g of sample was put into the Moisture Balance that had been prepared at 100°C for 10 minutes. The levels listed on the Moisture Balance were then recorded [10].

2.3. Maceration
The kratom stem extract and senggani flower extract were made by using the maceration method. 200 grams of simplicia each was put into a maceration vessel, soaked using 96% ethanol solvent until all samples were immersed and stirred for 30 minutes until completely mixed, after that, it was left for 24 hours and then filtered. The process was carried out for 3 x 24 hours [11]. The maceration results were filtered and ethanol filtrate was obtained. The ethanol filtrate was then concentrated at a temperature of 30-45°C by using rotary vacuum evaporator to obtain a thick ethanol extract of kratom stems and ethanol extract of senggani flowers. The yield was determined by using equation:

\[
\text{Yield} = \frac{W_{\text{concentrated}}}{W_{\text{simplicia}}} \times 100\% \tag{1}
\]

2.4. Phytochemical screening
Each medicinal plant contains a variety of organic compounds that are formed and contained in these plants. The content of active compounds contained in plants can be determined by separation, purification, and phytochemical screening. Phytochemical screening includes: flavonoid test, phenolic test, tannin test [12].

2.5. Determination of the total phenolic content of kratom stem extract

2.5.1. Determination of maximum wavelength. Prepare a total of 10 mg gallic acid, put into a 10 mL volumetric flask and dissolved in 10 mL of distilled water. Then 1 mL pipette was added and 0.4 Folin-Ciocalteu reagent was shaken, left for 3 minutes. Then add 4.0 mL of 7% Na₂CO₃ solution, shake until homogeneous and add distilled water to 10 mL, stand for 40 minutes [13]. Then the absorbance is measured at a wavelength of 600-850 nm [6].
2.5.2. Preparation of gallic acid standard curves with folin-ciocalteu reagent. 10 mg of gallic acid was diluted in 10 mL of distilled water on a volumetric flask. From this solution, 0.2; 0.4; 0.6; 0.8; and 1 mL were taken and mixed with distilled water up to 10 mL to make a standard concentration of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. 1 mL of each standard solution of gallic acid was mixed with 0.4 mL of Folin-Ciocalteu reagent. The mixture was shaken and left for 3 minutes and added with 4.0 mL of 7% Na$_2$CO$_3$ solution. The mixture was shaken until homogeneous and added with 10 mL of distilled water and then incubated for 40 minutes [13].

2.5.3. Determination of total phenolic content. 100 mg of ethanol extract of kratom stem and Senggani flower was dissolved in 100 mL of distilled water. 1 mL of samples was added with 0.4 mL of Folin-Ciocalteu reagent, and shaken the solution and left for 3 minutes. 4.0 mL of 7% Na$_2$CO$_3$ solution was added to the mixture and shaken until homogeneous. 10 mL of distilled water was added to the mixture and then incubated for 40 minutes [14]. The absorbance of the extract solution was measured using UV-Vis spectrophotometry with the results of the obtained wavelength of gallic acid. The measurements were made three times. Data analysis was performed using standard linear regression curve method based on absorbance data and concentration of gallic acid standard solution [6].

2.6. Determination of total flavonoid of ethanolic extract of kratom stem and ethanol extract of senggani flowers

2.6.1. Determination of maximum wavelength. The maximum wavelength is determined based on the Ahmad et al., journal with a modification of 10 mg of quercetin inserted into a 10 ml volumetric flask and dissolved in 10 ml of 96% ethanol [13]. 0.1 ml of the solution was added with 0.2 mL of aluminum chloride (AlCl$_3$) 10%, and added with 0.2 ml of potassium acetate 1 M and then added with distilled water up to 10 ml. The absorbance was measured at a wavelength of 400 - 800 nm using UV-Vis spectrophotometry [15].

2.6.2. Preparation of quercetin standard curves with folin-ciocalteu reagent. A standard quercetin curve was made based on the Ahmad et al., journal with a modified of 10 mg of quercetin was inserted into a 10 ml volumetric flask and dissolved in 10 ml of 96% ethanol for a concentration of 1000 ppm [13]. 1 ml of the solution was dissolved in 10 ml of ethanol 96% for a concentration of 100 ppm and then several standard concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm were made. 1 mL of each standard solution of quercetin was added with 0.2 ml of 10% aluminum chloride (AlCl$_3$), 0.2 ml of 1 M potassium acetate and added up to 10 ml with distilled water. The absorbance of all standards was measured at the quercetin wavelength using UV-Vis spectrophotometry that has been obtained.

2.6.3. Determination of total flavonoid. Determination of total flavonoid by colorimetric method based on the Ahmad et al., journal with modification, 100 mg of kratom stem and senggani flower ethanol extract dissolved in 100 ml of distilled water [13]. 0.1 ml of the samples was added with 0.2 ml of 10% AlCl$_3$, 0.2 ml of 1 M potassium acetate and then added with distilled water up to 10 mL. The absorbance was measured at a maximum wavelength of quercetin using UV-Vis spectrophotometry. Measurements were made 3 times. Data analysis was performed using standard linear regression curve method based on absorbance data and concentration of quercetin standard solution.

2.7. Determination of total tannin levels of ethanol extract of kratom stem and ethanol extract of senggani flowers

2.7.1. Determination of maximum wavelength. 0.01 gram of tannic acid was dissolved in 10 mL of distilled water. 1 mL of tannic acid was put into a 10 mL volumetric flask and then 0.4 mL of Folin-Ciocalteu reagent was added. The mixture was shaken homogeneously and left for 3 minutes and then added with 1 mL of 7% Na$_2$CO$_3$ solution, shaken homogeneously and added with distilled water up to
10 mL and then incubated for 40 minutes [16]. The absorbance was measured at 400-800 nm using UV-Vis spectrophotometry [17].

2.7.2. Preparation of tannic acid standard curves with folin-ciocalteu reagent. 0.01 grams of tannic acid was dissolved in 10 mL of distilled water for a concentration of 1000 ppm. Then several standard concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm were made. 1 mL of each concentration was put into a 10 mL volumetric flask and was added with 0.4 mL of the Folin-Ciocalteu reagent. The solution was shaken homogeneously and left for three minutes then added with 1 mL of 7% Na₂CO₃ solution, shaken until homogeneous and added with distilled water up to 10 mL and incubated for 40 minutes [16]. The absorbance was measured at a maximum wavelength of tannic acid using UV-Vis spectrophotometry.

2.7.3. Determination of tannin content. 0.1 gram of ethanol extract of kratom stem and Senggani flower was dissolved in 100 mL distilled water. 1 mL of the samples was added with 0.4 mL of Folin-Ciocalteu reagent, and shaken the solution homogeneously and left for three minutes and then added 1 mL of 7% Na₂CO₃ solution. The mixture was shaken homogeneously and added with distilled water up to 10 mL and then incubated for 40 minutes [16]. Data analysis was performed using standard linear regression curve method based on absorbance data and concentration of tannic acid standard solution.

3. Results and discussion

3.1. Plant determination results
Determination is carried out to find out the correct identity of the plants. Based on the letter of determination, the plant used is true kratom (Mitragyna speciosa Korth.) and Senggani (Melastoma malabathrium L.).

3.2. Water content
Determination of water content was important to gave the limit amount of water contained in simplicia, because the high amount of water can became a growth medium for bacteria and fungi that can damaged the compounds which contained in simplicia. The result of the water content for kratom stems was 3.8%. The result of the water content test for senggani flowers was 1%, this result showed that the simplicia was qualified for the water content requirements because the maximum water content limit for stem simplicia was not more than 10% [18].

3.3. Results of phytochemical screening
Phytochemical screening was aim to ensure the chemical compounds contained in the simplicia and to ensured that the extraction process in the concentration of the extract did not damage these compounds so it was carried out on the ethanolic extract of kratom stems and senggani flower extracts. The phytochemical screening data can be seen in Table 1.

| Sample        | Test       | Result   |
|---------------|------------|----------|
| Extract       | Flavonoid  | Yellow   | +        |
| Extract       | Fenolic    | Blackish green | + |
| Extract       | Tannin     | Blackish green | + |

In test phenolic shows the results of the positive things is because the phenolic reacted with FeCl₃ 1 % form the color red, purple, blue, or black are concentrated because of FeCl₃ 1% reacts with the group -OH aromatic. Addition of magnesium powder and hydrochloric acid to flavonoid testing will cause the reduction of flavonoid compounds that exist, causing red, yellow or orange that is characteristic of flavonoids. In flavonoid testing, it is positive in this test because there is a colour changing to yellow.
solution [19]. The positive results of the tannin test were shown by a blackish green color. The blackish green complex was due to the formation of complex compounds resulting from the reaction of tannins in samples with Fe$^{3+}$ ions from the addition of 1% FeCl$_3$ [20].

### 3.4. Percentage yield of extract

The purpose of extraction process is to attract the desired compound by using a suitable solvent, in the present study the extraction is done by maceration method, the advantage of this method is that it can prevent damage to the compound having thermolabile properties. A total of 200 g of simplicia was macerated using 1 L of 96% ethanol for 3 days and the solvent was replaced every 24 hours. Concentration of the liquid extract using a rotary vaporator with a temperature of 30-45°C and then stored in a water bath until it becomes a thick extract, the randemen value of kratom stem extract obtained is 44.5% while the yield value of senggani flower extract obtained is 52.5%.

### 3.5. Determination of maximum wavelength

Determination of the maximum wavelength is carried out to determine the wavelength that has the greatest absorbance. Maximum wavelength of gallic acid was 756.8 nm, quersetin was 435.5 nm, and tannic acid was 743.5 nm.

### 3.6. Absorbance of gallic acid, quersetin, and tannic acid standard

Standard curves were made with various concentrations of 20, 40, 60, 80, and 100 ppm and were measured at a maximum wavelength. The results are shown in table 2.

| Concentration (ppm) | Absorbance of Gallic Acid | Absorbance of quersetin | Absorbance of tannic acid |
|---------------------|---------------------------|-------------------------|---------------------------|
| 20                  | 0.0109                    | 0.25                    | 0.783                     |
| 40                  | 0.216                     | 0.341                   | 0.551                     |
| 60                  | 0.335                     | 0.477                   | 0.413                     |
| 80                  | 0.443                     | 0.623                   | 0.207                     |
| 100                 | 0.536                     | 0.756                   | 0.080                     |

### Table 3. Equation of calibration curve of gallic acid, quersetin, and tannic acid standard.

| Comparison Agent | Linear Regression | Correlation coefficient (r) |
|------------------|-------------------|-----------------------------|
| Gallic Acid      | $y = 0.0054x + 0.0036$ | 0.9986                     |
| Quersetin        | $y = 0.0065x + 0.1012$ | 0.9943                     |
| Tannic Acid      | $y = 0.0087x - 0.1175$ | 0.991                      |

Based on the table 3, it can be seen that correlation coefficient (r) value of gallic acid, quersetin, and tannic acid approaches 1 which indicates that the regression equation is linear.

### 4. Determination of phenolic, flavonoid, and tannin content

A total of 100 mg of kratom stem and senggani flowers ethanol extract were used as samples and three replications were made for data accuracy purposes. The absorbance data was entered into the linear regression that was obtained. Phenolic, flavonoid, and tannin content of kratom stem extract and senggani flower extract can be seen in Table 4.
Table 4. Phenolic, flavonoid, and tannin content of kratom stem extract and senggani flower extract.

|                | Kratom Stem | Senggani Flower |
|----------------|-------------|-----------------|
|                | Absorbance  | Content (mg/gram of Extract) | Absorbance | Content (mg/gram of Extract) |
| Fenolic        | 0.032       | 23.59           | 0.241       | 43.97           |
| Flavonoid      | 0.130       | 10.65           | 0.028       | 11.26           |
| Tannin         | 0.039       | 17.99           | 0.232       | 13.74           |

5. Conclusions
The total phenolic content in kratom stem extract was 23.59 mg / gram of extract, while in senggani flowers it was 43.97 mg / gram of extract. The total flavonoid level in kratom stem extract was 10.65 mg / gram of extract, while in senggani flowers it was 11.26 mg / gram of extract. The total tannin content in the kratom stem extract was 17.99 mg / gram of extract, while in the Senggani flower extract it was 13.74 mg / gram of extract. The phenolic and flavonoid content in the ethanol extract of senggani flowers were greater than the ethanol extract of kratom stems. Meanwhile, the tannin content in the senggani flower extract was lower than the ethanol extract of kratom stem.

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