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# Production of Biomass and Bioactive Compound as α-Glucosidase Inhibitor Activities Simplisia Cat Whiskers (*Orthosiphon aristatus*) at Fertilization and Differences Harvest Rotation Time

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***ABSTRACT***

*Cat whiskers are traditional medicinal plants with bioactive compounds like flavonoids like sinensetin. The development of cat whiskers as a medicinal plant is still constrained by the quality of the simplisia. Fertilization and harvesting are essential aspects of the cultivation of medicinal plants. The research aims to determine the proper fertilization and harvest rotation time differences to produce biomass production and bioactive compound simplisia in cat whisker leaves. The method used was a Randomized Complete Block Design composed of two factors. The first factor was harvest rotation, consisting of four and six levels. The second factor was fertilization, composed of three levels, namely 100 g Indigenous Arbuscular Mycorrhizal Fungi (AMF) inoculum, 2.1 g Urea (N fertilizer) & 2.7 g SP-36 (P fertilizer), and a combination of 100 g Indigenous AMF inoculum + 2.1 g Urea (N fertilizer) & 2.7 g SP-36 (P fertilizer). The results showed that the highest simplisia biomass production was obtained by giving a combination fertilizer (Indigenous AMF, N & P). In contrast, the highest simplisia bioactive compound production was obtained with indigenous Arbuscular Mycorrhizal Fungi (AMF) fertilizer or a combination fertilizer (Indigenous FMA, N & P). Production of biomass and bioactive compounds of cat whisker plants simplisia can be done by harvesting earlier every four weeks or extending the harvesting time to every six weeks.*



***Keywords:***
*Diabetes mellitus; medicinal plant; simplisia; sinensetin.*

## 1. Introduction

Medicinal plants can be utilized in part, all parts, or exudates of the plant used as a medicinal herb. Cat whisker is a medicinal plant commodity that was widely used in traditional medicine in Indonesia. Cat whiskers are diuretic, anti-microbial, anti-bacterial, antibiotic, and anti-inflammatory (Kusmala et al., 2022). Cat whiskers are used to cure diseases related to the kidney or bladder. The bioactive compound content of cat whiskers is flavonoid compounds such as sinensetin, eupatorin, and rosmarinic acid (Faramayuda et al., 2024), besides triterpenoid compounds such as ursolic acid, oleanolic acid, betulinic acid, hydroxybetulinic acid, maslinic acid, alpha-amyrin acid and beta-amyrin acid and diterpenoids (Wang et al., 2022).

Sinensetin from the flavonoid group is one of the phenol compounds found in cat whiskers. The activity of the α-glucosidase enzyme can be inhibited by sinensetin. Results by Widyawati (2014) stated that cat whisker extract 25 mg $ml^{-1}$ from several locations with water solvent can inhibit α-glucosidase ranging from 57.9-99.9%. Batubara et al. (2020) showed that 50% ethanol cat whiskers extract, as much as 62.5 mg $ml^{-1}$, was able to inhibit α-glucosidase activity up to 81.4%, while pure sinensetin could inhibit 32-89% with a concentration of 0.31-2.5%. Research on the ability of cat whisker extract to inhibit α-glucosidase enzyme has been done, but studies involving cultivation techniques have not been done.

Important aspects of medicinal plant cultivation are fertilization and harvesting. Fertilization has to do with the availability of nutrients during the formation process of plant bioactive compounds. The accumulation of bioactive compounds affects the plant's growth phase (Saleh et al., 2019). Harvesting cat whiskers by setting a harvest rotation will affect the plant's growth phase, harvested parts, and those left behind. Harvest rotation is essential for plants whose harvesting occurs once in a specific rotation.

In general, the development of cat whisker plants as medicinal plants is still constrained by the quality of simplisia, both in quantity and quality, from physical simplisia to the bioactive compound produced. Medicinal plants with good quality simplisia and high bioactive compound ingredients have no standardized SOP (Standard Operating Procedure). Fertilization and harvest rotation in cat whisker cultivation need to be researched on these two aspects. The research aims to determine the proper fertilization and harvest rotation time differences to produce biomass production and bioactive compound simplisia in cat whisker leaves.

## 2. Materials and Methods

### *2.1 Time and Place*

The research was conducted from March to August 2021. The research was held in Greenhouse, F7 Campus of Gunadarma University, Pharmacy Laboratory, F5 Campus of Gunadarma University, and Laboratory of Biopharmaca Study Center, IPB University.

### *2.2 Tools and Materials*

The tools used were polybags measuring 40 x 40 cm, Ohaus analytical balance, Memmert oven, High-Pressure Liquid Chromatography (HPLC) brand Hitachi UV-VIS L-2420, Scilogex centrifuge, and Dynamic HALO DB-20S UV VIS spectrophotometer. The substances used were Indigenous Arbuscular Mycorrhizal Fungi (AMF) soil inoculum, white flowering cat whisker (*Ortosipon aristatus*) plants aged 8 WAP (Week After Planting), α-glucosidase enzyme Sigma Aldrich G5003-100U, p-nitrophenyl-α-D glucopyranose Sigma Aldrich N1377-1G, Merck phosphate buffer, Merck sodium carbonate ($Na_2CO_3$), Whatman filter paper No. 1, Whatman 0. 45 µm, Urea fertilizer and SP-36 fertilizer.

### *2.3 Research Design*

The research design used was a Randomized Complete Block Design (RCBD) composed of two factors. The first factor was harvest rotation consisting of two levels, once: every four-week (W1) and once every six-week (W2). The second factor was fertilization composed of three levels, namely 100 g Indigenous AMF inoculum (P1), 2.1 g Urea (N fertilizer) & 2.7 g SP-36 (P fertilizer) (P2) and a combination of 100 g Indigenous AMF inoculum + 2.1 g Urea (N fertilizer) & 2.7 g SP-36 (P fertilizer) (P3). There were six treatment combinations, and they were repeated three times for 18 experimental units.

### *2.4 Procedure Methodology*

Research Implementation Procedure: 1) Preparation of planting material, the planting material used was eight-week-old cat whisker plants; 2) Planting media preparation, the soil was sterilized using an oven with a temperature of 150 °C for 2 hours; 3) AMF inoculation was done when moving the plant as much as 100 g; 4) N fertilization was obtained at a dose of 2.1 g in the form of Urea and P fertilization was obtained at a dose of 2.7 g in the form of SP-36; 5) Maintenance; 6) Harvesting of cat whiskers using pruning techniques. The height of the harvesting prune was 30 cm from the ground.

Observations in the study include a) Biomass Production Components, leaf composition, the wet and dry weight of leaves, the damp and dry weight of stems, total biomass, and leaf simplistic yield. b) Bioactive Compound Components, simplisia water content, sinensetin level of simplisia, and inhibition activity of α-glucosidase enzyme.

#### *2.4.1. Simplisia Water Content*

Simplisia water content was obtained from samples of cat whisker leaves, which were pulverized and filtered to get the finest samples. The samples were oven at 105 °C for 4-6 hours until constant (Susiani et al., 2017). Calculate the moisture content of simplisia with the formula, Sample water content = ((sample initial weight - sample final weight)/sample initial weight) x 100%.

#### *2.4.2. Sinensetin Level*

Sinensetin level was obtained from a pulverized sample of 1 g (dry weight) dissolved with 100 ml of methanol and shaken for 1 hour. Filter and evaporate the extract to leave 5 ml, then dilute into a 10 ml volumetric flask. The extract was 1 ml and diluted with methanol: water (6:4) mixed solvent into a 5 ml flask and filtered with Whatman paper 0.45 µm. The sample extract was injected into the HPLC device with the column used Linchrocart R125-4 with a volume of 20 ɥl and a flow rate of 1 ml for 1 minute, with a wavelength of 340 nm. The mobile phase used was a mixture of methanol:water: tetrahydrofuran (THF) with a ratio of 45:50:5 (Febjislami et al., 2018). Calculation of sinensetin content using the formula, Level (mg/g) = ((sample area / standard area) x (standard) (ppm) x (flask volume (ml) x dilution factor)) / (sample weight x 1000).

#### *2.4.3. Inhibition Activity of a-Glucosidase Enzyme*

The α-glucosidase enzyme inhibition activity was obtained from a pulverized sample of 0.5 g (dry weight). The sample was extracted with 20 ml of distilled water and boiled for

5 minutes. The extract was cooled and then filtered with filter paper using a vacuum filter. The filtered extract was centrifuged at 4 °C at 2,500 rpm for 15 minutes. The test continued with the preparation of a reaction mixture composed of blank (buffer), control A (buffer + enzyme), control B (extract + buffer), and sample (extract + enzyme) (Widyawati, 2014). The α-glucosidase enzyme used was derived from Saccharomyces cerevisiae type 1 with an activity of 0.5 units $ml^{-1}$ dissolved in 0.1 M phosphate buffer (pH 6.9). The reaction mixture was then incubated at 37 °C for 10 minutes, and 100 µl of 0.025 M p-NPG was added and incubated again at 37 °C for 20 minutes. After the second incubation, 350 µl of 2 M sodium carbonate ($Na_2CO_3$) solution was added and diluted by adding 1 ml of distilled water and then measuring the absorbance at a wavelength of 410 nm. Calculation of inhibitory activity using the formula, Inhibition (%) = ((control A-control B) - (sample-control B) / (control A-blanko)).

### *2.5 Statistical Analysis*

The data analysis method was obtained and analyzed using Analysis of Variance (ANOVA) at the level of α = 5%. If the analysis results show a significant effect, it will be continued with the Duncan Multiple Range Test (DMRT) at the level of α = 5%. Analysis using the SAS Windows 9.4 program.

## 3. Results and Discussion

### *3.1 Normality test*

The data normality test is used to see whether the data distribution is normal. The method used in testing the normality of data using the Shapiro-Wilk method on the treatment of harvest rotation, fertilization, and interaction on biomass production components and bioactive compound components of cat whisker plants. Mishra et al. (2019) stated that the Shapiro-Wilk method generally uses limited data for small samples (<50 samples) to produce accurate decision values.

**Table 1**. Data Normality test of cat whisker plants

| Observation Variable | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Harvest Rotation | | Fertilization | | | Interaction |
| | Four week | Six week | FMA Indigenous | N and P | FMA Indigenous, N & P | |
| | *P-value* | | | | | |
| **Biomass Production Components** | | | | | | |
| Young leaf composition | 0.32 | 0.17 | 0.21 | 0.29 | 0.59 | 0.41 |
| Adult leaf composition | 0.75 | 0.61 | 0.39 | 0.44 | 0.32 | 0.39 |
| Leaf wet weight | 0.70 | 0.76 | 0.07 | 0.72 | 0.33 | 0.45 |
| Leaf dry weight | 0.51 | 0.62 | 0.08 | 0.82 | 0.34 | 0.44 |
| Stem wet weight | 0.07 | 0.83 | 0.83 | 0.53 | 0.26 | 0.39 |
| Stem dry weight | 0.38 | 0.70 | 0.19 | 0.66 | 0.37 | 0.40 |
| Total biomass | 0.08 | 0.18 | 0.17 | 0.20 | 0.26 | 0.72 |
| **Bioactive Compound Components** | | | | | | |
| Leaf simplisia yield | 0.40 | 0.75 | 0.10 | 0.93 | 0.26 | 0.34 |
| Simplisia water content | 0.25 | 0.34 | 0.28 | 0.61 | 0.13 | 0.08 |

| Observation Variable | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Harvest Rotation | | Fertilization | | | Interaction |
| | Four week | Six week | FMA Indigenous | N and P | FMA Indigenous, N & P | |
| | *P-value* | | | | | |
| Sinensetin level of simplisia | 0.25 | 0.10 | 0.10 | 0.10 | 0.20 | 0.46 |
| Inhibition activity of α-glucosidase enzyme | 0.77 | 0.68 | 0.09 | 0.61 | 0.08 | 0.50 |
| $IC_{50}$ | 0.10 | 0.20 | 0.26 | 0.39 | 0.10 | 0.25 |

Remarks: *P-value > 0.05 (normal); P-value < 0.05 (not normal). Data on the composition of young leaf, adult leaf, wet weight of leaf, dry leaf, dry weight of stem, water content of simplisia, sinencetetin content of simplisia, and $IC_{50}$ were transformed using inverse transformation (1/x). Data on biomass production components and bioactive compound components were transformed using logarithmic transformations (Log x). Leaf wet weight data is converted using the quadratic transformation (x2) for purposes.*

The results of the normality test on biomass production components and bioactive compound components (Table 1) show that the significant values of harvest rotation, fertilization, and interaction have values greater than 0.05 (> 0.05), which means the values are normally distributed. In line with Sintia et al. (2022), the resulting hypothesis (H0) states that data is taken from a normally distributed population when p > 0.05; that is, H0 is accepted, and the data is said to be normally distributed. The data that meets the normality requirements can be used in parametric data testing.

### *3.2 Homogeneity test*

The homogeneity test is used to see if the data distribution has the same homogeneity. The data homogeneity test in this study used the Levene Test method to treat harvest rotation, fertilization, and interaction on biomass production components and bioactive compound components of cat whisker plants. The Levene Test method tests the equality of variance from several populations. The data tested using the Levene method was transformed to find the difference between each score and the group average (Sianturi, 2022).

**Table 2**. Data Homogeneity test of cat whisker plants

| Observation Variable | Treatment | | |
|---|---|---|---|
| | Harvest Rotation | Fertilization | Interaction |
| | *P-value* | | |
| **Biomass Production Components** | | | |
| Young leaf composition | 0.19 | 0.92 | 0.42 |
| Adult leaf composition | 0.26 | 0.54 | 1.13 |
| Leaf wet weight | 0.81 | 0.07 | 0.21 |
| Leaf dry weight | 0.99 | 0.09 | 0.18 |
| Stem wet weight | 0.51 | 0.09 | 0.21 |
| Stem dry weight | 0.74 | 0.22 | 0.10 |
| Total biomass | 0.23 | 0.57 | 0.27 |
| **Bioactive Compound Components** | | | |
| Leaf simplisia yield | 0.88 | 0.56 | 1.20 |

| Observation Variable | Treatment | | |
|---|---|---|---|
| | Harvest Rotation | Fertilization | Interaction |
| | *P-value* | | |
| Simplisia water content | 0.94 | 0.26 | 0.98 |
| Sinensetin level of simplisia | 0.28 | 0.97 | 0.87 |
| Inhibition activity of α-glucosidase enzyme | 0.12 | 0.08 | 1.18 |
| $IC_{50}$ | 0.13 | 0.06 | 1.47 |

Remarks: *P-value > 0.05 (homogen); P-value < 0.05 (not homogen). Data on the composition of young leaf, adult leaf, leaf wet weight, leaf dry weight, stem wet weight, total biomass, and bioactive compound components were transformed using logarithmic transformation (Log x). The dry weight stem, total biomass, and $IC_{50}$ were transformed using the inverse transformation (1/x).*

The homogeneity test results (Table 2) showed that the significant value of biomass production and bioactive compound components in each observation variable was more than 0.05 (> α 5%) in the harvest rotation treatment, fertilization, and interaction. A significant value > 0.05 indicates that the value has the same variation in the group (homogeneous). In line with the statement Zhou et al. (2023) comparison of two samples in a normal distribution, all tests have an error rate below 0.05 when the sample size is large. The data that meets the homogeneity requirements can be used in parametric data testing.

### *3.3 Leaf composition*

The composition values of young and adult leaves of cat whiskers obtained from the total harvest (Table 3) show that the composition of young leaves was influenced by fertilizer treatment. The six-week harvest rotation had the highest average value of young leaves compared to the four-week harvest rotation. The interaction between harvest rotation and fertilization did not significantly affect the composition of young leaves. Fertilization with a combination (Indigenous Arbuscular Mycorrhizal Fungi (AMF), N & P) increased young leaves about ±2-3 times higher than other fertilizers.

The biomass of young and adult leaves can affect the quality of simplisia medicine. In line with the statement of Hossain & Mizanur (2015), the amount of sinensetin produced by adult leaves was higher than that of young leaves in cat whisker plants. Adult leaves increase in production and growth with a longer harvest rotation, so the production of adult leaves tends to be more. In this study, leaf composition was calculated based on weight, while Nurhajijah (2014) was based on quantity. However, there are similarities in the results: the more extended the harvest age, the more adult leave of cat whiskers increased. Delyani (2016) added that short harvesting time produces shoots that have not formed branches, and young leaves are only formed on the shoots, so the harvested young leaves have a lower value.

The composition of adult leaves was influenced by harvest rotation and fertilization treatments. The interaction between harvest rotation and fertilization did not significantly affect the composition of adult leave. The number of adult leaves resulted from the six-week rotation treatment and combined fertilization (Indigenous AMF, N & P) (Table 3). That was due to the amount of biomass harvested.

**Table 3.** Composition of young and adult leaves of cat whisker with different harvest rotations and fertilization

| Treatment | Young Leaves | Adult Leaves |
|---|---|---|
| | Leaf composition of 6 buds/branches (g) | |
| Harvest Rotation | | |
| Four Week | 5.91 (0.63) a | 6.61 (0.24) b |
| Six Week | 6.91 (0.45) a | 18.10 (0.13) a |
| Fertilization | | |
| Indigenous AMF | 3.23 (0.80) b | 4.62 (0.30) b |
| N & P | 5.53 (0.51) b | 10.82 (0.16) ab |
| Indigenous AMF, N & P | 10.46 (0.31) a | 21.62 (0.11) a |
| Interaction | ns | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of logarithmic inverse transformations (1/x).*

This study's highest composition of young and adult leaves resulted from a combination fertilizer (Indigenous AMF, N & P). The combination fertilizer was applied because the availability of macro and micronutrients was sufficient. Camenzind et al. (2014) stated that nitrogen can be absorbed optimally in the soil by the roots of the mycorrhiza. Laturmas et al. (2017) added that applying N and P fertilizers formulated with AMF will make it easier for roots to reach nutrients to help form vegetative tissues such as cell division and elongation. That was related to the formation of plant organs, especially leaves.

### *3.4 Wet and dry weight of leaves*

The wet and dry weight values of cat whisker leaves obtained from the total of all harvest (Table 4) showed that the wet and dry weight of leaves was influenced by fertilizer treatment. The six-week harvest rotation has the largest average value of leaf damp weight compared to a four-week harvest rotation. The six-week harvest rotation also increased the dry weight of the leaves by three times and had the most significant average value compared to the four-week harvest rotation.

The interaction between harvest rotation and fertilization did not significantly affect the wet and dry weight of the leaves. Fertilization with a combination of (Indigenous AMF, N & P) increased the wet and dry weight of the leaves by about ±2.2-3.1 and ±2.1-3.2 times greater than other fertilizer treatments, respectively.

The wet and dry weight of leaves in the fertilization treatment increased due to the role of each fertilizer, which provided nutrients for plants. Plants with sufficient nutrients will experience more optimal development than those without nutrients. Fertilization with AMF was able to better absorb nitrogen elements in the soil. Nitrogen element has the function of vegetative growth for plants to increase leaf formation. The results by Hartoyo & Trisilawati (2021) stated that the use of AMF significantly affected plant growth increment, leaf fresh weight, leaf area, and plant root system. White flowering clones responded best to AMF inoculation (leaf fresh weight increased by 41.1% to 89.59%). In line with results by Luturmas et al. (2017), the application of AMF and N & P fertilizer was practical in the growth of jabon seedlings, especially on the increase in height, diameter, and shoot dry weight.

**Table 4.** Wet and dry weights of cat whisker leaves with different harvest rotations and fertilization

| Treatment | Wet Weight | Dry Weight |
|---|---|---|
| | g plant$^{-1}$ | |
| Harvest Rotation | | |
| Four Week | 21.24 (0.07) a | 4.01 (0.39) a |
| Six Week | 22.51 (0.07) a | 4.45 (0.37) a |
| Fertilization | | |
| Indigenous AMF | 12.00 (0.10) b | 2.24 (0.59) b |
| N & P | 16.59 (0.07) ab | 3.37 (0.33) ab |
| Indigenous AMF, N & P | 37.03 (0.05) a | 7.08 (0.23) a |
| Interaction | ns | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of quadratic transformations ($X^2$) and inverse transformations (1/x).*

The dried leaves of cat whisker are the main medicinal ingredient, so the dry weight of the leaves of the cat whisker plant has a vital role in the medicinal trade industry. In this study, the proportion between leaves and stems of cat whisker plants showed higher leaf production than stems because leaves indicate the output of cat whisker plant simplisia. Bonifasius (2014) and Zainuddin et al. (2023) stated that the higher the proportion of leaves, the higher the productivity of cat whisker plants.

### *3.5 Wet and dry weight of stems*

**Table 5.** Wet and dry weights of cat whisker stems with different harvest rotations and fertilization

| Treatment | Wet Weight | Dry Weight |
|---|---|---|
| | g plant$^{-1}$ | |
| Harvest Rotation | | |
| Four Week | 4.94 (0.37) a | 1.10 (1.96) a |
| Six Week | 8.14 (0.35) a | 1.14 (1.95) a |
| Fertilization | | |
| Indigenous AMF | 3.26 (0.43) b | 0.53 (2.70) b |
| N & P | 3.49 (0.41) b | 0.62 (1.97) b |
| Indigenous AMF, N & P | 12.87 (0.23) a | 2.21 (1.21) a |
| Interaction | ns | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of logarithmic transformations (Log x) and inverse transformations (1/x).*

The wet and dry weight values of cat whiskers stems obtained from the total harvest (Table 5) show that the dry weight of the stems is influenced by fertilizer treatment. The six-week harvest rotation has the most significant average value of wet and dry stem weight compared to a four-week harvest rotation. The interaction between harvest rotation and fertilization had no significant effect on wet and dry stem weights. Fertilization with a combination of (Indigenous AMF, N & P) increased the wet and dry weight of the stem by about ±3.7-3.9 and ±3.6-4.2 times greater than other fertilization, respectively.

The wet and dry weight of the stem using fertilization (Indigenous AMF, N & P) was improved because the fertilization applied with a mixture of mycorrhiza helps plants carry out physiological and biochemical processes. Based on Haryadi (2015) statement, plants that absorb many nutrients will have optimal vegetative growth and produce greater plant weight. This is in line with the statement of Tarfeen et al. (2023), where the content of N fertilizer available in sufficient quantities can help form cells, tissues, and plant organs as a whole, especially plant organs in stems and leaves. Weight gain occurs due to enough nutrients in the soil (Delyani, 2016).

### *3.6 Total biomass*

The total biomass was obtained from the total measurement of crown dry weight and root dry weight. The total biomass value of cat whiskers obtained from the total harvest (Table 6) shows that the total biomass was influenced by fertilization treatment. The six-week harvest rotation has the most significant average total biomass value compared to a four-week harvest rotation. The interaction between harvest rotation and fertilization did not significantly affect the roots' wet and dry weight. Combination fertilization (Indigenous AMF, N & P) increased total biomass by about ±1.70-1.73 times greater than other fertilizations.

**Table 6.** Total biomass of cat whisker plant with different harvest rotations and fertilization

| Treatment | Total Biomass |
|---|---|
| | --------------- g plant$^{-1}$ ---------------- |
| Harvest Rotation | |
| Four Week | 47.81 (1.60) a |
| Six Week | 54.71 (1.64) a |
| Fertilization | |
| Indigenous AMF | 41.11 (1.54) b |
| N & P | 41.76 (1.56) b |
| Indigenous AMF, N & P | 70.93 (1.76) a |
| Interaction | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of logarithmic transformations (Log x).*

The pruning process applied to cat whisker plants is a way of harvesting to gain economically valuable harvested biomass. The increase in total biomass was caused by a combination of fertilization, which can improve the nutritional conditions of soil deficient in nutrients, especially phosphate, water, and carbohydrates, thereby increasing vegetative growth and biomass harvesting of cat whisker plants. Mpongwana et al. (2023) stated in the research that inoculation AMF and rhizobia influence and increase the biomass of legume plants. Plants that absorb nutrients increase total plant biomass because photosynthetic activity occurs optimally (Zhao et al., 2022).

### *3.7 Leaf simplisia yield*

The yield value of cat whiskers leaf simplicia obtained from the total harvest (Table 7) shows that the yield of simplicia leaves was influenced by fertilizer treatment. The interaction between harvest rotation and fertilization did not significantly affect leaf yield. N and P fertilization produced the highest yield value compared to other fertilizers. The yield of leaf simplistic based on fertilization treatment ranged from 18.60-20.79%, indicating that from 100 g wet weight of leaves, it will produce 18-20 g dry weight of leaves.

The yield of leaf simplisia in this study has a narrower range than the research of Nurhajijah (2014), where the range of leaf simplisia yields is 11.41-29.19% (wider) in two harvests. Meanwhile, the results by Delyani (2016) obtained a narrower leaf simplistic yield than the current research results, with a range of 14.15-15.39% in six harvests. The difference in distance is because the harvesting time of the two researchers is different from the current study.

**Table 7**. Simplisia yield of cat whisker plants leaves with different harvest rotations and fertilization

| Treatment | Leaf Simplisia Yield |
|---|---|
| | ------------------- % ------------------- |
| Harvest Rotation | |
| Four Week | 19.83 (1.30) a |
| Six Week | 19.66 (1.29) a |
| Fertilization | |
| Indigenous AMF | 18.60 (1.27) b |
| N & P | 20.79 (1.32) a |
| Indigenous AMF, N & P | 19.84 (1.30) ab |
| Interaction | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of logarithmic transformations (Log x).*

The yield of leaf simplisia generally has results inversely proportional to the water content of the simplisia, where the higher the water value, the lower the yield value. The reduction in yield is due to water evaporation during the drying process (Aisyiatussupriana, 2018). The yield of simplisia from the sample is needed to determine the amount of extract obtained during the extraction process. In their research, Warnis & Angelina (2022) stated that the water content of simplisia, yield, and extract water content have a relationship where the water content of simplisia is inversely proportional to the yield and directly proportional to the extract water content.

### *3.8 Simplisia water content*

The water content value of cat whiskers Simplicia obtained from the total harvest (Table 8) shows that the harvest rotation treatment influenced the water content of Simplicia. The interaction between harvest rotation and fertilization had no significant effect on the water content of simplisia. The six-week harvest rotation increased the highest value of simplisia water content compared to the four-week harvest rotation.

**Table 8.** Simplisia water content of cat whisker plants with different harvest rotations and fertilization

| Treatment | Water Content |
|---|---|
| | ------------------- % ------------------- |
| Harvest Rotation | |
| Four Week | 5.56 ± 1.06 (0.69) b |
| Six Week | 9.99 ± 1.11 (0.93) a |
| Fertilization | |
| Indigenous AMF | 8.01 ± 1.09 (0.83) a |
| N & P | 5.98 ± 1.07 (0.66) a |

| Treatment | Water Content |
|---|---|
| | ------------------- % ------------------- |
| Indigenous AMF, N & P | 9.32 ± 1.11 (0.94) a |
| Interaction | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of logarithmic transformations (Log x) and inverse transformations (1/x).*

This study's value of simplisia water content ranged from 5.56-9.99%, meaning that this value has met the standard requirements for simplisia water content. The water content of leaf simplistic for cat whiskers is not more than 13% or 14% (Delyani et al., 2017). The water content value of simplisia can affect the compound's storage time and quality. Delyani (2016) added that the average value of water content of simplisia obtained based on pruning height and fertilizer application is 7.7%.

### *3.9 Sinensetin level of simplisia*

The level of sinensetin in this study was obtained using the HPLC method, namely comparing the area of the peak of sinensetin compound from the chromatography of sinensetin standards with the results by chromatography of cat whiskers extract samples.

**Table 9.** Sinensetin level of cat whisker plants with different harvest rotations and fertilization

| Treatment | Sinensetin Level | |
|---|---|---|
| | mg $g^{-1}$ BK | % |
| Harvest Rotation | | |
| Four Week | 0.49 a | 0.05 (21.12) a |
| Six Week | 0.50 a | 0.05 (20.93) a |
| Fertilization | | |
| Indigenous AMF | 0.51 a | 0.05 (20.62) a |
| N & P | 0.43 a | 0.04 (24.30) a |
| Indigenous AMF, N & P | 0.56 a | 0.06 (18.16) a |
| Interaction | ns | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of logarithmic transformations (Log x) and inverse transformations (1/x).*

Table 9 shows the value of sinensetin content of cat whiskers simplisia obtained from all harvests. The sinensetin value was not affected by harvest rotation and fertilization treatments. The interaction between harvest rotation and fertilization had no significant effect on sinensetin values.

The amount of sinensetin in this study ranged from 0.04-0.06% (equivalent to 0.04-0.06 mg $ml^{-1}$), which inhibited the α-glucosidase enzyme by 11.98-35.25% (Table 9). The capability of cat whisker extract to inhibit α-glucosidase enzyme activity was due to the involvement of several compounds, one of which was a phenol compound. Phenol compounds are the primary source of inhibiting the activity of α-glucosidase and pancreatic lipase enzymes (Limcharoen et al., 2022). Faramayuda et al. (2024) stated that the activity of phenol content in plants in inhibiting α-glucosidase was due to its activity that binds to proteins.

### *3.10 Inhibition activity of a-glucosidase enzyme*

The inhibitory activity and $IC_{50}$ values of the cat whiskers α-glycosidase enzyme obtained from cat whiskers leaf extract (Table 10) show that the $IC_{50}$ was influenced by harvest rotation treatment. The interaction between harvest rotation and fertilization did not significantly affect the inhibition activity and $IC_{50}$ of the α-glycosidase enzyme**.**

The average inhibition value in the harvest rotation and fertilization treatment ranged from 11.98-35.25% (Table 10). The average inhibition value in this study was lower than that in the research of Widyawati (2014) and Delyani (2016), namely with an inhibition value of 83.48% and 74.76%, respectively. The inhibition value can be differentiated based on the plant accession, harvesting age, part of the leaf on the plant used, harvesting method, and differences in fertilizer application. The four-week harvest rotation had a higher $IC_{50}$ value than the six-week harvest rotation. The $IC_{50}$ value indicates the concentration of the compound that can reduce 50% of enzyme activity. The higher the $IC_{50}$ value, the weaker the enzyme activity ability (Delyani, 2016).

**Table 10.** Inhibition value and $IC_{50}$ α-glucosidase enzyme of cat whisker plants with different harvest rotations and fertilization

| Treatment | Inhibition Value (%) | $IC_{50}$ (mg $ml^{-1}$) |
|---|---|---|
| Harvest Rotation | | |
| Four Week | 11.98 (0.69) a | 107.09 (0.07) a |
| Six Week | 35.25 (0.78) a | 47.04 (0.34) b |
| Fertilization | | |
| Indigenous AMF | 33.35 (0.37) a | 64.89 (0.05) a |
| N & P | 16.73 (1.53) a | 90.60 (0.53) a |
| Indigenous AMF, N & P | 20.77 (0.30) a | 75.70 (0.05) a |
| Interaction | ns | ns |

Remarks*: The numbers in the same column and followed by the same letter are not significantly different at the 5% DMRT test level, ns = not significantly different at the 5% F test, and numbers in parentheses are the result of logarithmic transformations (Log x) and inverse transformations (1/x).*

## 4. Conclusion

Based on the research results that have been carried out, it can be concluded that the highest simplisia biomass production was obtained by giving a combination fertilizer (Indigenous Arbuscular Mycorrhizal Fungi (AMF), N & P). In contrast, the highest simplisia bioactive compound production was obtained with indigenous AMF fertilizer or a combination fertilizer (Indigenous AMF, N & P). Production of biomass and bioactive compounds of cat whisker plants can be done by harvesting earlier every four weeks or extending the harvesting time to every six weeks.

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**References**

Aisyiatussupriana, S. (2018). The effect of drying time on some quality components of the peel of melinjo (*Gnetum gnemon* L.). Thesis. Mataram University. Mataram.

Batubara, I., K. Komariah., A. Sandrawati., and W. Nurcholis. (2020). Genotype selection for phytochemical content and pharmacological activities in ethanol extracts of fifteen Orthosiphon aristatus (Blume) Miq types. Leaves using chemometric analysis. *Scientifc Reports*, 10: 20945. https://doi.org/10.1038/s41598-020-77991-2.

Bonifasius. (2014). Growth, production, and bioactive sinensetin levels of cat's whisker (*Orthosiphon aristatus* Bl. Miq) at various doses of N, P, and K fertilization. B. Thesis, IPB University.

Camenzind, T., S. Hempel., J. Homeier., S. Horn., A. Velescu., W. Wilcke., and M.C. Rillig. (2014). Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Global Change Biology*, 20(12): 3646–3659. https://doi.org/10.1111/gcb.12618.

Delyani, R. (2016). Biomass, sinensetin content, and α-glucosidase inhibitory activity of cat's whisker (*Orthosiphon aristatus*) with different fertilization and harvesting methods. M.S. Thesis, IPB University.

Delyani, R., A. Kurniawati., M. Melati., and D.N. Faridah. (2017). Simplicia production of cat whisker with different fertilization method and cutting height in three-week harvest interval. *Journal Horticulture Indonesian*, 8(3): 209–217. https://doi.org/10.29244/jhi.8.3.209-217.

Faramayuda, F., S.R. Suryani., S.J. Karmila., A.S. Windyaswari., and R.K. Guntina. (2024). Extraction time effect on active compound levels in cat whiskers (*Orthosiphon aristatus* (Blume) Miq.). *International Journal of Applied Pharmaceutics*, 16(1): 1–10. https://doi.org/10.22159/ijap.2024.v16s1.01.

Febjislami, S., M. Melati., A. Kurniawati., and Y. Wahyu. (2018). Agronomy character and sinensetin content of several java's tea plant accession (*Orthosiphon stamineus*). *Journal Horticulture Indonesian,* 9(3): 206–215. http://doi.org/10.29244/jhi.9.3.206-215.

Hartoyo, B., and O. Trisilawati. (2021). Diversity of arbuscular mycorrhiza fungi (amf) in the rhizosphere of sugarcane. *IOP Conference Series: Earth and Environmental Science*, 653: 1–7. https://doi.org/10.1088/1755-1315/653/1/012066.

Haryadi, D., H. Yetti., and S. Yoseva. (2015). Effect of some types fertilizer on the growth and production of kailan (*Brassica alboglabra* L.). *Online Journal of Faperta Students*, 2(2): 1–10.https://jom.unri.ac.id/index.php/JOMFAPERTA/article/view/8399/8068

Hossain, M.A., and R.S.M. Mizanur. (2015). Isolation and characterisation of flavonoids from the leaves of medicinal plant *Orthosiphon Stamineus*. *Arab Journal Chem*, 8(2): 218–21. https://doi.org/10.1016/j.arabjc.2011.06.016.

Kusmala, Y.Y., N.A. Fathiyya., N.M.Z. Khanza., A.M. Fatimah., and S. Riyanti. (2022). Potentials of the cat's whisker plants (*Orthosiphon aristatus*) for kidney health. *Journal of Health and Dental Sciences*, 2(3): 387–404. https://doi.org/10.54052/jhds.v2n3.p387-404.

Laturmas, F.Y.R., S.W. Budi., and I. Mansur. (2017). Effectiveness of arbuscula mycorrhizal fungi (AMF) nitrogen and phosphate fertilizer toward jabon (*Anthocephallus cadamba* Roxb.) seedling growth. *Journal of Tropical Silviculture*, 1(8): 20–25. https://doi.org/10.29244/j-siltrop.8.1.20-25.

Limcharoen, T., P. Pouyfung., N. Ngamdokmai., A. Prasopthum., A.R. Ahmad., W. Wisdawati., W. Prugsakij., and S. Warinhomhoun. (2022). Inhibition of α-glucosidase and pancreatic lipase properties of *mitragyna speciosa* (korth.) Havil. (kratom) leaves. *Nutrients*, 14(19): 3909. https://doi.org/10.3390/nu14193909.

Mishra, P., C.M. Pandey., U. Singh., A. Gupta., C. Sahu., and A. Keshri. (2019). Descriptive statistics and normality tests for statistical data. *Ann Card Anaesth*, 22(1): 67–72. https://doi.org/10.4103/aca.ACA_157_18.

Mpongwana, S., A. Manyevere., J. Mupangwa., C.T. Mpendulo., and C.V. Mashamaite. (2023). Optimizing biomass yield of three herbaceous forage legumes through dual inoculation of arbuscular mycorrhizal fungi and rhizobia. *South Africa Journal of Botany*, 159: 61–71. https://doi.org/10.1016/j.sajb.2023.06.006.

Nurhajijah. (2014). Growth, production, and sinensetin content of cat's whisker (*Orthosiphon aristatus* Bl. Miq.) plants at various harvest ages. B. Thesis, IPB University.

Saleh, H.A.R., Y.I. El-Nashara., M.F.S. El-Dind., and Y.H. Dewira. (2019). Plant growth, yield, and bioactive compound of two culinary herbs are affected by substrate type. *Scientia Horticulturae*, 243: 464–471. https://doi.org/10.1016/j.scienta.2018.08.047.

Sianturi, R. (2022). Homogeneity test as a requirement for analysis testing. *Journal of Education, Social Science, and Religion*, 8(1): 386-397. https://doi.org/10.53565/pssa.v8i1.507.

Sintia, I., M.D. Pasarella., and D.A. Nohe. (2022). Comparison of consistency levels of normality distribution tests in the case of unemployment rates in java. *Prociding of National Seminar on Mathematics, Statistics and Its Applications*, *Samarinda, Indonesia*, (2): 322–333.

Susiani, E.F., A. Guntari., and Kintoko. (2017). The influence of drying temperature against flavonoid total extract ethanol leaves cucumber soul (*Orthosiphon aristatus* (bl) miq). *Borneo Journal of Pharmascientech,* 1(2): 1–8. https://doi.org/10.51817/bjp.v1i2.86.

Tarfeen, N., K.U. Nisa., S. Hassan., A. Manzoor., Humairah., and Z. Sultan. (2023). Integrated nutrient management strategies for improving crop yield. *Sustainable Plant Nutrition*, 12: 283–297. https://doi.org/10.1016/B978-0-443-18675-2.00003-1.

Wang, Q., J. Wang., N. Li., J. Liu., J. Zhou., P. Zhuang., and H. Chen. (2022). A systematic review of orthosiphon stamineus benth. In the treatment of diabetes and its complications. *Molecules*, 27(2): 444. https://doi.org/10.3390/molecules27020444.

Warnis, M., and E. Angelina. (2022). Comparison of total flavonoid content of sambung nya leaf extract (*Gynura procumbens* L.) from simplisia with different drying methods. *Journal of Pharmaceutical and Health Research*, 3(3): 88–94. https://doi.org/10.47065/jharma.v3i3.2772.

Widyawati, R. (2014). Identification of the ability of cat whisker extracts from various regions on the island of Java as inhibitors of alpha amylase and alpha glucosidase enzyme activities. B. Thesis, IPB University.

Zainuddin, I.M., M. Salsabila., M.G. Muktabara., and D. Sumardib. (2023). Effect of pgpm and goat manure doses on biomass yield and rosmarinic acid content in cat's whiskers plant (*Orthosiphon aristatus* Bl. Miq). *Current Research on Biosciences and Biotechnology*, 4(2): 289–298. https://doi.org/10.5614/crbb.2023.4.2/LIGQIUMZ.

Zhao, Y.Y., A. Cartabia., I. Lalaymia., and S. Declerck. (2022). Arbuscular mycorrhizal fungi and production of secondary metabolites in medicinal plants. *Mycorrhiza*, 32: 221–256. https://doi.org/10.1007/s00572-022-01079-0.

Zhou, Y., Y. Zhu., and W.K. Wong. Statistical tests for homogeneity of variance for clinical trials and recommendations. *Contemp Clinical Trials Communications*, 33: 101119. https://doi.org/10.1016/j.conctc.2023.101119.