

COMPARISON OF POLYPHENOL LEVELS OF CALLUS AND WILD TYPE OF CAT'S WHISKERS PLANT (*Orthosiphon aristatus* (Blume) Miq.) PURPLE VARIETIES

Fahrauk Faramayuda*¹, Soraya Riyanti¹, Ari Sri Widyaswari¹, Zaini Alfahmi¹, Sultan Salahudin Jamal¹, Totik Sri Mariani², Elfahmi^{3,4}, Sukrasno³

¹Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

²School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia

³School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia

⁴Biosciences and Biotechnology Research Center, Bandung Institute of Technology, Bandung, Indonesia

*corresponding author

Email: fahrauk.faramayuda@lecture.unjani.ac.id

Abstract. The cat's whiskers plant (*Orthosiphon aristatus* (Blume) Miq) that grows in Indonesia has white, purple, and whit-purple flowers. The quality of the secondary metabolite content of each cat whiskers plant is different because it depends on environmental influences such as rainfall or soil mineral content. Plant tissue culture methods such as callus or cell culture are used to overcome these obstacles because these methods do not depend on the environment and can produce secondary metabolites such as those produced by the original plant. Cat whiskers contain the main secondary metabolite compounds: rosmarinic acid, eupatorin, and sinensetin. Rosmarinic acid belongs to the phenolic group, while sinensetin and eupatorin are flavonoids. This study aimed to determine the total levels of polyphenols and flavonoids in purple cat whiskers and determine the ratio of secondary metabolites of cat whiskers callus with wild-type plants, determination of polyphenol content using the Folin Ciocalteau reagent. Determining flavonoid levels is based on colorimetry using $AlCl_3$ reagent, specific for flavone and flavonol compounds. The levels of polyphenols contained in the ethanolic extract of callus and cat whiskers of the purple variety were 16.056 ± 0.204 mgQE/g and 30.780 ± 0.425 mgQE/g, while the polyphenol content of the callus ethyl acetate extract and the purple variety of cat whiskers leaves was $15,489 \pm 0.425$ mgQE/g and $13,262 \pm 0.044$ mgQE/g. The levels of flavonoids contained in the ethanol extract of the cat whiskers leaf were 1.39 ± 0.03 mgQE/g and callus 0.16 ± 0.02 mgQE/g in the ethyl acetate extract of the leaves of the cat whiskers 2.16 ± 0.012 mgQE/g and callus 2.24 ± 0.010 mgQE/g. The polyphenols and flavonoids in the callus ethyl acetate extract were more significant than the ethyl acetate extract of the purple variety (wild type) cat whiskers leaf.

Keywords: callus; flavonoids; polyphenols; aristatus; quantitative analysis

1. Introduction

Indonesia is an area with various traditional medicines or herbal medicines that people can appropriately use. Sources of traditional medicinal ingredients and natural medicinal ingredients have been used for generations as traditional medicinal ingredients. One of the plants that can become traditional medicine is the cat's whiskers (*O. aristatus*).

O. aristatus is popular in herbal medicine (Shibuya *et al.*, 1999; Faramayuda *et al.*, 2021^a; Faramayuda *et al.*, 2021^b) by the people of Southeast Asia and is widely used as a traditional medicine with the efficacy for urinary tract infections, acute and chronic kidney infections, urinary stones, and gout rheumatism. (Awale *et al.*, 2001, Faramayuda *et al.*, 2021^c; Faramayuda *et al.* 2021^d)

O. aristatus comes from Latin and consists of orthos and siphon, straight and cylindrical. (Ameer *et al.*, 2012) Furthermore, it is part of the Labiatae or Lamiaceae (Keng & Siong, 2006). The varieties of cat whiskers that grow in Indonesia are white flowers, purple flowers, and white flowers with purple patterns. The most widely grown cat whiskers are purple and white varieties with purple patterns, but the most widely used for treatment are white cat whiskers with purple patterns (Faramayuda *et al.*, 2021^e; Faramayuda *et al.*, 2021^f)

The secondary metabolite content in the cat's whiskers plant contains large amounts of flavonoids, polyphenols, essential oils, and potassium. Specifically, cat whiskers contain bioactive components, namely minerals, most of which are potassium minerals, lipophilic flavones (sinensetin and isosinensetin), flavonol glycosides, caffeic acid (rosmarinic acid), essential oils, diterpenes (Awale *et al.*, 2001) orthosiphonol d, orthosiphonol E (Takeda *et al.*, 1993), triterpenes and chromenes such as methylpariochromene A A novel component 5, 6, 7, 8-tetra hydroxy-6-methoxyflavone was isolated from this plant (Hossain *et al.*, 2008). In 2018, Cai research reported that the levels of sinensetin in the leaves of the cat whiskers were 2.719 mg/g, the levels of rosmarinic acid in the leaves were 19.861 mg/g and the levels of eupatorin in the leaves were 4.731 mg/g. (Cai *et al.*, 2018).

Flavonoids have many health benefits, some of which can be used as antioxidants and anti-inflammatory (Tahir *et al.*, 2017). Phenolic compounds and flavonoids have activity as antioxidants. Phenolic compounds and flavonoids are chemical compounds that have conjugated double bonds and chromophore groups. Chemical compounds with conjugated double bonds and chromophore groups can also be determined using the UV-Visible spectrophotometric method (Sari *et al.*, 2018). Plant tissue culture can produce many plants continuously in a relatively short time under controlled conditions, regardless of the season and the weather throughout the year (Garcia-Gonzales *et al.*, 2011). There is a method that can increase the levels of secondary metabolites, especially polyphenols and flavonoids, namely plant tissue culture. Plant tissue culture can increase flavonoid levels so that high levels of flavonoids are produced in plant extracts.

The cat whiskers' great potential (*Orthosiphon aristatus* (Blume) Miq .) plant, research on this plant continues to grow. One of the studies that have been carried out is planting callus on plant tissue culture media *Murashige and Skoog* with the addition of growth regulators, including giving concentrations of 2,4-D and BAP on callus formation (Faramayuda *et al.*, 2020). 2,4-D induced and regulated callus somatic embryogenesis (Bhatia, 2015; Miransari, 2016). 6-benzyl amino purine (BAP) can modify apical dominance by promoting axillary bud formation (Bhatia, 2015).

Based on the chemical content and biological activity, the cat whiskers' is a plant that can be used for various treatments. Based on the literature search, no scientific research has compared the levels of polyphenols and flavonoids from callus whiskers of the purple cat variety. In this study, a comparison of polyphenols and flavonoids from the purple variety of the cat whiskers variety was carried out. In this research, the AlCl₃ and 2,4 DNP methods are used. According to research (Chang *et al.*, 2002), the colorimetric method using aluminum chloride reagent to determine flavonoid content was proven to be specific only for flavones and flavonols. Meanwhile, the 2,4 dinitrophenylhydrazine method to determine the flavanone and flavanone groups. The results of this study are expected to be the basis for the development of secondary metabolite production, especially sinensetin, a polyphenol group with a plant tissue culture approach.

2. Methods

2.1 Cat Whisker Leaf Determination

The purple of cat whiskers (*Orthosiphon aristatus* (Blume) Miq.) leaves was determined at the Central Laboratory of Padjadjaran University, located at Jl. Bandung Sumedang, Jatinangor, Sumedang Regency, West Java.

2.2 Material Collection and Processing

The part of the purple cat whiskers (*Orthosiphon aristatus* (Blume) Miq.) used in this study was the leaf part obtained from the Manoko Experimental Garden, Lembang, Bandung. The results of plant determination can be seen in appendix 1. While the purple clone cat Whisker callus (*Orthosiphon aristatus* (Blume) Miq.) was obtained from the Plant Tissue Culture Laboratory Bandung Institute of Technology.

The plant parts used in this study were the leaves and callus of the cat's whiskers. Purple leaves cat whiskers have been obtained, then washed and aerated in a place not exposed to direct sunlight, followed by drying in the drying cupboard at 50 °C up, easily destroyed material. Meanwhile, the cat's whiskers callus that has been collected is dried by drying in direct sunlight until the callus is dry. After the two samples were dry, they were reduced in size by grinding them into powder.

Raw material from the leaves of the cat's whiskers and the powder from the callus of the cat's whiskers. The leaves of the cat's whiskers that have been collected are sorted first and then washed with running water to remove dirt on the surface of the leaves. Drying the leaves and callus aims to remove the water content in the sample to inhibit the growth of microorganisms and prevent damage to the sample so that the sample can be stored for a longer time. After the two samples were dry, they were reduced in size by grinding them into powder. The reduction in size to powder aims to expand the surface of the sample so that the surface in contact with the solvent will be larger to facilitate the extraction step.

2.3 Determination of Phytochemical Profile of Cat's Whisker Leaf and Callus (Kemenkes RI, 2017)

2.3.1 Identification of Alkaloids

Alkaloid testing using Dragendorff and Mayer reagents (Kemenkes R I, 2017).

2.3.2 Identification of Flavonoids

The result of a yellow to red color is formed on the amyl alcohol layer, which indicates the presence of flavonoid compounds (Kemenkes RI, 2017).

2.3.3 Identification of Tannins and Polyphenols

A total of 1 g of each sample was heated in a water bath. The filtrate is divided into two equal parts. Green-blue black color is formed, which indicates the presence of polyphenol compounds (Kemenkes RI, 2017).

2.3.4 Identification of Saponins

Saponins were indicated by foam formation with a height of at least 1 cm and were persistent for several minutes (Kemenkes RI, 2017).

2.3.5 Identification of Monoterpenoids and Sesquiterpenes

The occurrence of colors indicates the presence of monoterpenoid-sesquiterpenoid compounds (Kemenkes RI, 2017).

2.3.6 Identification of Steroids and Triterpenoids

The Liebermann-Bouchard reagent is dripped on the residue. The formation of purple color indicates the presence of triterpenoid compounds, and the green-blue color indicates the presence of steroid compounds (Kemenkes RI, 2017).

2.3.7 Identification of Quinone

The formation of a yellow to red color indicates the presence of quinone compounds (Kemenkes RI, 2017).

2.3.8 Extract Preparation

A total of 1 g of callus powder and 1 g of leaf raw material were extracted in each vial by maceration method.. 15 mL of 96% ethanol solvent was added to the first raw material and callus, and 15 mL of raw material ethyl acetate solvent was added and the second callus, then the vial was put into an orbital shaker 200 rpm for 24 hours. The extraction process was repeated twice with the same type and amount of solvent. All the macerate was collected, then concentrated by aerating until the sample was concentrated.

2.4 Determination of Flavonoid Levels Aluminum Chloride Method (Chang *et al.*, 2002)

2.4.1 Quercetin Calibration Curve

A calibration curve, 25 mg of quercetin, was first weighed into a 25 mL volumetric flask and dissolved in 25 mL of pro-analytical ethanol (1000 µg/mL), then made standard solutions with

various specific concentrations. Each standard solution was pipetted with 0.5 mL and added with 1.5 mL of pro-analytical ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of potassium acetate, and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the mixture was measured at a predetermined wavelength with a UV-Visible spectrophotometer (Table 1).

Table 1. Absorbance Quercetin

Concentration ($\mu\text{g/mL}$)	Absorbance Value = 433 nm
60	0.227
80	0.285
100	0.361
120	0.428
140	0.532

2.5 Preparation of Callus Ethanol Extract Test Solution and Cat Whisker Leaf Ethanol Extract

Callus and leaves of the purple cat whiskers were taken at 1.0 g and 10 mL of pro-analytical ethanol and 10 mL of pro-analytical ethyl acetate in a volumetric flask.

2.6 Determination of Flavonoid Concentration of Ethanol Extract of Callus and Cat Whisker Leaf with Aluminum Chloride (AlCl_3) Method (Azizah *et al.*, 2014)

The test solution was diluted to 10,0 g/mL. 1.0 mL of extract was added to 10 mL of ethanol in a volumetric flask. 0.5 mL of the solution was pipetted, then 1.5 mL of pro-analytical ethanol was added, 0.1 mL of 10% aluminum chloride (AlCl_3), 0.1 mL of potassium acetate, and 2.8 mL of distilled water were added. Incubated at room temperature (30 minutes), the absorbance of the mixture was measured at a predetermined wavelength with a UV-Visible spectrophotometer.

Table 2. Absorbance of the gallic acid

Concentration ($\mu\text{g/mL}$)	Absorbance value = 758.5 nm
80	0.464
100	0.58
120	0.638
160	0.842
200	1.033

2.7 Determination of Polyphenol Levels with Folin Ciocalteu (Fidrianny *et al.*, 2013)

2.7.1 Calibration curve gallic acid

10 mg of gallic acid was dissolved in 10 mL of Methanol: Aqua distillate (50:50 v/v). Then, several variations of concentration were made. Then 5 mL of Folin-Ciocalteu reagent (1:10 in aqua distillate) was added, and 4 mL of Na_2CO_3 1M solution was added. Furthermore, UV-Visible spectrophotometry measured the absorption at a 400-800 nm wavelength (Table 2).

2.7.2 Preparation of Test Solution

100 mg of purple cat whiskers callus extract was dissolved in 10 mL of methanol: aqua distillate (50:50 v/v).

2.7.3 Determination of Polyphenol Levels

The test solution (0.5 mL) was separately added with 5 mL Folin-Ciocalteu reagent (1:10 in aqua distillate), and then 4 mL of Na_2CO_3 1M 4 mL solution was added. The test was carried out three times with repetition. Furthermore, UV-Visible spectrophotometry measured the absorption at its maximum wavelength of 764.8 nm.

3. Results and Discussion

In this study, the first step was to determine the plant from the purple of the cat whiskers (*Orthosiphon aristatus* (Blume) Miq.) at the Central Laboratory of Padjadjaran University, which is located at Jl. Bandung Sumedang, Jatinangor, Sumedang Regency, West Java.

Secondary metabolite compounds from raw material and callus can be identified by phytochemical screening. Phytochemical screening aims to determine the content of secondary metabolites in raw material and purple cat whiskers callus. Secondary metabolites in raw material and cat whiskers callus tested included alkaloids, polyphenols, tannins, flavonoids, quinones, saponins, and sesquiterpenoids, as well as steroids and triterpenoids. The results of screening on raw material and callus can be seen in [Table 3](#).

The alkaloid test showed positive results on raw material and callus using Dragendorff's reagent because there was a yellow-orange to red brick precipitate, while Mayer's reagent did not show positive results because there was no white precipitate or turbidity in the solution. The addition of an alkaloid reagent will trigger a reaction between nitrogen and metal in the alkaloid, which will form an insoluble form that occurs in an acidic environment. However, this method has a weakness, namely that these reagents can precipitate alkaloids and precipitate several types of compounds, such as proteins and coumarins. This reaction is known as a false positive. However, the literature strengthens the results, which state that the cat's whiskers contain alkaloid compounds ([Ameer et al., 2012](#)).

In the experiment, raw material flavonoid compounds and callus showed positive results due to the formation of a yellow to red color on the amyl alcohol layer, which had previously been shaken vigorously and reacted with magnesium powder and 2N hydrochloric acid. Adding HCl is because the alkaloids are alkaline. They are usually extracted with acid-containing solvents ([Faramayuda et al., 2021⁸](#)). The addition of Mg powder itself is used to speed up the reaction, and in an acidic environment, it induces a reduction reaction of the carbonyl group on the number three

C atom into an alcohol group to form a colored hydroxy compound, and the color formed will then be attracted by the amyl alcohol.

Table 3. Screening for Phytochemical Raw material and Callus of Purple Cat Whisker

No	Compound Groups	Reagen	Results	
			Raw material	Callus
1.	Alkaloids	Mayer	-	-
		Dragendorff	+	+
2.	Polyphenol	FeCl ₃	+	-
3.	Tannins	1% gelatin	-	-
		Steasny	-	-
4.	Flavonoids	Amyl Alcohol	+	+
5.	Quinone	5% KOH	-	-
6.	Saponins	dilute HCl	+	+
7.	Steroids & Triterpenoids	Lieberman Bouchard	+	-
8.	Monoterpenes & Sesquiterpenes	Vanillin-SO ₄	+	+

Description :

(+) : positive contains the group

(-) : negative contains the group

The test results for polyphenol compounds showed positive results on raw material and negative results on the callus. The callus did not detect the presence of steroid groups. The growth regulators used had not succeeded in modifying the biosynthetic pathway of steroid compounds (Bhatia, 2015). The group of polyphenolic compounds is indicated by a strong green, red, purple, blue, or black color with FeCl₃ reagent. Phenol compounds will form Fe³⁺ complex compounds with coordination bonds so that a blue-black color change occurs.

In the tannin compound test, adding 1% gelatin will cause a white precipitate in the solution that has been reacted, but raw material and callus did not give positive results to the gelatin reagent. While adding steasny will cause a pink precipitate in the solution, raw material and callus did not show positive results for the steasny reagent.

Quinone secondary metabolites were not detected in raw material and callus because there was no change in the color of the solution to a yellow-brown color after adding 5% KOH. The presence of quinone compounds is indicated by their ability to form colored salts. This colored salt is formed between hydroquinone and a robust alkaline solution.

In the secondary metabolite test, saponins showed positive results on raw material and callus because the foam was formed on the test results after adding dilute HCl and shaking vigorously, indicating that there are glycosides that can form foam in water. Glycosides function as polar groups, while steroids and terpenoids are non-polar groups. Compounds have polar and non-polar groups due to the presence of these groups, which can act as an active surface in forming foam. The hydrophilic group binds to water, while the hydrophobic group faces inward because of the fear of water.

The test results for monoterpenoids and sesquiterpenoids gave positive results, indicated by the presence of colors. Monoterpenoid and sesquiterpenoid compounds from raw material and

callus reacted with 10% Vanillin reagent in H₂SO₄, forming different colors from the previous ones, purple and red. The color formed from each compound will be different because the two compounds are essential oils consisting of various compounds. Terpenoid compounds in essential oils generally consist of compounds with a total of 10 C atoms, also called monoterpenes and 15 C atoms, or are called sesquiterpenes (Faramayuda *et al.*, 2021^g).

Tests on steroids and triterpenoids showed positive results on raw material, while callus showed negative results. A positive result is indicated by forming a green-blue color on the cup after giving the reagent. However, the cat's whiskers callus itself showed negative results. Based on phytochemical studies, there is a close relationship between saponins and the triterpenoid group. If there are positive results in saponins, it is indicated by the formation of foam, and on the results of triterpenoid screening with positive results, it can be seen that cat whiskers contain steroids (Faramayuda *et al.*, 2021^g).

Determination of polyphenol content was carried out using the Folin-Ciocalteu method. The principle of this method is that the hydroxyl groups in phenolic compounds react with Folin-Ciocalteu reagents to form a blue molybdenum-tungsten complex measured in a spectrophotometer (Bhaskar, 2011). This method can be used because the Folin-Ciocalteu method has several advantages, such as being simple, fast, and accurate, and the chromophore's absorption is at a high wavelength so that it can reduce interference from the sample matrix. The basic mechanism of the Folin-Ciocalteu method of measuring polyphenols in natural materials is an oxidation/reduction reaction in which the phenolic group will be oxidized, and metal ions will be reduced. The comparison used in testing polyphenol levels is gallic acid because gallic acid is a derivative of hydroxybenzoic acid, which is classified as a simple phenolic acid and a standard with stable and pure substance availability (Sari & Ayuchecaria, 2017).

Table 4. Comparison of Polyphenol Levels of Callus and Wild Type of Cat Whiskers

Sample	Polyphenol content (mg GAE/g extract)
Callus Ethanol Extract	16.056±3.204
Leaf Ethanol Extract	30.780 ± 2.425
Callus Ethyl Acetate Extract	15.489 ± 0.425
Leaf Ethyl Acetate Extract	13.262 ± 0.044

At the measurement stage, the maximum wavelength is carried out in the range of 400-800 nm because of that wavelength. It can provide a colored solution that has an absorption in the visible or visible light region. The maximum wavelength produced was 758.5 nm at 80 g/mL. The maximum wavelength obtained was used to measure the absorption of the calibration curve in the purple clone callus extract, and cat whiskers leave. The results of the polyphenol content of the extract and leaves of the purple cat whiskers can be seen in Table 4.

Determination of flavonoid content in callus and stems of purple varieties using the Aluminum Chloride (AlCl_3) method (Azizah *et al.*, 2014). Based on this principle, the comparison used is quercetin because quercetin is a flavonoid flavonol group that has a ketone group on the C-4 atom and a hydroxyl group on the neighboring C-3 or C-5 atom (Chang *et al.*, 2002). marker of the presence of flavonoids because of their widely distributed presence in plants. The reaction between quercetin and AlCl_3 . The results of flavonoid levels from the leaf extract and callus of the purple cat whiskers can be seen in Table 5.

Table 5. Measurement of Flavonoid Levels

Sample	Flavonoid Level (mg QE/g extract)
Callus Ethanol Extract	0.1629 ± 0.027
Leaf Ethanol Extract	1.3918 ± 0.038
Callus Ethyl Acetate Extract	2.2427 ± 0.010
Leaf Ethyl Acetate Extract	2.1629 ± 0.012

The results obtained from the determination of flavonoid levels showed that the flavonoid content of the ethanol extract from the callus of the cat's whiskers was smaller than the ethanolic extract of the leaves of the cat's whiskers. However, the ethyl acetate extract showed that cat whiskers callus flavonoid content was higher than cat whiskers. This can happen because the secondary metabolite content of flavonoids from cat whiskers is sinensetin, a poly ethoxy flavone compound (Berim & Gang, 2016). Less polar flavonoid aglycones such as isoflavones, flavanones, and flavones and flavanols tend to be more soluble in semi-polar solvents (Berim and Gang, 2016). Therefore, the ethyl acetate extract showed higher levels of flavonoids because, in the extraction process, secondary metabolites were withdrawn from callus and leaves using an appropriate solvent. Furthermore, the levels of flavonoids from each leaf extract and callus were analyzed statistically using SPSS version 25 software with non-parametric statistical tests using the *Kruskal-Wallis Test* method. The Kruskal-Wallis Test test shows the probability value obtained is 0.016, with a significant difference because of the Asymp.Sig value <0.05 ($p < 0.05$). Therefore, the results of the determination of flavonoid levels showed that between leaves and callus and between variations of extracts showed significantly different results.

4. Conclusion

Callus ethyl acetate extract's polyphenol and flavonoid levels were more significant than leaf ethyl acetate extract. The tissue culture of the cat's whiskers plants successfully increased the levels of secondary metabolites. Cat whiskers callus can be developed to the cell suspension culture stage to produce marker compounds.

Acknowledgments

Research institute and community service University of Jenderal Achmad Yani for their support in this study.

References

- Ameer, O. Z., Salman, I. M., Asmawi, M. Z., Ibraheem, Z. O., & Yam, M. F. (2012). *Orthosiphon stamineus*: traditional uses, phytochemistry, pharmacology, and toxicology. *Journal of medicinal food*, 15(8), 678-690. <https://doi.org/10.1089/jmf.2011.1973>
- Awale, S., Tezuka, Y., Banskota, A. H., Kouda, K., Tun, K. M., & Kadota, S. (2001). Five novels highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar. *Journal of Natural Products*, 64(5), 592–596. <https://doi.org/10.1021/np000607t>
- Azizah, D. N., Kumolowati, E., & Faramayuda, F. (2014). Determination of Flavonoid Levels AlCl₃ Method in Methanol Extract of Cocoa Fruit Peel (*Theobroma cacao* L.). *Kartika Scientific Journal of Pharmacy*, 2(2), 45–49. <https://doi.org/10.26874/kjif.v2i2.14>
- Berim, A., & Gang, D. R. (2016). Methoxylated flavones: occurrence, importance, biosynthesis. In *Phytochemistry Reviews*, 15(3), 363-390. <https://link.springer.com/article/10.1007/s11101-015-9426-0>
- Bhaskar, A., Nithya, V., & Vidhya, V. G. (2011). Phytochemical screening and in vitro antioxidant activities of the ethanolic extract of *Hibiscus rosa sinensis* L. *Scholars Research Library Annals of Biological Research*, 2 (5), 653–661. www.scholarsresearchlibrary.com
- Bhatia, S. (2015). Chapter 2 - Plant Tissue Culture, 31–107 in S. Bhatia, K. Sharma, R. Dahiya, & T. Bera, eds., *Modern Applications of Plant Biotechnology in Pharmaceutical Sciences*, Academic Press: Boston. <https://doi.org/10.1016/c2014-0-02123-5>
- Cai, X., Xiao, C., Xue, H., Xiong, H., Hang, Y., Xu, J., & Lu, Y. (2018). A comparative study of the antioxidant and intestinal protective effects of extracts from different parts of Java tea (*Orthosiphon stamineus*). *Food Science and Nutrition*, 6(3), 579–584. <https://doi.org/10.1002/fsn3.584>
- Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, 10(3), 178-182. <https://www.jfda-online.com/journal/vol10/iss3/3/>
- Faramayuda, F., Mariani, T. S., Elfahmi and Sukrasno. (2020). Short Communication : Callus Induction in Purple and White-Purple Varieties of *Orthosiphon aristatus* (Blume) Miq. *Biodiversitas*, 21(10), 4967–4972. <https://doi.org/10.13057/biodiv/d211063>
- Faramayuda, F., Mariani, T. S., Elfahmi, & Sukrasno. (2021a). Phytochemical analysis of callus two varieties *Orthosiphon aristatus* (Blume) miq on murashige and skoog media: a strategic step of secondary metabolite production. *International Journal of Applied Pharmaceutics*, 13(2), 71–77. <https://doi.org/10.22159/ijap.2021.v13s2.14>
- Faramayuda, F., Mariani, T. S., Elfahmi, E., & Sukrasno, S. (2021b). Potential of *Orthosiphon aristatus* blume miq as antiviral : A review. *Tropical Journal of Natural Product Research*, 5(March), 410–419. <https://pesquisa.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/resource/pt/covidwho-1261520>
- Faramayuda, F., Mariani, T. S., Elfahmi, & Sukrasno. (2021c). Effects of 6-benzyl amino purine and naphthalene acetic acid on shoot and root induction in purple variety *Orthosiphon aristatus*. *Plant Cell Biotechnology and Molecular Biology*, 22(May), 362–371. <https://orcid.org/0000-0002-4411-5550>
- Faramayuda, F., Mariani, T. S., Elfahmi, & Sukrasno. (2021d). Identification of secondary metabolites from callus *Orthosiphon aristatus* (Blume) miq by thin layer chromatography. *Sarhad Journal of Agriculture*, 37(3), 1081–1088. <https://orcid.org/0000-0002-4411-5550>
- Faramayuda, F., Mariani, T. S., Elfahmi, & Sukrasno. (2021e). Micropropagation and secondary metabolites content of white-purple varieties of *Orthosiphon aristatus* Blume miq. *Pakistan Journal of Biological Sciences*, 24(8), 858–867. <https://doi.org/10.3923/pjbs.2021.858.867>
- Faramayuda, F., Mariani, T. S., Elfahmi, & Sukrasno. (2021f). Chemical compound identification of two varieties cat whiskers (*Orthosiphon aristatus* Blume Miq) from in vitro culture. *Sarhad Journal of Agriculture*, 37(4), 1355-1363.

<https://dx.doi.org/10.17582/journal.sja/2021/37.4.1355.1363>

- Faramayuda, F., Mariani, T.S., Elfahmi, & Sukrasno. (2021g). A comparative pharmacognostic study of the two *Orthosiphon aristatus* (blume) miq. varieties. *Journal of Experimental Biology and Agricultural Sciences*, 9(2), 228-223. [https://doi.org/10.18006/2021.9\(Spl-2-ICOPMES_2020\).S228.S233](https://doi.org/10.18006/2021.9(Spl-2-ICOPMES_2020).S228.S233)
- Fidrianny, I., Windyaswari, A. S., & Wirasutisna, K. R. (2013). DPPH Scavenging Activity of Various Extracts of Sweet Potatoes Leaves with Varying Tubers Colors, 3(2), 133–145. <https://www.semanticscholar.org/paper/DPPH-Scavenging-Activity-of-Variou-Extracts-of-Fidrianny-Windyaswari/01474b9822c1917f149c8d91bfcc231a8c01ff51>
- Garcia-Gonzales, R., Quiroz, K., Carrasco, B., & Caligari, P. (2011) Plant tissue culture: Current status, opportunities and challenges, *Ciencia e Investigación Agraria*, 37, 5–30. <https://doi.org/10.4067/S0718-16202010000300001>
- Hossain, M. A., Ismail, Z., Rahman, A., & Kang, S. C. (2008). Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth. *Industrial Crops and Products*, 27(3), 328–334. <https://doi.org/10.1016/j.indcrop.2007.11.008>
- Kemenkes, R. I. (2017). *Farmakope Herbal Indonesia (II)*. Jakarta, Indonesia: Kementerian Kesehatan RI.
- Keng, C. L., & Siong, L. P. (2006). Morphological Similarities and Difference between the Two Varieties of Cat's Whiskers (*Orthosiphon stamineus* Benth.) grown in Malaysia. *International Journal of Botany*. <https://dx.doi.org/10.3923/ijb.2006.1.6>
- Miransari, M. (2016). 6 - Soybeans and Plant Hormones, 131–156 in Mohammad Miransari, ed., *Environmental Stresses in Soybean Production*, Academic Press: San Diego. https://www.researchgate.net/publication/308566231_Environmental_Stresses_in_Soybean_Production_Soybean_Production
- Sari, A. K., Alfian, R., Musiam, S., Prasdianto, & Renny. (2018). Determination of Total Phenolic and Flavonoid Levels of Yellow Wood (*Arcangelisia flava* Merr) Methanol Extract Using Uv-Visible Spectrophotometric Method. *Journal of Indonesian Pharmacists*, 1(2), 210–217. <https://e-jurnal.stikes-isfi.ac.id/index.php/JIFI/article/view/260>
- Sari, A. K., & Ayuhecara, N. (2017). Determination of Total Phenolic Content and Total Flavonoid Extract of Black Rice (*Oryza Sativa* L) from South Kalimantan. *Scientific Journal of Ibn Sina*, 2(2), 327–335. <https://doi.org/10.36387/jiis.v2i2.112>
- Shibuya, H., Ohashi, K., & Kitagawa, I. (1999). Search for pharmacochemical leads from tropical rainforest plants. *Pure and Applied Chemistry*, 71(6), 1109–1113. <https://www.degruyter.com/document/doi/10.1351/pac199971061109/pdf>
- Tahir, M., Muflihunna, A., & Syafrianti, S. (2017). Determination of Total Phenolic Content of Patchouli Leaf Ethanol Extract (*Pogostemon Cablin* Benth.) Using Uv-Vis Spectrophotometry Method. *Journal of Indonesian Fitofarmaka*, 4(1), 215–218. <https://doi.org/10.33096/jffi.v4i1.231>
- Takeda, Y., Matsumoto, T., Terao, H., Shingu, T., Futatsuishi, Y., Nohara, T., & Kajimoto, T. (1993). Orthosiphon D and E, minor diterpenes from *Orthosiphon stamineus*. *Phytochemistry*, 33(2), 411–415. [https://doi.org/10.1016/0031-9422\(93\)85530-5](https://doi.org/10.1016/0031-9422(93)85530-5)