

Total Flavonoid Content and Antioxidant Activity of Three Fractions *Vernonia amygdalina* Del. from Bali

Kandungan Flavonoid Total dan Aktivitas Antioksidan Tiga Fraksi dari *Vernonia amygdalina* Del. Asal Bali

Ni Ketut Esati^{1*}, Indah Sri Wiratini², Kadek Duwi Cahyadi¹

¹Department of Pharmacy, Faculty of Pharmacy and Health Sciences, Universitas Pendidikan Nasional, Denpasar 80224, Bali, Indonesia

²Department of Pharmacy, Sekolah Tinggi Farmasi Mahaganesha, Denpasar 80226, Bali, Indonesia

*Email: esati@undiknas.ac.id

ABSTRACT

The antioxidant was commonly used in daily life. It was a compound that was used to inhibit free radicals. The antioxidant could reduce the negative impacts of free radicals, such as damage to cell components. The public has widely used herbal plants as antioxidants. One plant known to have antioxidant activity was African leaves (*Vernonia amygdalina* Del.). African leaves from Bali were studied in this research. Known that a plant of the same species but in a different place of life can provide different secondary metabolite content depending on the environmental conditions in which the plant lives. This research aimed to determine total flavonoid levels and antioxidant activity fractions (n-hexane; ethyl acetate; aqueous) of African leaves extract from Tabanan (Bali). Extraction was carried out by maceration method using ethanol. The total flavonoid content of each fraction was tested using the colorimetric method (AlCl₃ reagent), while the antioxidant activity used the DPPH radical reduction method. The results of this study obtained total flavonoid levels in the ethyl acetate, n-hexane, and aqueous fractions, respectively, were 12.668%; 1.469%; and 0.031%. The ethyl acetate fraction provided the highest flavonoid content. The antioxidant activity was shown from the IC₅₀ value, reducing the value caused the antioxidant activity to become higher. The IC₅₀ were 82.596 mg/L; 121.077 mg/L; and 70.101 mg/L for the ethyl acetate, n-hexane, and aqueous fractions, respectively. Therefore, it can be concluded that the aqueous fraction of African leaves extract provides better antioxidant activity than others.

Keywords: *Vernonia amygdalina* Del.; total flavonoid; antioxidants; fraction

Received: 30 November 2022; **Accepted:** 13 April 2026; **Available online:** 29 July 2026

1. INTRODUCTION

Many people use antioxidants in their daily life. Antioxidants are compounds that function as free radical scavengers, so they can reduce the negative effects of free radicals. Therefore, antioxidants from outside the body are needed to assist in counteracting free radicals by donating one electron to unstable reactive free radicals. In addition, antioxidants can also

chelate transition metal ions, such as Fe and Cu, so that new free radicals are not formed through chain reactions (Yuslianti, 2019). The use of herbal plants as antioxidants has been widely carried out by the community. One of the plants known to have antioxidant activity is African leaves (*Vernonia amygdalina* Del.). Based to Hasibuan et al. (2018), the n-hexane, ethyl acetate, and ethanol fraction of African

leaves from Medan City, North Sumatra are known to have antioxidant activity based on the IC₅₀ value, which is 297.33 ± 0.46 g/mL; 177.99 ± 0.32 g/mL; 37.92 ± 1.03 g/mL (Hasibuan et al., 2014). Secondary metabolites known to act as antioxidants in the African leaves are flavonoids. Flavonoids have four mechanisms of action as antioxidants, namely capturing free radicals, binding metals so that metals are not oxidized, suppressing enzymes associated with the formation of free radicals, and stimulating the production of endogenous antioxidants (Banjarnahor & Artanti, 2014).

Based on Suhendy et al. (2022), African leaves extract from Jatinangor City, West Java has a total flavonoid content of 0.20 gQE/100 g (n-hexane extract); 0.58 gQE/100 g (ethyl acetate extract); 0.31 gQE/100 g (methanol extract), which correlates to the EC₅₀ value of each African leaves extract, which is 717.50 g/mL (n-hexane extract); 740.28 g/mL (ethyl acetate extract); 1800.46 g/mL (methanol extract) (Suhendy et al., 2022).

However, differences in total flavonoid content in the African leaves can be influenced by several factors, such as environment, geography, and season. In environmental conditions with high temperatures, plants will increase ROS in plants causing cell damage. Plants adapt by producing compounds that are antioxidants as a form of defense against the environment, such as flavonoids so that high-temperature environmental conditions can increase flavonoid content in plants (Utomo et al., 2020). It was known that the quality of the active compounds contained in the plants will be influenced by land conditions, climate and the conditions in the environment where the plants live. This was also confirmed by Sholekah (2017), that secondary metabolites were formed from primary metabolites

through various metabolic pathways which were influenced by several environmental factors. These factors such as light, temperature, pH, altitude and temperature influenced the phytochemical content. The phytochemical content such as flavonoids and beta carotene from a plant will differ in each region, influenced by environmental factors.

Based on research by Esati et al. (2021), stated that there was flavonoid content in the n-hexane, ethyl acetate, and African leaves aqueous fraction from Tabanan, Bali, but the total flavonoid content had not been determined and had not been tested for its antioxidant activity. Therefore, the researcher wants to continue This study was conducted because of the reported content of flavonoid compounds, so it is necessary to measure total flavonoid content and test the antioxidant activity of African leaves from Tabanan (Bali), then related to the effect of flavonoid compounds on antioxidant activity.

2. MATERIALS AND METHODS

2.1. Materials

African leaves (*Vernonia amygdalina* Del.) from Bali, 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder (EMSURE®), quercetin (EMSURE®), powder Mg (EMSURE®), and other reagent in laboratory grade. Separating funnel (HERMA), TLC elution vessel (CAMAG), TLC plate (Merck TLC silica gel G₆₀ F₂₅₄), UV-Vis spectrophotometer (THERMO SPECTRO GENESIS 10V-VIS), centrifugator (IPC), and evaporator (IKA).

2.2. Methods

2.2.1 Extraction of African leaves

A total of 480 g of African leaves powder was macerated with 1.5 L of 96% ethanol for 72 hours, then filtered to obtain the filtrate. The residue obtained was carried out with the same treatment. The filtrate is collected and evaporated (Depkes RI, 2000).

2.2.2 Flavonoid phytochemical screening in African leaves extract

A total of 0.2 g of extract was added to 10 mL of aquadest, then heated for 5 minutes, cooled and filtered. Take 5 mL of the filtrate, then add 0.1 g of Mg powder, 1 mL of HCl, and 2 mL of amyl alcohol. The mixture is shaken and allowed to separate (Depkes RI, 1995).

2.2.3 Fractionation

A total of 10 g of African leaves extract was dissolved in 50 mL of aquadest and 20 mL of 96% ethanol, then filtered. The filtrate was put into a separating funnel, then added *n*-hexane, shaken until homogeneous. Allow to stand until a layer of *n*-hexane and aqueous is formed. The aqueous layer was partitioned using ethyl acetate with the same treatment. Each layer was evaporated to obtain three types of fractions, i.e., *n*-hexane, ethyl acetate, and aqueous (Rukmana, 2019).

2.2.4 Identification of flavonoids

The African leaves extract fraction was analyzed using the TLC method with a mixture of eluents *n*-butanol-glacial acetic acid-water with ratio 4:1:5. The fraction was spotted on a silica gel plate and eluted. The elution results were detected under UV lamps at 254 nm and 365 nm, then the spots were spotted using NH₃ vapor (Markham, 1988).

2.2.5 Determination of total flavonoid content

The quercetin main standard solution was prepared in a series of 10-100 mg/L. Standard working solution of 40 mg/L quercetin was pipetted 0.5 mL, added 1.5 mL of 96% ethanol, 0.1 mL of AlCl₃, 0.1 mL of 1M potassium acetate, and added aquadest up to the mark in a 5 mL volumetric flask. It was left for 30 minutes, then the absorption spectrum was observed at 200-800 nm using a UV-Vis spectrophotometer.

A total of 25 mg of each fraction was put into a 25 mL volumetric flask, then dissolved in 96% ethanol to the mark. Pipette 0.5 mL of each fraction and the working standard solution of quercetin, add 1.5 mL of 96% ethanol, 0.1 mL of AlCl₃, 0.1 mL of 1M potassium acetate, and add aquadest up to the mark in a 5 mL volumetric flask. It was allowed to stand for 30 minutes, then the absorbance was measured at the maximum wavelength. The blank used was a mixture of 1.5 mL of 96% ethanol, 0.1 mL of AlCl₃, 0.1 mL of 1M potassium acetate, and added aquadest to mark the limit in a 5 mL volumetric flask (Depkes RI, 2017).

2.2.6 Antioxidant activity test

Antioxidant activity test using DPPH radical reduction method. Measurement of the maximum wavelength of the 40 mg/L DPPH solution in the range of 400-800 nm using a UV-Vis spectrophotometer was carried out. African leaves extract fractions were made in several concentration series, namely the *n*-hexane fraction (50, 100, 150, 200, 250 mg/L), while the ethyl acetate and aqueous fractions (20, 40, 60, 80, 100 mg/L). Quercetin was used as a positive control, made in several concentration series, namely 2, 3, 4, 5, and 6 mg/L. A total of 2 mL of 40 mg/L DPPH solution was added with 2 mL of fraction solution of each concentration, incubated for 30 minutes at room temperature, and in a dark container.

The negative control used was 2 mL of 40 mg/L DPPH solutions added with 2 mL of 96% ethanol. The mixture of fraction and negative control solution was measured for absorbance at the maximum wavelength of DPPH. The test was replicated three times. The absorbance obtained is used to determine the percent attenuation, then plotted with the concentration to determine the IC₅₀ and AAI values (Rukmana, 2019).

2.2.7 Data analysis

Total flavonoid content was statistically analyzed with the help of SPSS using the One-Way ANOVA test and the post-hoc test using the Bonferroni test. IC₅₀ and AAI values were processed using Microsoft Excel

3 RESULTS AND DISCUSSION

3.2 Extraction and screening flavonoid phytochemicals

The extraction results of 480 g of dried African leaves simplicia (*Vernonia amygdalina* Del.) by maceration and remaceration methods one time using 96% ethanol as solvent are shown in **Table 1**. Extracts were identified qualitatively with

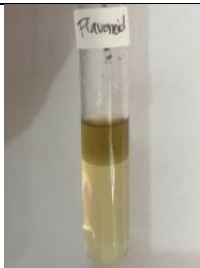
flavonoids which showed positive results containing flavonoids are presented in **Table 2**.

In this study, the maceration method was used because maceration is an extraction method that does not use high temperatures so that it is effective on thermolabile compounds, then followed by remaceration to extract compounds that are still left behind during the maceration process. This step was carried out for 72 hours to obtain the optimal compound. This is supported by Ningrum (2017), who said that the maceration time of 72 hours resulted in an increase in extract yield compared to 24 hours and 48 hours.

Table 1. The yield of African leaves extract

Sample	Extract weight (g)	Yield percentage (w/w)
480 g of African leaves simplicial	31.8	6.625

Table 2. Qualitative identification of flavonoid compound in African leaves extract

Test	Reagent	Result	
		Color	Picture
Shinoda	Mg powder, HCl (p), amyl alcohol	The yellow color on the amyl alcohol layer	

However, from the extract data, obtained a low percent yield (6.625%). This is thought to be influenced by the amount of solvent used is not sufficient in attracting compounds in the African leaves extract. Based on Depkes RI (2017), the ratio of simplicia and solvent during extraction is 1:10, while in this study a ratio of 1:3 was used. The extract was tested for phytochemical screening as a preliminary test to determine the presence of flavonoid

compounds contained in the African leaves with the Shinoda test using Mg powder, HCl, and amyl alcohol. Mg and HCl aim to reduce the benzopyran core in the flavonoid structure so that a yellow flavilium salt is formed (Ergina, 2014), as shown in **Table 2**. This is in accordance with research by Kharimah *et al.* (2016), which showed that African leaves extract from West Java was positive for flavonoids. The content of secondary

metabolites in plants is influenced by several factors, such as geographical influences, temperature, and nutrients where plants grow (Cannel, 2005).

2.3. Fractionation and identification of flavonoid

The results of the fractionation of 10 g extract using the liquid-liquid extraction method using *n*-hexane, ethyl acetate, and water as solvents are shown in Table 3. Each fraction was then identified as its flavonoid content by the thin layer chromatography (TLC) method, then evaporated with NH_3 , as presented in **Fig. 1**. The extract was fractionated with three solvents with different

polarities, then identified flavonoids using TLC. This was done to ensure that the fractions of African leaves extract from Tabanan (Bali) contain flavonoid compounds. In **Fig. 1**, it can be seen that there are differences in color spots in the fractions of African leaves extract and quercetin as a comparison. Based on Markham (1988), the difference in color spots is thought to be due to the presence of different types of flavonoids. The spots in the *n*-hexane and ethyl acetate fractions there are orange spots on a 365 nm UV lamp suspected of the presence of flavonols, while in the aqueous fraction there are light blue spots indicating the presence of isoflavones.

Table 3. The yield of African leaves extract fractions

Sample	Fraction weight (g)	Yield percentage % (w/w)
Aqueous fraction	0.9158	9.158
Ethyl acetate fraction	0.6399	6.399
<i>N</i> -hexane fraction	3.0046	30.046

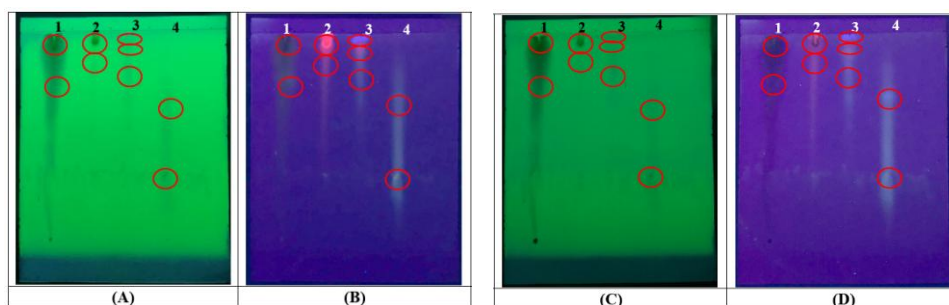


Figure 1. The result of flavonoid identification by the TLC method

Description:

A = TLC plate before evaporation of NH_3 on a 254 nm UV lamp; B = TLC plate before evaporation of NH_3 on a 365 nm UV lamp; C = TLC plate after evaporation of NH_3 on a 254 nm UV lamp; D = TLC plate after evaporation of NH_3 on a 365 nm UV lamp; spots from quercetin (1); *n*-hexane fraction (2); ethyl acetate fraction (3), and aqueous fraction (4)

2.4. Determination of total flavonoid content

Determination of total flavonoid content using quercetin as a standard solution and obtained the maximum wavelength of quercetin is 430 nm. The linear regression equation obtained from the standard solution of quercetin was used to calculate the total flavonoid content of each African leaves extract fraction with the results presented in **Table 4**. Determine of total flavonoid content in each African leaves extract fraction using the colorimetric method with an AlCl_3 reagent. The principle of this method is the formation of a stable complex with C-4 keto groups, as well as at C-3 or C-5 hydroxyl groups of flavones and flavonols which produces a yellow color. AlCl_3 reagent forms an acid labile complex with ortho-hydroxyl groups on the A- or B- ring of flavonoid compounds. The addition of potassium acetate aims to detect the presence of a free 7-hydroxyl group in the sample (Markham, 1988). The linear regression equation obtained is $y = 0.067x + 0.0139$ with an R^2 value of 0.9901, while the

r-value is 0.995 which is close to the value of one. This shows that there is a positive relationship between the concentration (x) and the absorbance (y) of the solution on a linear line. The higher the concentration of quercetin solution, the higher the absorbance obtained (Gandjar & Rohman, 2018). Based on statistical analysis, a significant value was obtained ($<0,005$), indicating that there was an average difference between the African leaves extract fractions.

The ethyl acetate fraction had the highest total flavonoid content compared to the *n*-hexane and aqueous fractions (Table 4). Similar to Suhendy *et al.* (2022) stated that the total flavonoid content in the ethyl acetate extract of African leaves from West Java (0.584 ± 0.050 gQE/100g) was higher than that of the *n*-hexane extract (0.197 ± 0.063 gQE/100g) and methanol (0.315 ± 0.007 gQE/100g). This is presumably because it is influenced by the semi-polar nature of ethyl acetate, so it can attract flavonoids with different polarities according to the principle of “like dissolves like” (Seidel, 2005).

Table 4. Total flavonoid content of African leaves extract fractions

Sample	Absorbance	Total flavonoid content $\pm$ SD (% b/b)	Sig.*
Aqueous fraction	0.016	0.031 ± 0.079	
<i>n</i> -Hexane fraction	0.112	1.469 ± 0.048	<0.05
Ethyl acetate fraction	0.863	12.668 ± 0.127	

*One-way ANOVA test, there are significant differences (<0.05)

2.5. Antioxidant activity test

The results of the antioxidant activity test showed that the maximum wavelength of DPPH was 517 nm which was used to measure the absorbance of the sample on a UV-Vis spectrophotometer. The results of IC₅₀ values are presented in **Tables 5-8**. Each African leaves extract fraction was tested for its antioxidant activity using the DPPH radical

reduction method. According to Maesaroh et al. (2018), the DPPH radical reduction method is the most effective method compared to the FRAP and FIC methods because it has high sensitivity. This can be seen based on the lowest LoQ and LoD values, which indicate that the DPPH radical can still be reduced by samples at low concentrations.

Table 5. Antioxidant activity of aqueous fraction of African leaves extract

Concentration (mg/l)	Average of absorbance	Inhibition percentage	Regression linear equation	IC ₅₀ (mg/L)	Category*
20	0.398	34.647	Y = 0.3156x + 27.876 R ² = 0.9628	70.101	Moderate
40	0.360	40.941			
60	0.324	46.798			
80	0.305	49.973			
100	0.233	61.686			

Table 6. Antioxidant activity of *n*-hexane fraction of African leaves extract

Concentration (mg/l)	Average of absorbance	Inhibition percentage	Regression linear equation	IC ₅₀ (mg/L)	Category*
50	0.374	38.588	Y = 0.1504x + 31.79 R ² = 0.9642	121.077	Weak
100	0.306	49.808			
150	0.290	52.381			
200	0.245	59.770			
250	0.175	71.210			

Table 7. Antioxidant activity of ethyl acetate fraction of African leaves extract

Concentration (mg/l)	Average of absorbance	Inhibition percentage	Regression linear equation	IC ₅₀ (mg/L)	Category*
20	0.444	27.094	Y = 0.364x + 19.935 R ² = 0.9516	82.596	Moderate
40	0.386	36.563			
60	0.361	40.777			
80	0.333	45.320			
100	0.249	59.113			

Table 8. Antioxidant activity of quercetin

Concentration (mg/l)	Average of absorbance	Inhibition percentage	Regression linear equation	IC ₅₀ (mg/L)	Category*
2	0.497	18.391	Y = 14.023x – 5.9112 R ² = 0.9812	3.987	Very strong
3	0.368	39.628			
4	0.294	51.779			
5	0.210	65.572			
6	0.149	75.534			

*The IC₅₀ category is based on (4) which states that values >250 mg/l (very weak), 100-250 mg/l (weak), 50-100 mg/l (moderate), and 10-50 mg/l (very strong) (Phongpaichit, et al., 2007)

The principle of the DPPH radical reduction method is that when the DPPH solution reacts with an antioxidant compound, the compound will donate its hydrogen atom to the DPPH. The electrons on the nitrogen atom of DPPH are reduced by accepting a hydrogen atom from the reacted antioxidant compound. The DPPH radical is called a stable free radical because one free atom has moved to another atom. If the DPPH solution is mixed with antioxidant compounds that donate hydrogen atoms, it can cause the violet color of the DPPH solution to disappear. This shows that antioxidant compounds reacted with DPPH have the ability to reduce the activity of DPPH free radicals (Yuslianti, 2019).

Antioxidant activity test in this study was carried out using a mixture of 40 mg/L DPPH solution, then incubated for 30 minutes. According to Molyneux (2004), 30 minutes is the optimal time for DPPH radicals to react with the sample. The mixture will experience a color change from dark purple to pale purple or pale yellow. This is because the sample has donated a hydrogen atom to the DPPH radical, resulting in a reduced form of the DPPH radical (Maesaroh et al., 2018). The aqueous fraction of African leaves extract from Tabanan (Bali) has better antioxidant activity (IC_{50}) than the ethyl acetate and *n*-hexane fractions (Table 5). The lower IC_{50} value indicates that at low concentrations, the sample has been able to reduce DPPH radicals by 50% (Molyneux, 2004).

Decrease in IC_{50} value was caused many factors, not only total flavonoid content in the extract, there were other factors that affect the antioxidant activity of the African leaves extract fractions, such as the effect of solvent polarity and other secondary metabolites that are thought to act as antioxidants.

Based on Gaffar et al. (2022) stated that the antioxidant activity of *n*-hexane extract of African leaves was low because non-polar solvents contained few compounds that could donate electrons or hydrogen atoms. Water solvents have high polarity, so they can attract polar secondary metabolites, such as alkaloids, tannins, flavonoids, and saponins found in the African leaves (Alara, 2017). This is still a presumption due to the limitations of this study, namely not carrying out further purification of flavonoid compounds in each fraction of African leaves extract from Tabanan (Bali).

4. CONCLUSIONS

Based on the research that has been done, it can be concluded that: there are differences in the total flavonoid content of the fractions of African leaves extract from Tabanan (Bali) with the total flavonoid content of each fraction, i.e., 1.469 %w/w (*n*-hexane fraction), 23.939 %w/w (ethyl acetate fraction), and 0.376 %w/w (aqueous fraction). There are differences in antioxidant activity in the fractions of African leaves extract from Tabanan (Bali) with IC_{50} values for each fraction, i.e., 121.077 mg/L (*n*-hexane fraction), 82.596 mg/L (ethyl acetate fraction), and 70.101 mg/L (aqueous fraction), while quercetin as a comparison has an IC_{50} value of 3.987 mg/L.

ACKNOWLEDGMENT

Funding: This work was supported by the the Chemical Laboratory of Mahaganisha College of Pharmacy, Bali-Indonesia

LIST OF REFERENCES

- Alara O. W., Abdurahman N. H., Mudalip S. K. A. & Olalere O. A. (2017). Phytochemical and Pharmacological Properties of *Vernonia amygdalina*: A Review. *JCEIB*, 2017(2), 80-96.

- Banjarnahor S. D. S. & Artanti N. (2014). Antioxidant Properties of Flavonoids. *Med J Indones*, 23(4), 239-244.
- Cannel R. J. P. (2005). *Follow-Up of Natural Product Isolation*, in Sarker SD, Latif Z, Gray AI (eds). Natural products isolation 2nd edition. Totowa NJ, Humana Press.
- Depkes RI (Departemen Kesehatan Republik Indonesia). (2000). *Parameter Standar Umum Ekstrak Tumbuhan Obat*. Jakarta: Departemen Kesehatan Republik Indonesia.
- Depkes RI (Departemen Kesehatan Republik Indonesia). (1995). *Farmakope Indonesia*, 4th edition. Jakarta: Departemen Kesehatan Republik Indonesia.
- Depkes RI (Departemen Kesehatan Republik Indonesia). (2017). *Farmakope Herbal Indonesia*, 2nd edition. Jakarta: Departemen Kesehatan Republik Indonesia.
- Ergina, Nuryanti S. & Pursitasari I. D. (2014). Uji Kualitatif Senyawa Metabolit Sekunder pada Daun Palado (*Agave angustifolia*) yang Diekstraksi dengan Pelarut Air dan Etanol. *J Akad Kim*, 3(3), 165-172.
- Esati, N., Budiarta, I., Cahyadi, K. & Lestari, G. (2021). Isolasi Dan Identifikasi Senyawa Flavonoid Fraksi Etil Asetat Ekstrak Daun Afrika (*Vernonia amygdalina* Del.). *Jurnal Ilmiah Ibnu Sina*, 6(2), 350-360.
- Gaffar S., Nugraha M. Y., Hafiz E., Wiraswati H. L. & Herlina T. (2022). Aktivitas Antioksidan dan Sitotoksik Terhadap Sel Kanker HeLa dari Ekstrak Daun *Vernonia amygdalina* (Asteraceae). *Chimica et Natura Acta*, 10(1), 6-14.
- Gandjar I. G., Rohman A. (2018). *Kimia Farmasi Analisis*. Yogyakarta: Penerbit Pustaka Belajar.
- Hasibuan P. A. Z., Harahap U., Sitorus P., & Satria D. (2018). *Proceedings of BROMO Conference: Evaluation of Antioxidant and Cytotoxic Activities of Vernonia amygdalina Del. Leaves*. Surabaya, Indonesia. SCITEPRESS - Science and Technology Publications.
- Kharimah N. Z., Lukmayani Y., & Syafnir L. (2016). Identifikasi Senyawa Flavonoid pada Ekstrak dan Fraksi Daun Afrika (*Vernonia amygdalina* Del.). *Prosiding Farmasi*, 2(2), 703-709.
- Maesaroh K., Kurnia D., & Anshori J. A. (2018). Perbandingan Metode Uji Aktivitas Antioksidan DPPH, FRAP, dan FIC Terhadap Asam Askorbat, Asam Galat, dan Kuersetin. *Chimica et Natura Acta*, 6(2), 93-100.
- Markham K. R. (1988). *Cara Mengidentifikasi Flavonoid*. diterjemahkan dari Bahasa Inggris oleh K. Padmawinata. Indonesia: Penerbit ITB Press.
- Molyneux P. (2004). The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin J Sci Technol*, 2004(26), 211-219.
- Ningrum M. P. (2017). *Pengaruh Suhu dan Lama Waktu Maserasi terhadap Aktivitas Antioksidan Ekstrak Metanol Rumpun Laut Merah (Eucheuma cottonii) (skripsi)*. Malang: Universitas Brawijaya.
- Phongpaichit S., Nikom J., Rungjindamai N., Sakayaroj J., Hutadilok-Tawatana N., Rukachaisirikul V., et al. (2007). Biological Activities of Extracts from Endophytic Fungi Isolated from *Garcinia* Plants: Biological Activities of Extracts from Endophytic Fungi. *FEMS Immunol Med Microbiol*, 51(3), 517-525.
- Rukmana H. (2019). *Uji Aktivitas Antioksidan Ekstrak Etanol dan Fraksi N-Heksana serta Etil Asetat Daun Sirsak (Annona muricata L.) dengan Metode 1,1-difenil-2-pikrilhidrazil (DPPH) (skripsi)*. Medan: Universitas Sumatera Utara.
- Seidel V. (2005). *Initial and Bulk Extraction*, in Sarker SD, Latif Z, Gray AI. Natural products isolation 2nd edition. Totowa NJ, Humana Press.
- Sholekah, F. F. (2017). Perbedaan Ketinggian Tempat Terhadap Kandungan Flavonoid dan Beta Karoten Buah Karika (*Carica pubescens*) Daerah

-
- Dieng Wonosobo. *Prosiding Seminar Nasional. Jurusan Pendidikan Biologi. Fakultas MIPA, Universitas Negeri Yogyakarta.*, p. 8.
- Suhendy H., Alif A., & Rahmiyani I. (2022). Korelasi Kadar Fenolik dan Flavonoid Total terhadap Aktivitas Antioksidan Beberapa Ekstrak Daun Afrika (*Vernonia amygdalina* Delile.) menggunakan Metode FRAP (*Ferric Reducing Antioxidant Power*). *Medical Sains: Jurnal Ilmu Kefarmasian*, 7(2), 225-236.
- Utomo D. S., Kristiani E. B. E., & Mahardika A. (2020). Pengaruh Lokasi Tumbuh Terhadap Kadar Flavonoid, Fenolik, Klorofil, Karotenoid, dan Aktivitas Antioksidan pada Tumbuhan Pecut Kuda (*Stachytarpheta Jamaicensis*). *Bioma*, 22(2), 143-149.
- Yuslianti E. R. (2019). *Pengantar Radikal Bebas dan Antioksidan*, 1st edition. Yogyakarta: Deepublish.