Trad. Med. J., January-April 2022
Vol. 27(1), p 51-59
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 13-01-2022
Revised : 23-03-2022
Accepted : 26-03-2022

Evaluation of Total Flavonoid, Total Phenolic, and Antioxidant Activity of *Etlingera elatior* (Jack) R.M.Sm Flower, Fruit, and Leaf

Ulya Safrina*, Wardiyah, Harpolia Cartika
Department of Pharmacy, Politeknik Kesehatan Kemenkes Jakarta II, Jakarta Pusat, DKI Jakarta, Indonesia

ABSTRACT

Etlingera elatior (*E. elatior*) plant has long been used as a kitchen spice and daily food. *E. elatior* has potential as an antioxidant because it contains polyphenol and flavonoid compounds widely found in the leaves, fruit, and flowers. This study measures the total flavonoid content, total phenol content, and antioxidant activity using the ABTS method. Total phenol content was determined by the Follin-Ciocalteu method and total flavonoid content was determined by the Aluminium Chloride method. The results showed that the total flavonoid content from the highest to the lowest value from *E. elatior* was fruit extract, leaf extract, and flower extract, respectively. The value of total flavonoid content was 8.38 ± 0.15; 4.86 ± 0.10; and 2.60 ± 0.04 % w/w Catechin Equivalent (CE). The total phenol content from the highest to the lowest value from *E. elatior* was fruit extract, leaf extract, and flower extract, respectively. The total phenolic content values were 54.48 ± 1.89, 46.20 ± 0.83, and 4.80 ± 0.53 % w/w Gallic Acid Equivalent (GAE). The highest to lowest antioxidant activity values (IC_{50}) were leaf extract at 58.82 ppm (strong activity), fruit extract at 103.05 ppm (moderate activity), and flower extract at 251.40 (weak activity), respectively.
Keywords: *Etlingera elatior*; total phenol; total flavonoid; antioxidant activity

INTRODUCTION

Antioxidants are natural compounds that can prevent oxidation reactions. These compounds can protect the body's cells from damage caused by free radicals. Herbal plants are sources of natural antioxidants. Therefore, they can fulfill the body's antioxidant needs (Ulewicz-Magulska and Wesolowski, 2019). In its performance, these natural compounds inhibit oxidation reactions by binding to free radical molecules and maintaining the genetic structure in normal conditions (Lingga, 2012). Antioxidant compounds commonly found in foodstuffs are vitamin E, vitamin C, beta-carotene, selenium, superoxide dismutase (SOD), and flavonoids. Natural antioxidants are dominated by plants and generally contain phenolic compounds such as phenolic acids, flavonoids, lignin, stilbenes, and tannins, which are spread throughout the plant (Silvia *et al*., 2016; Ulewicz-Magulska and Wesolowski, 2019).

Extracts are liquid, viscous, or dry preparations resulting from the extraction process of a matrix or simplicial using an appropriate method (Endang, 2019). Antioxidant compounds from plants can be obtained by extraction using a solvent. In this case, the type of solvent influences the antioxidant activity obtained.

One of the plants that are efficacious as antioxidants are *E. elatior*. Traditionally, *E. elatior* has long been used and utilized by the community as medicine and food flavoring. The compound content of *E. elatior* leaves consists of saponins, flavonoids, and chlorogenic acid. *E. elatior* has various kinds of pharmacological activities such as antioxidant, antibacterial, larvicidal, and repellent (Farida and Maruzy, 2016).

In a previous study, antioxidant activity was tested in the aqueous extract of *E. elatior* leaves using the DPPH method (2,2-diphenyl-1-picrylhydrazil), which gave an IC_{50} value of 24.39 mg/L, which was classified as very strong (Ningtyas, 2011). Meanwhile, the antioxidant activity of 96% ethanol extract of *E. elatior* leaves obtained an IC_{50} value of 4.7645 ppm, classified as having very strong antioxidant activity with total phenolic content of 48.223 mg GAE/gram (Handayani *et al*., 2014; Pramiastuti *et al*., 2018). In addition, the antioxidant activity of the leaf, flower, and stem fraction of *E. elatior* was tested using the ferric thiocyanate method. The method is based on the formation of peroxide, which results from the oxidation of linoleic acid. The study results on *E. elatior* leaves showed that the type of fraction had a significant effect on total phenol and antioxidant activity. The ethyl acetate fraction gave the total phenol and antioxidant values, respectively, ranging from 522.08 to 1776.08 mg/100 g and 62.30 to 73.87%. Meanwhile, the ethanol fraction ranged from 854.10 to 4851.30 mg/100 g and 47.47 to 75.07% (Naufalin and Rukmini, 2011).

One of the antioxidant testing methods is ABTS (2,2-Azinobis 3-Ethyl Benzothiazoline 6 Sulfonic Acid). ABTS is a method used to test

***Corresponding author : Ulya Safrina**
Email : ulya.syafrina@poltekkesjkt2.ac.id

antioxidants in plants. The advantages of the ABTS method are reacting quickly with antioxidants, can be used at different pH levels, and being soluble in water and organic solvents.

Based on previous studies, the potential of the *E. elatior* plant as an antioxidant has been proven in previous studies using *E. elatior* leaf extract using the DPPH method. In addition, testing was carried out on the *E. elatior* leaf fraction using the ferric thiocyanate method. Thus, the ABTS method encouraged the authors to research different antioxidant activities by determining the total phenolic and flavonoid levels in flower, fruit, and leaf extracts. A compound is proven to function as an antioxidant by testing at least 3 test methods.

METHODOLOGY

Source of plant material

The samples used in this research were the leaf, fruit, and flowers of *E. elatior* obtained from the gardens of local farmers in the Samadua region, South Aceh Regency, Aceh, on April 22, 2021. The plant parts were then inspected at the Laboratory of the Biological Research Center, LIPI, Indonesia, to prove the correctness of the plants used in this study. The results showed that the sample used was the correct *E. elatior* plant, with the certificate number for the determination result being B-147/V/DI.05.07/10/2021.

Source of chemical material

The chemicals used in this study were ABTS, potassium persulfate, Catechin, Folin-Ciocalteu (Sigma Aldrich), Gallic Acid (MP Biomedicals), AlCl3, Ethanol, Acetic acid, Ferric chloride (Merck), and Aquadest.

Extraction of E. elatior leaf, fruit, and flower extract

Fresh *E. elatior* leaf, flowers, and fruit were cut into pieces, dried, then powdered. Extraction was carried out by the maceration method using ethanol as a solvent. *E. elatior* leaf, flowers, and fruit powder (100 g) were weighed and put into a maceration chamber with the addition of 70% ethanol as much as 750 mL. The powder was soaked for 6 hours while stirring every 30 minutes, then held for 3 days at room temperature (Maserate I). After three days, the preparations were separated and added with 250 ml of 70% ethanol. After being dissolved, 70% ethanol was allowed to be held for 2 days at room temperature (Maserate II). Maserates I and II were mixed, then the extraction solution was filtered using the Büchner funnel. The filter results were then evaporated in a rotary evaporator until it became a thick extract (Sivanandham, 2015; Wardiyah *et al*., 2021). The thick extract obtained was then measured for its water content using a moisture analyzer.

Phytochemical screening of E. elatior leaf, fruit, and flower extract

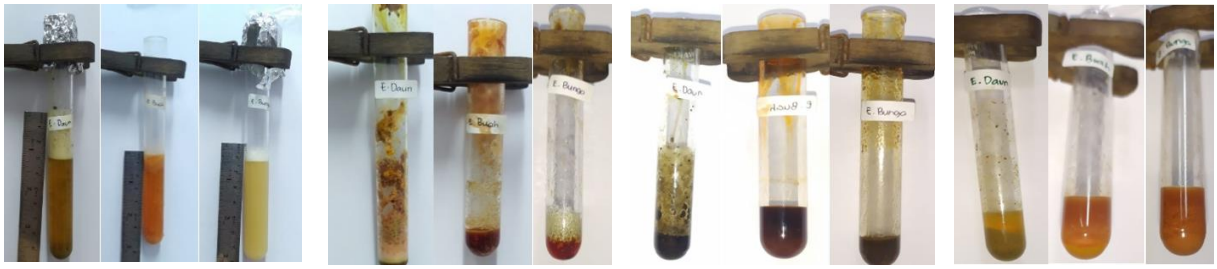
E. elatior leaf, flower, and fruit extracts were screened qualitatively for phenolic compounds, flavonoids, saponins, and alkaloids groups. Identification of flavonoids was carried out by adding 2 mg of magnesium powder and 2 mL of concentrated hydrochloric acid into the extract and then shaking it with 10 mL of amyl alcohol. A positive reaction is indicated by the orange, yellow, or red color on the amyl alcohol layer (Harborne, 2012). Identification of alkaloids was carried out with 1 gram of extract with three drops of 10% ammonia and 1.5 mL of chloroform, then shaken. The chloroform layer was taken and then dissolved in 1 mL of 2 N sulfuric acid, then shaken. After that, the mixture was added with Meyer's reagent. The mixture contains alkaloids if there is a white precipitate (Departemen Kesehatan Republik Indonesia, 1989). Saponin identification was carried out by placing 1 gram of extract into a test tube, then adding 20 mL of hot water, cooling it, then shaking vigorously vertically for 10 seconds. If the foam is formed as high as 1 to 10 cm, which is stable for no less than 10 minutes and does not disappear with one drop of 2 N hydrochloric acid, it indicates the presence of saponins (Departemen Kesehatan Republik Indonesia, 1989). Identification of phenol by placing the extract into a test tube and then add two drops of 5% $FeCl_3$. If a greenish, red-purple, blue, or black color is formed in the mixture, indicating the presence of phenolic compounds (Harborne, 2012).

Determination of total phenolic content

Sample solutions (250 and 500 µL) and standard solutions of gallic acid (25,50, 100, 150, and 200 µL) were pipetted into a test tube, and 4 mL of distilled water were added. Then, 250 µL of Follin-Ciocalteu reagent was added and shaken. After being allowed to stand for 8 minutes at room temperature, 750 µL of 20% Sodium Carbonate was added and shaken homogeneously. Next, the mixture was allowed to stand for 2 hours at room temperature and the absorbance was measured at a wavelength of 765 nm (Singleton *et al*., 1999).

Determination of total flavonoid content

E. elatior leaf, fruit, and flower extracts and standard solutions of quercetin were made in various concentrations of 205.2 to 680 ppm. Each concentration was taken 5 mL and put in a 10.0 mL volumetric flask. Then, 0.3 mL of 5% $NaNO_2$ and 0.3



(a) (b) (c) (d)

Figure 1. Results of phytochemical screening of *E.elatior* leaf, fruit, and flower extracts sequentially. (a) Saponin test with aquadest, (b) Flavonoid test with Mg powder and concentrated HCl, (c) Phenolic test with FeCl3 reagent, (d) Alkaloid test with Dragendorf and Mayer reagents.

Table I. Phytochemical Screening of *E. elatior* Leaf, Fruit, and Flower Extracts Qualitatively

Sample	Flavonoid	Phenol	Alkaloid	Saponin
***E. elatior* Leaf Extract**	+++	+++	++	+++
***E. elatior* Fruit Extract**	+++	+++	+++	-
***E. elatior* Flower Extract**	+++	+++	+++	+++

Notes : + Weak; ++Strong; +++Very strong, -Negative/none

mL of 10% $AlCl_3$ were added to the sample solution. Then, the sample solution was incubated at room temperature for 5 minutes. Next, 2 ml of 1 M NaOH was added and distilled water up to 10.0 mL. The sample solution was measured using a UV/Vis Spectrophotometer at a wavelength of 415 nm.

Evaluation of antioxidant activity using ABTS method

The testing procedure was carried out based on previous research (Arnao, 2000; Wardiyah *et al*., 2021). *E. elatior* leaf, fruit, and flower extract sample solutions were made with 25 to 400 ppm varying concentrations. Vitamin C standard solution was made with a concentration variation of 0.625 to 5 ppm. The ABTS solution and the sample were pipetted in a 1:1 ratio into a 96-well microplate, then homogenized. The absorbance of the sample solutions and standard solutions was then measured with a microplate reader at a wavelength of 516 nm.

RESULT AND DISCUSSION

Phytochemical Screening of *E. elatior* Leaf, Fruit and Flower Extracts

Phytochemical screening was carried out simply using color reagents using qualitative analysis methods. The results of phytochemical screening of *E. elatior* flower, leaf, and stem extracts are in Table I.

Phytochemical screening aims to determine the class of compounds in the extracts of leaves, fruits, and flowers of *E. elatior*. Phytochemical screening tests were carried out on four main compounds: flavonoids, phenols, alkaloids, and saponins. The results of phytochemical screening on *E. elatior* leaf and flower extracts showed positive results for flavonoid, alkaloid, saponin, and phenolic compounds. This is in line with other studies showing the same results: *E. elatior* leaves contain many flavonoid and saponin compounds (Nisrina Effendi *et al*., 2019; Roslim and Umam, 2021). In addition, the phytochemical screening of *E. elatior* fruit extract showed positive results containing flavonoids, alkaloids, and phenolics. This is in line with other studies that show the phytochemical content of *E. elatior* fruit from Nagan Raya, Aceh, which contains alkaloids, phenols, flavonoids, tannins, and terpenoids. *E. elatior* flower parts also contain alkaloids, phenols, flavonoids, saponins, tannins, and terpenoids (Ernilasari *et al*., 2021). The difference between the results of this study and previous studies is the intensity of the color produced during testing. The more intense the color produced, the more secondary metabolites in the extract (Das and Gezici, 2018). Differences in secondary metabolites in plants are influenced by variations in plant growth height, light, climate, temperature, groundwater, soil fertility, and salinity (Giweli *et al*., 2013; Liu *et al*., 2016).

Table II. Comparison of Total Phenolic Content in *E. elatior* Leaf, Fruit, and Flower Extracts (n = 3 replication)

No	Part of Plants	Average Total Phenolic Content in Extract (% w/w Gallic Acid Equivalent)
1	*E. elatior* Fruit	54.48 ± 1.89
2	*E. elatior* Leaf	46.20 ± 0.83
3	*E. elatior* Flower	4.80 ± 0.53

Table III. Comparison of Total Flavonoid Content in *E. elatior* Leaf, Fruit, and Flower Extracts (n = 3 replication)

No	Part of Plants	Average Total Flavonoid Content in Extract (% w/w Catechin Equivalent)
1	*E. elatior* Fruit	8.38 ± 0.15
2	*E. elatior* Leaf	4.86 ± 0.10
3	*E. elatior* Flower	2.60 ± 0.04

Total Flavonoid and Total Phenol Content of *E. elatior* Leaf, Fruit, and Flower Extract

Measurement of total phenolic in extracts of leaves, fruit, and flowers of *E. elatior* using gallic acid standard. The results of total phenolic measurements can be seen in Table II.

The total phenolic content of *E. elatior* leaf, fruit, and flower extract was tested using the Follin-Ciocalteu method, with gallic acid as the standard. The test results are expressed in % mg/g gallic acid equivalent. The total phenolic content was obtained from the linear regression equation y = 0.0517x + 0.2155, R^2 = 0.9918 (Appendix 8). From table 5, it can be seen that the highest to lowest total phenolic content were fruit extract, leaf extract, and *E. elatior* flower extract with values of 54.48 ± 1.89, 46.20 ± 0.83, and 4.80 ± 0, 53. This result is different from several other studies on several etlingera species. The ethanol extract of *E. elatior* leaves showed a higher total phenolic content than the fruit ethanol extract (Ahmad *et al*., 2015; Isyanti *et al*., 2019; Shahid-Ud-Daula *et al*., 2019). This can happen because the fruit and leaves are parts of the plant that are easier to get light and sunlight, so the concentration of phenolic acids and flavonoids is greater in the fruit and leaves than the flower parts (Shahid-Ud-Daula *et al*., 2019). Another study also showed the same results regarding the total phenolic content of *E. elatior* flower extract, where the total phenolic content was lower than that of the leaves and stems. This is because the leaves contain many polar compounds and chlorophyll (Nuryanti *et al*., 2021). The high temperature during simplicial drying can also affect the total phenolic content in the *E. elatior* flower extract. The flower parts contain many volatile essential oils, which can evaporate when drying simplicial. This is in line with other studies, which showed the total phenolic content of fresh *E. elatior* flower simplicial was higher than that of dried *E. elatior* flower simplicial (Nuryanti *et al*., 2021). In this study, dry simplicial was used.

Measurement of total flavonoids in the extract of leaves, fruit, and flowers of *E. elatior* was using catechin standards. The measurement of total flavonoids can be seen in Table III.

Testing the total flavonoid content of *E. elatior* leaf, fruit, and flower extracts were carried out using catechins as a standard. The test is expressed in % mg/g Catechin equivalent (CE). From table 6, the total flavonoid content from the highest to the lowest is *E. elatior* fruit extract, leaf extract, and flower extract with a value of 8.38 ± 0.15; 4.86 ± 0.10; and 2.60 ± 0.04. This is following previous research, which states that the *E. elatior* plant's total flavonoid and phenolic content are primarily contained in the leaves, flowers, stems, and rhizomes (Mai, 2009). The results of the total flavonoid content were linear with the results of the total phenolic content. Extracts that have a high total phenolic content also contain high total flavonoids. Plants that contain lots of flavonoids can function as a source of antioxidants that can increase the antioxidant capacity of organisms and fight lipid peroxidation (Khorasani Esmaeili *et al*., 2015).

The difference in the amount of total phenolic content and total flavonoid in each part of the plant is influenced by various environmental factors, such as light and ultraviolet radiation, temperature, lack of water in the soil, salt content in the soil, soil composition, differences in plant age, metal content, and other chemical factors. (Li *et al*., 2020). The different types of solvents used in the extraction process also affect the content of metabolite compounds (Herni *et al*., 2018). Extraction of phenolic compounds using water as a solvent resulted in a total phenolic content of 15.9% more than ethanol (Ghasemzadeh *et al*.,

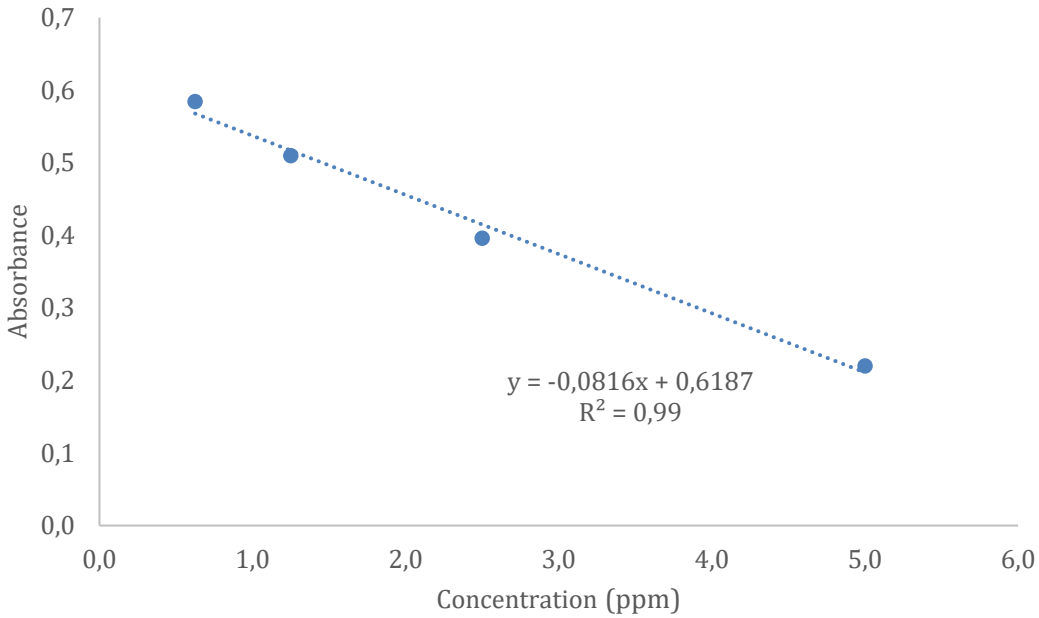


Figure 2. Vitamin C Standard Solution Calibration Curve

2015). The solubility of polyphenolic compounds is better in ethanol solvents than in water solvents. The simplicial drying process can also damage some phenolic compounds because, in dry conditions, phenolic compounds are trapped in plant cells and cannot be extracted (Nuryanti *et al*., 2021). However, the high temperature during the extraction process can increase the solubility of phenolic compounds. Since high temperatures can cause phenolic compounds to come out of plant cell walls, more phenolic compounds are extracted (Wazir *et al*., 2011). Thus, the drying time and temperature need to be validated during the drying process so that the bioactive components contained in the extract are not damaged.

Phenolic compounds and flavonoids are metabolites in many plants and have been reported to have effective antioxidant activity due to their specific redox characteristics. Flavonoids belong to the phenolic group that can effectively reduce Reactive Oxygen Species (ROS). Differences in the amount of flavonoid content in plants can be caused by different types of flavonoid compounds contained in these plants. Less polar flavonoid compounds (isoflavones, flavanones, flavones, and flavonols) are better extracted with non-polar solvents, and more polar flavonoid compounds (glycosides and aglycones) are better extracted using polar solvents (alcohol, water-alcohol) (Aryal *et al*., 2019; Muflihah *et al*., 2021).

Antioxidant Activity of *E. elatior* Leaf, Fruit, and Flower Extracts

Measure antioxidant activity in extracts of leaves, fruit, and flowers of *E. elatior* using the ABTS method with Vitamin C as a standard. The percentage value inhibition and IC_{50} of antioxidants were obtained from the linear regression equation of the standard curve of the standard and the sample. Antioxidant activity testing was conducted to measure *E. elatior* leaf, fruit, and flower extracts' relative antioxidant ability to reduce free radicals in the reagents. Antioxidant testing using ABTS reagents is the direct production mechanism of ABTS•+, which is blue or green due to the oxidation reaction of ABTS with potassium persulfate, followed by a reduction reaction due to the presence of hydrogen donor antioxidants (Al-Mansoub *et al*., 2021). The ABTS method is used because free radicals are more stable when receiving hydrogen ion donors from antioxidants. Thus, the blue color of ABTS disappears. The ABTS method is more sensitive to determining antioxidant activity because the kinetic reaction is faster and detects antioxidants better than the DPPH method. ABTS reagent is also soluble in water and organic solvents. Therefore, it can determine both hydrophilic and lipophilic antioxidants (Lee *et al*., 2015; Shah and Modi, 2015).

The IC_{50} value was obtained from the calibration curve of the vitamin C standard

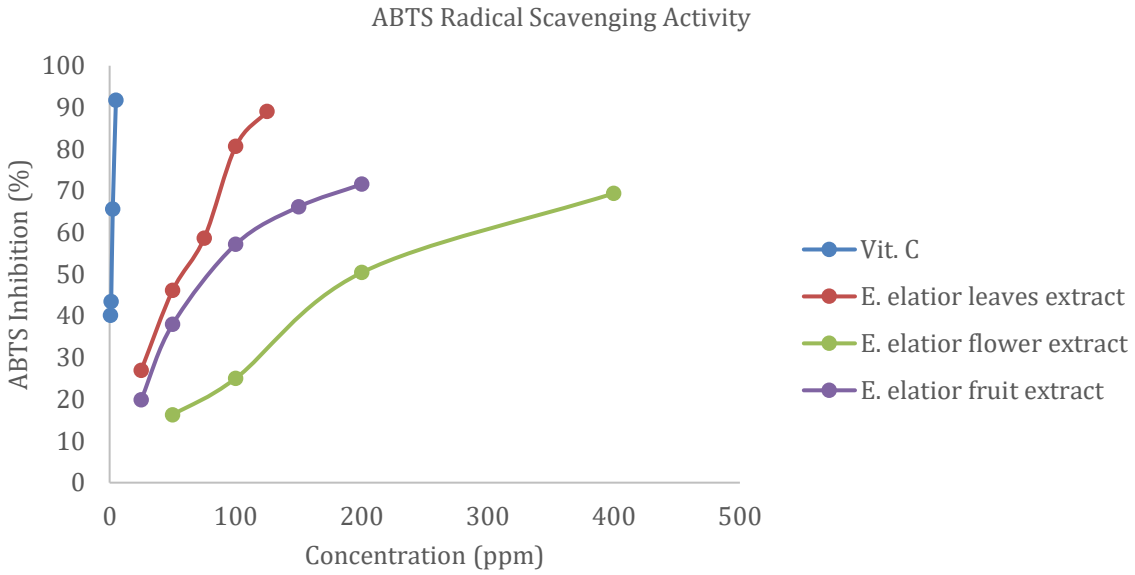


Figure 3. ABTS radical scavenging activity

Table IV. Value of IC_{50} *E. elatior* Leaf, Fruit and Flower Extract (n = 3 replication)

No	Part of Plants	Value of IC_{50} (ppm)	Activities
1	*E. elatior* Fruit	58.82	Strong
2	*E. elatior* Leaf	103.05	Medium
3	*E. elatior* Flower	251.40	Weak
4	Vitamin C	1.51	Very Strong

solution. The calibration curve was obtained from the concentration vs. absorbance plot of the sample so that the linear regression equation y = -0.0816x + 0.6187 with R^2 = 0.9900 (Figure 2) was obtained.

After obtaining the calibration curve, the percentage of ABTS radical scavenging activity was calculated using the formula. The results of percentage ABTS inhibition vs. concentration are in Figure 3. The percentage ABTS radical scavenging activity is sorted from highest to lowest: *E. elatior* leaf extract, fruit extract, and flower extract, respectively. The difference in the results of free radical reduction is due to the difference in the total phenol and flavonoid content in the plant parts. The highest total phenol and flavonoid content was in the *E. elatior* fruit extract, but the highest % ABTS reduction was in the *E. elatior* leaf extract. This can happen because the leaves of *E. elatior* contain other non-polar compounds that have antioxidant activity, such as steroids and terpenoids (Wardiyah *et al*., 2021). Further research needs to be done to see other compounds responsible for antioxidants besides phenols and flavonoids in the *E. elatior*.

The results of antioxidant activity were obtained in IC_{50} values, as shown in Table 4. The highest antioxidant activity was found in *E. elatior* leaf extract with an IC_{50} value of 58.82 ppm and classified as strong activity. This is in line with previous studies, which showed that the ethanolic extract of *E. elatior* leaves has strong antioxidant activity with an IC_{50} value of 23.45 ug/mL (Wardiyah *et al*., 2021). This difference in the value of antioxidant activity may occur due to differences in the number of secondary metabolite compounds contained in the plant parts since the *E. elatior* plants used in this study, and previous studies were harvested in different areas. In addition, variations in plant age, growing location, soil composition, and weather also affect the levels of secondary metabolites (Li *et al*., 2020).

The positive control used in this study was vitamin C. Vitamin C is a hydrophilic compound and has very strong antioxidant activity. The IC_{50} value is 1.51 ppm. The use of positive control aims

to compare the antioxidant activity of *E. elatior* leaf, fruit, and flower extracts with positive controls.

In previous studies, the antioxidant activity of *E. elatior* leaf was tested using the DPPH and ABTS methods. The results of previous studies showed that the antioxidant activity of *E. elatior* leaf was in the very strong range in both methods. This is different from the current study, where *E. elatior* leaf was only in the strong range. This can occur due to differences in the location of plant growth used as samples in two studies (Li *et al*., 2020). In this study, the highest antioxidant activity was shown by the fruit *extract E.* elatior. The advantage of this study is to use several parts of *the E. elatior* plant to compare the value of the antioxidant activity. ABTS method can measure hydrophilic and lipophilic antioxidant compounds *in E. elatior*. However, this study has several limitations, such as antioxidant testing using the ABTS method, which is an in vitro test model so it cannot describe all antioxidant activities *in E. elatior*. In addition, antioxidant activity is also influenced by the solvent used during extraction (Floegel *et al*., 2011). It needs to be considered for further research in the selection of appropriate methods and solvents.

CONCLUSION

E. elatior fruit extract has the highest total phenol and flavonoid content compared to other plant parts, but the highest antioxidant activity is obtained from *E. elatior* leaf extract. This can happen because the leaves of *E. elatior* contain other non-polar compounds that have antioxidant activity, such as steroids and terpenoids.

ACKNOWLEDGEMENT

The authors would like to thank the Politeknik Kesehatan Kemenkes Jakarta II, Indonesia, for funding this research. The authors also thank all parties involved in this research.

REFERENCES

Ahmad, A.R., Juwita, J., Ratulangi, S.A.D., Malik, A., 2015. Penetapan Kadar Fenolik dan Flavonoid Total Ekstrak Metanol Buah dan Daun Patikala (Etlingera elatior (Jack) R.M.SM). Pharm. Sci. Res. 2, 1–10.

Al-Mansoub, M.A., Asif, M., Revadigar, V., Hammad, M.A., Chear, N.J.-Y., Hamdan, M.R., Majid, A.M.S.A., Asmawi, M.Z., Murugaiyah, V., 2021. Chemical composition, antiproliferative and antioxidant attributes of ethanolic extract of resinous sediment from Etlingera elatior (Jack.) inflorescence. Braz. J. Pharm. Sci. 57.

Arnao, M., 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. Trends Food Sci. Technol. 11, 419–421.

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., Koirala, N., 2019. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants Basel Switz. 8, E96. h

Das, K., Gezici, S., 2018. Review article Plant secondary metabolites, their separation, identification and role in human disease prevention. Ann. Phytomedicine Int. J. 7, 13–24.

Departemen Kesehatan Republik Indonesia, 1989. Materia Medika Indonesia Jilid V. Departemen Kesehatan Republik Indonesia, Jakarta.

Endang, H., 2019. Analisis Fitokimia. EGC.

Ernilasari, E., Walil, K., Fitmawati, F., Roslim, D.I., Zumaidar, Z., Saudah, S., Rayhannisa, R., 2021. Antibacterial activity of leaves, flowers, and fruits extract of Etlingera elatior from Nagan Raya District, Indonesia against Escherichia coli and Staphylococcus aureus. Biodiversitas J. Biol. Divers. 22.

Farida, S., Maruzy, A., 2016. Kecombrang (Etlingera elatior): Sebuah Tinjauan Penggunaan Secara Tradisional, Fitokimia Dan Aktivitas Farmakologinya. J. Tumbuh. Obat Indones. 9, 19–28.

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S.I., Chun, O.K., 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J. Food Compos. Anal. 24, 1043–1048.

Ghasemzadeh, A., Jaafar, H.Z.E., Rahmat, A., Ashkani, S., 2015. Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of Etlingera elatior (Jack) R.M.Sm grown in different locations of Malaysia. BMC Complement. Altern. Med. 15, 335.

Giweli, A., Dzamic, A., Sokovic, M., Ristic, M.S., Janackovic, P., Marin, P.D., 2013. The chemical composition, antimicrobial and antioxidant activities of the essential oil of Salvia fruticosa growing wild in Libya. Arch. Biol. Sci. 65, 321–329.

Handayani, V., Ahmad, A.R., Sudir, M., 2014. Uji Aktivitas Antioksidan Ekstrak Metanol Bunga dan Daun Patikala (Etlingera elatior

(Jack) R.M.Sm) Menggunakan Metode DPPH. Pharm. Sci. Res. 1, 86–93. https://doi.org/10.7454/psr.v1i2.3321

Harborne, J.B., 2012. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Springer Science & Business Media.

Herni, K., Subarnas, A., Diantini, A., Iskandar, Y., Marliani, L., S.A, N., 2018. Activity-guided Isolation of Antioxidant Phenolic Compound from Etlingera elatior Leaves. Res. J. Chem. Environ. 22, 43–46.

Isyanti, M., Andarwulan, N., Nur Faridah, D., 2019. Karakteristik Fisik dan Fitokimia Buah Kecombrang (Etlingera elatior (Jack) R.M. Sm). War. Ind. Has. Pertan. 36, 96.

Khorasani Esmaeili, A., Mat Taha, R., Mohajer, S., Banisalam, B., 2015. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from In Vivo and In Vitro Grown Trifolium pratense L. (Red Clover). BioMed Res. Int. 2015, e643285.

Lee, K.J., Oh, Y.C., Cho, W.K., Ma, J.Y., 2015. Antioxidant and Anti-Inflammatory Activity Determination of One Hundred Kinds of Pure Chemical Compounds Using Offline and Online Screening HPLC Assay. Evid. Based Complement. Alternat. Med. 2015, e165457.

Li, Y., Kong, D., Fu, Y., Sussman, M.R., Wu, H., 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem. 148, 80–89.

Lingga, L., 2012. The Healing Power of Antioxidant. PT Elex Media Komputindo, Jakarta.

Liu, W., Yin, D., Li, N., Hou, X., Wang, D., Li, D., Liu, J., 2016. Influence of Environmental Factors on the Active Substance Production and Antioxidant Activity in Potentilla fruticosa L. and Its Quality Assessment. Sci. Rep. 6, 28591.

Mai, C.W., 2009. Antiproliferative and Apoptotic Studies of the Standardised Extracts of Etlingera elatior on Human Colorectal Carcinoma Cells. Malays. J. Chem. MJChem 11, 136–142.

Muflihah, Y.M., Gollavelli, G., Ling, Y.-C., 2021. Correlation Study of Antioxidant Activity with Phenolic and Flavonoid Compounds in 12 Indonesian Indigenous Herbs. Antioxidants 10, 1530.

Naufalin, R., Rukmini, H., 2011. Potensi Antioksidan Hasil Ekstraksi Tanaman Kecombrang (Nicolaia speciosa Horan) Selama Penyimpanan.

Ningtyas, R., 2011. Uji antioksidan dan antibakteri ekstrak air daun kecombrang (etlingera elatior) (Jack) R.M.Smith) sebagai pengaw*et al*ami terhadap escherichia coli dan staphylococus aureus.

Nisrina Effendi, K., Fauziah, N., Wicaksono, R., Erminawati, Arsil, P., Naufalin, R., 2019. Analysis Of Bioactive Components And Phytochemical Of Powders Stem And Leaves Of Kecombrang *(Etlingera elatior)*. IOP Conf. Ser. Earth Environ. Sci. 406, 012003.

Nuryanti, S., Latifasari, N., Naufalin, R., Wicaksono, R., Erminawati, E., 2021. Antioxidant Activity and Total Phenol Extract of Kecombrang Flower, Stem and Leaves with Different Types of Solutions. Molekul 16, 110.

Pramiastuti, O., Zen, D.A., Prastiyo, B.A., 2018. Penetapan Kadar Total Fenolik Dan Uji Aktivitasantioksidan Ekstrak Etanol 96% Daun Kecombrang (Etlingera Elatior) Dengan Metode 2,2-Difenil-1-Pikrilhidazil (DPPH). J. Farm. Sains Indones. 1, 42–55.

Roslim, D.I., Umam, A.H., 2021. A phytochemical screening of Bakkala (Etlingera elatior) originated from suakbugis, Aceh, Indonesia and its potential in ethnobotany. Int. J. Herb. Med. 9, 37–42.

Shah, P., Modi, H.A., 2015. Comparative Study of DPPH, ABTS and FRAP Assays for Determination of Antioxidant Activity 3, 7.

Shahid-Ud-Daula, A.F.M., Kuyah, M.A.A., Kamariah, A.S., Lim, L.B.L., Ahmad, N., 2019. Phytochemical and pharmacological evaluation of methanolic extracts of Etlingera fimbriobracteata (Zingiberaceae). South Afr. J. Bot. 121, 45–53.

Silvia, D., Katharina, K., Hartono, S.A., Anastasia, V., Susanto, Y., 2016. Pengumpulan Data Base Sumber Antioksidan Alami Alternatif Berbasis Pangan Lokal Di Indonesia 18.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants using a folin-ciocalteu reagent, in: Methods in Enzymology, Oxidants and Antioxidants Part A. Academic Press, pp. 152–178.

Sivanandham, V., 2015. Phytochemical Techniques - A Review. World J. Sci. Res. 1, 80–91.

Ulewicz-Magulska, B., Wesolowski, M., 2019. Total Phenolic Contents and Antioxidant Potential of Herbs Used for Medical and Culinary Purposes. Plant Foods Hum. Nutr. 74, 61–67.

Wardiyah, W., Safrina, U., Prihandiwati, E., Niah, R., 2021. Phytochemical Contents and Antioxidant Activities of Etlingera elatior

Leaf Extract and Fractions. Trop. J. Nat. Prod. Res. 5, 1439–1444.

Wazir, D., Ahmad, S., Muse, R., Mahmood, M., Shukor, Y., 2011. Antioxidant activities of different parts of Gnetum gnemon L. J. Plant Biochem. Biotechnol. 20, 234–240.