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

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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–Ciocalteau 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 (IC₅₀) 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-Maguliska and Wesolowski, 2019). In its performance, these natural compounds inhibit oxidation reactions by binding to free radicals and 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-Maguliska 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 is *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 IC₅₀ value of 24.39 mg/mL which was classified as very strong (Ningtyas, 2011). Meanwhile, the antioxidant activity of 96% ethanol extract of *E. elatior* leaves obtained an IC₅₀ value of 4.7645 ppm, classified as having very strong antioxidant activity with total phenolic content of 48.223 mg GAE/gram (Farida, 2014; Pramiatistuti et al., 2016). 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% (Naufalni and Rukmini, 2011).

One of the antioxidant testing methods is ABTS (2,2-Azinoxylo-3-Ethyl Benzothiazoline 6 Sulfonic Acid). ABTS is a method used to test...
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.05.07/18/2021.

**Source of chemical material**

The chemicals used in this study were ABTS, potassium persulfate, Catechin, Folin-Ciocalteu (Sigma Aldrich), Gallic Acid (MP Biomedicals), AICl3, 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 residues were then evaporated in a rotary evaporator until it became a thick extract (Sivanandhan, 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 g 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% FeCl3, 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 was added. Then, 250 µL of Folin-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% NaNO2 and 0.3
mL of 10% AlCl₃ 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 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 I. Phytochemical Screening of *E. elatior* Leaf, Fruit, and Flower Extracts Qualitatively

<table>
<thead>
<tr>
<th>Sample</th>
<th>Flavonoid</th>
<th>Phenol</th>
<th>Alkaloid</th>
<th>Saponin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. elatior</em> Leaf Extract</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><em>E. elatior</em> Fruit Extract</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td><em>E. elatior</em> Flower Extract</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

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

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.
The total phenolic content of *E. elatior* leaf, fruit, and flower extract was tested using the Folin-Ciocalteu method, with gallic acid as the standard. The results of total phenolic measurements can be seen in Table II.

The total phenolic content 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 Esmæelî 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 (Herzi et al., 2018).

<table>
<thead>
<tr>
<th>No</th>
<th>Part of Plants</th>
<th>Average Total Phenolic Content in Extract (% w/w Gallic Acid Equivalent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E. elatior</em> Fruit</td>
<td>54.48 ± 1.89</td>
</tr>
<tr>
<td>2</td>
<td><em>E. elatior</em> Leaf</td>
<td>46.20 ± 0.83</td>
</tr>
<tr>
<td>3</td>
<td><em>E. elatior</em> Flower</td>
<td>4.80 ± 0.53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>Part of Plants</th>
<th>Average Total Flavonoid Content in Extract (% w/w Catechin Equivalent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E. elatior</em> Fruit</td>
<td>8.38 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td><em>E. elatior</em> Leaf</td>
<td>4.86 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td><em>E. elatior</em> Flower</td>
<td>2.60 ± 0.04</td>
</tr>
</tbody>
</table>
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 flavonoids) are better extracted using non-polar solvents (alcohol, water-alcohol) (Aryal et al., 2019; Muфиhah 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 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 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 $R^2 = 0.99$ (Figure 2) was obtained. After obtaining the calibration curve, the percentage of ABTS radical scavenging activity was calculated using the formula. The results of
Evaluation of Total Flavonoid, Total Phenolic, and Antioxidant Activity

The 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

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

<table>
<thead>
<tr>
<th>No</th>
<th>Part of Plants</th>
<th>Value of IC$_{50}$ (ppm)</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. elatior Fruit</td>
<td>58.82</td>
<td>Strong</td>
</tr>
<tr>
<td>2</td>
<td>E. elatior Leaf</td>
<td>103.05</td>
<td>Medium</td>
</tr>
<tr>
<td>3</td>
<td>E. elatior Flower</td>
<td>251.40</td>
<td>Weak</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin C</td>
<td>1.51</td>
<td>Very Strong</td>
</tr>
</tbody>
</table>
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.

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