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Original Research Article

Phytochemical Contents and Antioxidant Activities of *Etlingera elatior* Leaf Extract and Fractions

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ABSTRACT

The plant, *Etlingera elatior* has long been utilized by people as medicines and flavouring agents. Polyphenols and flavonoids are antioxidant metabolites found in the leaves of the plant. This research was aimed at determining the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of the ethanol extract, water, ethyl acetate, and n-hexane fractions from *E. elatior* leaves. Extracts were prepared from the leaves of *E. elatior* and were fractionated. TPC and TFC of the extracts and fractions were determined spectrophotometrically by utilizing the Folin-Ciocalteu and aluminium chloride reagents, respectively. Also, the antioxidant activity of the extract and fractions was tested by two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). The results indicated that the ethyl acetate fraction had the highest level of TPC (5.60 ± 0.02% w/w Gallic Acid Equivalent [GAE]) and TFC (14.66 ± 0.67% w/w catechin equivalent [CE]). In both the DPPH and ABTS procedures, the level of inhibition towards free radical was obtained in the hexane fraction with IC_{50} values of 39.35 µg/mL and 13.04 µg/mL, respectively. This result was the highest compared to the ethanol extract, water fraction, and ethyl acetate fraction. However, all antioxidant tests revealed that the value of IC_{50} was less than 50 µg/mL, indicating that both the extract and fractions of *E. elatior* has strong antioxidant activity.

***Keywords*:** *Etlingera elatior*, Total phenol content, Total flavonoid content, DPPH, ABTS.

Introduction

An antioxidant is a chemical compound that prevents cell damage due to free radicals. The free radicals result in the occurrence of oxidative stress reaction, a condition where there are more free radicals than antioxidant compounds in the body. This condition causes several problems such as premature aging, cancer, or degenerative diseases to the body. Naturally, the body can produce antioxidants. However, excessive exposure to free radicals causes instability of the natural antioxidant in the body thereby necessitating the intake of exogenous antioxidants.[1] Naturally, the sources of antioxidants are fruits or plants that are consumed daily by humans. Vitamins C, E, beta carotene, and flavonoid are some examples of natural sources of antioxidant. The natural sources of antioxidant are dominated by plants and generally contain phenolic compounds that are contained in all parts of the plant.[2]

Traditionally, *Etlingera elatior* has long been utilized by people as medicines and flavoring agents. *E. elatior* is an herb that originated from Southeast Asia. It is known as 'torch ginger' (*jahe obor*) or lily red ginger (*jahe merah lily*) which are part of *zingiberaceae*. In Indonesia, it is known as *kecombrang* or *honje*, whereas in Malaysia, it is called *bunga kantan*.[3] *E. Elatior* has synonyms of *Alpinia elatior*, *Elettaria speciosa*, *Nicolaia elatior*, *Nicolaia speciosa*, and *Phaeomeria speciose*.[4]

Empirically, this plant has been widely used for traditional medicines and cosmetics. *E. elatior* flowers are commonly used for food seasoning, while the leaves are utilized as materials for water immersion to eliminate body odor, especially for mothers in the postnatal period.[5] The phytochemical content of *E. elatior* flower extract is known to consist of polyphenols, tannins, saponins, flavonoids, glycosides, steroids, and terpenoids.[3,6] Moreover, extract of the leaves contain flavonoids, quercetin, and chlorogenic acid. Kaempferol and quercetin are a class of flavonoids found in the leaves.[7] Other metabolites found in the leaves are ergosterol 5,8-peroxide, cytostenone, isoquercetin, kaempferol 3-glucoronide, catechins, and desmethoxycurcumin.[8] The total phenolic and flavonoid contents from *E. elatior* were mostly found in the flowers, leaves, stems, and rhizome.[9] Torch ginger or lily red ginger has several pharmacological properties such as antioxidant, antibacterial, anti-fungal, tyrosinase inhibition, and cytotoxic activities.[5,10]

The method of testing antioxidant activities is very crucial as it is not only sufficient to utilize one method to test the contents of the secondary metabolites from a complex plant.[11] Several methods that are known for antioxidant activity testing are 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC).[12] To obtain a more reliable result, there must be at least two testing methods. Each of the methods has its respective strengths. ABTS is generally used for an antioxidant test that generates proton (proton donating antioxidant), whereas DPPH is a method that is often used for smaller samples and with more sensitive results. ABTS and DPPH are examples of antioxidant testing methods through scavenging activity mechanisms. The DPPH procedure utilizes spectrophotometer to measure the antioxidant ability to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH), a radical that is not found in biological system.[11,12] The antioxidant ability is observed from a color change of the sample solution from purple to yellow.[13]

Previous research on the antioxidant activity testing of *E. elatior* leaves and methanol extract using the DPPH method yielded an IC_{50}

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value of 30.65 g/mL, classifying it as a strong antioxidant.[14] The test result of the total phenol from the *E. elatior* fresh leaf extract showed a quite high value (3,550 ± 304 mg GAE/100 g). The antioxidant activity testing that was conducted with the DPPH method generated the IC_{50} value that was stated in ascorbic acid equivalent antioxidant capacity (AEAC) in mg AA/100 g. Testing with the DPPH method, an antioxidant value of 3,750 ± 555 mg AA/100 g was generated. Meanwhile, when the FRAP technique was employed, a value of 19.6 ± 2.1 mg GAE/g was recorded.[7] The antioxidant activity from *E. elatior* by reducing the free radicals in the human physiological system refers to the secondary metabolite content of such plants that is relatively high with phenolic and flavonoid compounds.[15,16] Phenolic compounds including phenolic acid, polyphenol, and flavonoid commonly found in plants that function in preventing oxidative damage and are developed to be antioxidant compounds that are utilized by humans.[17]

There have been several investigations on *E. elatior* involving the plant parts of flowers, stems, leaves, and rhizomes. The previous study showed methanol leaves extract of *Etlingera* species has high values of total phenolic content. *E. elatior has* the highest TPC value among other species of *Etlingera*. There is a correlation between TPC and antioxidant activity, the species that have high TPC values also have high antioxidant values.[7] However, the antioxidant activity testing of the extracts and fractions in different solvents is limited. To learn more about the TPC, TFC, and antioxidant activity of *E. elatior*, the ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction of *E. elatior* leaves were analyzed.

Materials and Methods

Source of plant material

The plant material used for this study was *E. elatior* leaves which were obtained from the Herb and Medicinal Plant Research Center (*Balai Penelitian Tanaman Rempah dan Obat* / BALITRO) in Bogor, Indonesia, on 5th November 2020. The samples were identified at the Research Centre for Biology of LIPI, Indonesian Institute of Sciences, Indonesia. The voucher number of the plant sample is 1098/IPH.1.01/If.07/XI/2020.

Sources of chemical materials

The chemical materials used in this research which include DPPH, ABTS, trolox, catechin, Folin-Ciocalteu were purchased from Sigma-Aldrich (Indonesia), while the 98% gallic acid was obtained from MP Biomedicals (Indonesia). Also, aluminum chloride, sodium carbonate, sodium acetate anhydrous, acetic acid, and ferric chloride were procured from Merck (Indonesia). Other chemical reagents used include 96% ethanol, ethyl acetate, n-hexane, and aquadest.

Preparation of Etlingera elatior leaf extract

Fresh *E. elatior* leaves were cut, dried, and then pulverized. The extraction was carried out with maceration using 96% ethanol solvent. A total of 300 g of *E. elatior* leaf powder were weighed and poured into a maceration container with the addition of 2.25 L of 96% ethanol. *Simplicia* was then soaked for 6 hours while being stirred every 30 minutes with a minimum of 5 minutes stirring, and then left to stand for 3 days (Macerate 1). After few days, the preparation was separated and added to 750 mL of 96% ethanol. Afterward, the mixture was left to stand for 2 days (Macerate 2). Macerates 1 and 2 were mixed, and then the extraction solution was filtered utilizing filter paper. The filtrates were collected in a container and then concentrated in a water bath at 50ºC until they became viscous.[18]

Fractionation of Etlingera elatior leaf extract

The *E. elatior* leaves extract was fractionated using the liquid-liquid extraction method. A 30 g weight of *E. elatior* leaves ethanol extract was dissolved in 100 mL of water. It was then poured into a separating funnel with the tap closed and topped with 300 mL of n-hexane. The funnel was closed, gently shaken and the excess vapour pressure was occasionally released. This step resulted in 2 layers, the water layer and the n-hexane layer. The n-hexane layer was then separated by transferring it to an Erlenmeyer flask. This procedure was repeated 5 times by adding more n-hexane into the remaining water layer in the funnel. Afterwards, the n-hexane fraction was evaporated using a water bath at a temperature of 50^0C. The same process was repeated to obtain an ethyl acetate fraction. Lastly, the remaining water fraction was evaporated using a water bath at the same temperature until a water fraction was obtained.[18]

Determination of total phenolic content

Determination of the total phenolic content of the *E. elatior leaf extract* was performed by the Singleton and Rossi method.[19] An aliquot (one mL) of extract, the three fraction solutions, and standart solution of gallic acid were (5, 6, 8, 9, 10, 11 mg/L) was added to 10 ml volumetric flask, containing four mL of demineralized water. 0,2 mL of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After five min, 0.2 mL of 15% Sodium Carbonate was added to the mixture and fixed with addition of water until 10.0 mL.The sample incubated at room temperature for 15 minutes. The absorbance was measured at the maximum absorption wavelength of 750 nm using a UV-Visible spectrophotometer (Shimadzu). Gallic acid was utilized as a standard reference and values were expressed as mg gallic acid equivalent/g extract.[19]

Determination of total flavonoid content

The leaves extract, the three fraction solutions, and standard solution of catechin were prepared in 12, 15, 18, 21, and 24 mg/L. The samples (5 mL each) were taken from each concentration and poured into a 10.0 mL volumetric flask. 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ were added to each of the samples in the flask. Then the sample solutions were incubated at room temperature for 5 minutes. Following the incubation, 2 mL of 1 M NaOH was added and the total volume was made up to 10.0 mL with water.[20] The absorbance of the sample was measured at a wavelength of 415 nm. The results were expressed as catechin equivalent (CE)% w/w.[21]

Evaluation of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant activity testing was performed using the DPPH method as described by Blois.[22]

Several samples with concentrations ranging from 6.25 to 100 µg/mL, as well as Trolox (2, 4, 6, and 8 µg/mL) were tested. The sample was mixed with DPPH solvent in a ratio of 1:4 and placed onto a 96-well transparent polystyrene microplate, which was subsequently homogenized. The mixture was incubated for 30 minutes at a temperature of 37^oC. Absorbance was measured with a UV-Visible Spectrophotometer (Shimadzu©) at a wavelength of 516 nm. Trolox used as the positive control was treated the same way as the sample.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay

Sample solvents with concentrations ranging from 6.25 to 50 µg/mL were prepared from the extract and fractions. Also, Trolox was prepared at 2, 4, 6, and 8 µg/mL concentrations. ABTS solvent and the sample were mixed in a ratio of 1:1 and placed into a 96-well clear polystyrene microplate which was then homogenized. The absorption of the mixture was then measured with a UV-Visible Spectrophotometer (Shimadzu©) at a wavelength of 516 nm. Trolox used as the positive control was treated the same way as the sample.[23]

Statistical analysis

All the experiments were replicated three times and the values were presented as mean ± standard deviation (SD). The results of the TPC and TFC were compared statistically using paired sample t-test with significantly different at a level of $P<0.05$. Also, a percentage of radical scavenging activity was calculated using the results of each antioxidant testing method. The regression equation, $y = a + bx$ was derived by plotting the concentration (µg/mL) against the percentage of radical scavenging activity. The IC_{50} value was used to calculate the number of sample concentrations required to achieve 50% radical scavenging activity.

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Results and Discussion

The total phenol content was determined using the Folin-Ciocalteu method, which is based on the ability to reduce the phenol hydroxyl group using gallic acid as a standard, and the test result was expressed in% w/w GAE (Gallic Acid Equivalent). The total value of phenol (Table 1) was obtained from the calibration curve of y = 0.070x – 0.037, with R^2 = 0.998 (Figure 1). Based on the results obtained for the phytochemical contents, the total phenolic contents of the 96% ethanol extract, water, ethyl acetate, and hexane fractions were 3.70 ± 0.06, 3.44 ± 3.34, 4.74 ± 0.13, 5.60 ± 0.02, and 3.44 ± 3.34% w/w GAE, respectively. On the other hand, the total flavonoid content was measured using the calibration curve with the linear regression equation of y = 0.025x + 0.066 and R^2 = 0.989 as shown in Figure 2 and the flavonoid content was presented in Table 1. The highest level of total flavonoid content was obtained in the ethyl acetate fraction (14.66 ± 0.67% w/w CE), followed by the n-hexane faction (11.43 ± 0.05% w/w CE), ethanol extract (10.77 ± 0.32% w/w CE), and water fraction (8.65 ± 0.88% w/w CE).

According to Table 1, the ethyl acetate fraction had the highest total phenolic content of 5.60% w/w GAE, while the n-hexane fraction had the lowest total phenolic content of 3.44% w/w GAE. There was a significant difference (P < 0.05) between the total amount of phenol in the leaf extract and the fractions of *E. elatior*. Because the phenolic compound belongs to the polar compound group, polar solvents such as methanol, ethanol, acetone, and ethyl acetate are frequently used in the extraction procedure. Ethanol is a typical solvent for polyphenol extraction that is also safe for humans. Methanol is more effective for polyphenol extraction because of its low molecular mass, whereas acetone water solvent is better for flavanol compound extraction because of its bigger molecular mass.[24]

Flavonoid level is determined using total flavonoid testing and catechin as a reference. The % w/w CE (Catechin Equivalent) was used to express the measurement. The standard was catechin, and the measuring method utilizes $AlCl_3$ colorimetry. Catechin was chosen as the standard because it is a phenolic compound with two rings (A and B) and one hydropyran group (ring C) that possesses antioxidant properties.[25] The present study employed a maximum wavelength of 510 nm. The $NaNO_2$ reagent for determining total flavonoids are specific for routine, luteolin, and catechin, but it could also assist detects additional flavonoids on a wavelength of 415 nm. The addition of $AlCl_3$ could result in a bio-chromic shift towards the visible spectrum which is indicated by a solution with a yellower color, whereas the addition of NaOH allows the detection of the presence of 7-hydroxyl group and to maintain the wavelength within the area of 380-560 nm.[26] Furthermore, the 5 minutes incubation carried out was necessitated so that the reaction occurred smoothly by providing the maximum color intensity.

The ethyl acetate fraction had the maximum flavonoid content with a value of 14.66% w/w CE, while the water fraction had the lowest flavonoid content with a value of 8.65% w/w CE, as shown in Table 1. The total amount of flavonoid in the leaf extract and fractions of *E. elatior* differs significantly (P < 0.05). Because it can attract the component group of alkaloids, flavonoids, saponins, tannins, polyphenols, and triterpenoids, ethyl acetate might be used as a solvent for *E. elatior* leaf extract.[27] In this study, the ethyl acetate fraction of *E. elatior* leaves extract contained the highest phenol and flavonoid content. This is because polyphenols and flavonoids are generally polar, so they expectedly dissolve easier in polar or semipolar solvents.[28]

These results are in line with previous studies, where leaves of wild and cultivated *Etlingera* species contain more antioxidants than other plant parts. The level of TPC in *E. elatior* leaves is influenced by the presence of other compounds, such as tannin. Because tannin is a hydrophilic compound, so it can be extracted with polar solvent and can be read at the time of measurement. The difference in solvent also affects the TPC and TFC value, where the most to the least efficient solvent are methanol, 50% methanol-water, ethyl acetate, and dichloromethane. In addition, differences in total phenolic contents and total flavonoid contents in *E. elatior* leaves can be caused by environmental factors, such as plant age, differences in growing places, soil composition, weather, and UV radiation.[7,29,30]

Phenol is a phenolic compound with at least one hydroxyl group, which makes all phenolic compounds a reducing agent. Reduction refers to electron transfer, where this electron is provided by the phenol as a hydrogen atom with a single atom (H•). This atom is generated from the bond split of O – H with a process where each fragment maintains one of the electrons. This H• will reduce the oxidation amount caused by free radicals.[31,32] Even at low concentrations, flavonoids may contribute to the antioxidant properties of the plant's secondary metabolite. The conjugated double bond, the hydroxyl group on C4' and C3', and catechol in ring B in the flavonoid structure all contribute to this.

By shifting the electron clouds around the aromatic ring and electron donors, this structure turns flavonoid into a target for free radical attack, limiting chain reactions and stabilizing radical form.[29] Such observation indicates that the largest secondary metabolite occurred at the semipolar compound. However, further review of the secondary metabolite that has antioxidant activities requires an antioxidant testing by using DPPH and ABTS methods.

E. elatior leaves has been reported contain high flavonoids, such as kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside, and quercetin 3-rhamnoside. *E. elatior* leaves showed high antioxidant activity than other parts. With promising antioxidant activity, *E.elatior* has great potential to be developed into herbal products and applicable to the food and nutraceutical industries.

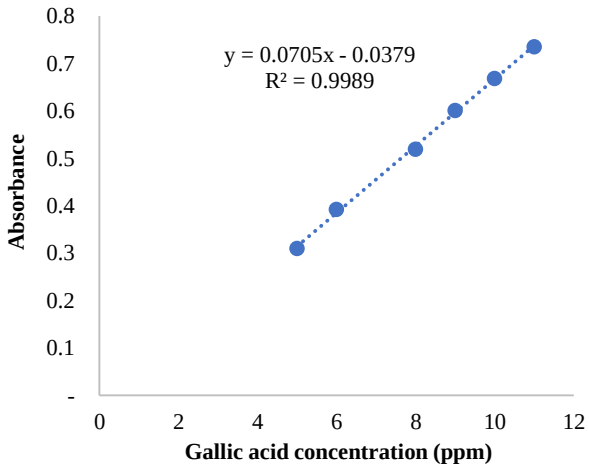

Figure 1: Calibration curve of the gallic acid standard at a wavelength of 765 nm

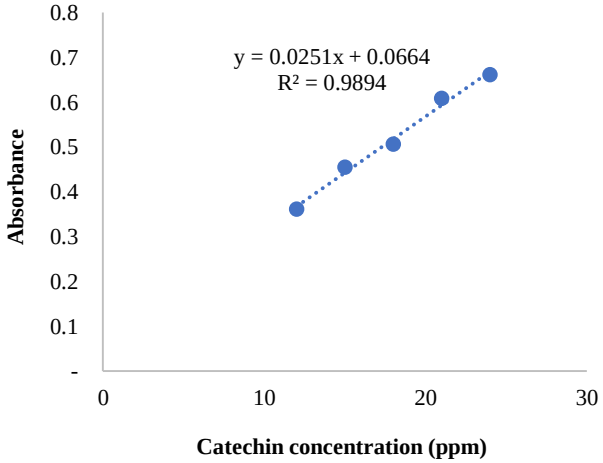

Figure 2: Calibration curve of Catechin at a wavelength of 510 nm

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Nowadays, *E. elatior* leaves are used as food flavouring and traditional medicine, but hardly any research has been done on their antioxidant activity.[7]Table 2, Figure 3, and Figures 4 revealed the results obtained from the experiment. Using the DPPH method, the antioxidant activity testing of the 96% ethanol extract, water, ethyl acetate, and hexane fractions with a final concentration of 50 µg/mL revealed inhibition of the DPPH radical with values of 59.89 ± 0.55, 55.57 ± 1.74, 58.36 ± 2.47, and 61.09 ± 2.08%, respectively. Also, the IC_{50} values obtained for the 96% ethanol extract, water, ethyl acetate, and hexane fractions were 44.86, 45.31, 40.48, and 39.35 µg/mL, respectively compared to the IC_{50} of the reference (Trolox) which had a value of 3.49 µg/mL. Radical scavenging activity of the DPPH from various fractions and extracts of *E. elatior* is presented in Figure 3. There was an increase in DPPH radical scavenging activity of all the samples with a corresponding increase in sample concentration. DPPH radical scavenging activities were in the order of n-hexane > ethyl acetate fraction > ethanol extract > water fraction.

The results of the antioxidant activity testing obtained were in agreement with the observations made when the ABTS method was used. With a final concentration of 25 µg/mL of 96% ethanol extract, water, and ethyl acetate fractions, the radical scavenging activity values recorded were 51.69 ± 0.70, 46.4 ± 0.70, and 62.91 ± 0.97%, respectively. A radical scavenging activity value of 50.38 ± 0.88% was obtained for the n-hexane fraction with a concentration of 12.5 µg/mL. In comparison with the standard reference, Trolox, at a concentration of 4 µg/mL concentration generated radical scavenging activity of 53.49 ± 0.32%. The IC_{50} value of the 96% ethanol extract, water, ethyl acetate, and hexane fractions were 23.45, 28.29, 18.23, and 13.04 µg/mL, respectively compared to the Trolox which had an IC_{50} value of 7.33 µg/mL. Also, there was an increase in the ABTS radical scavenging activity in all the samples with a corresponding increase in sample concentration (Figure 4). The ABTS radical scavenging activity was in the order of n-hexane fraction > ethyl acetate fraction > ethanol extract > water fraction, which was in agreement with the observations made when the DPPH method was employed.

The highest radical scavenging activities were obtained in the n-hexane fraction in both the DPPH and ABTS methods compared to the three other test samples, while the lowest value was recorded for the water fraction. The antioxidant activity correlates with the total flavonoid and phenolic contents. However, the highest radical scavenging activity in this research is different from the sample with the highest total flavonoid and phenol contents, (ethyl acetate fraction). On the two approaches employed for antioxidant activity testing, the fractionation process on the ethanol extract of *E. elatior* did not result in a substantial change in antioxidant activity, where the activities were still in the high range. This could be due to a compound found in ethanol extract that, while being in a solvent with different polarity, tends to work in synergy.[33] The antioxidant activity test on the 96% ethanol extract, water, ethyl acetate, and n-hexane fractions inhibited the DPPH radicals with IC_{50} values of 44.86, 45.31, 40.48, and 39.35 µg/mL, respectively compared to the Trolox 3.49 µg/mL (Table 2). Based on this observation, the less non-polar the solvent used, the lower the IC_{50} value. The IC_{50} of a substrate is the concentration at which 50% of DPPH activities (color) are lost. Therefore, IC_{50} becomes a reference to evaluate the secondary metabolite effectiveness of *E. elatior* leaves, which can inhibit DPPH activity by up to 50%. The higher the free radical scavenging activity, the lower the IC_{50} value.[27] Such a result demonstrates that a non-polar chemical, such as the hexane fraction, has the potential to play a critical role,[32] although it has the lowest total phenolic and flavonoid concentrations. However, this shows that a small amount of non-polar compounds can have strong antioxidant properties (<50 µg/mL).[32] Because non-polar compounds with antioxidant activity belong to the steroid and terpenoid families, more research is needed to examine isolates and compounds with antioxidant properties.[31] Triterpenoid or steroid antioxidant mechanisms include capturing/scavenging reactive species such as superoxide and metal chelation (Fe^{2+} and Cu^{2+}).[34]

In this research, the ABTS method was also utilized to determine the antioxidant activity. The principle of antioxidant activity testing with the ABTS method is the ABTS cation decolorization to measure the antioxidant capacity that is directly reacting with the ABTS cation radicals. ABTS is a nitrogen-centered radical with a blue-green color that, when reduced by an antioxidant, transforms into a non-radical state, transforming from colored to non-colored. Because the ABTS technique is light-sensitive, even the synthesis of ABTS•- requires a 12- to 16-hour incubation period in the dark.[33] The antioxidant testing with the ABTS method is proportionate to DPPH, specifically the n-hexane fraction, which has a very strong antioxidant activity (< 50 µg/mL). Both the DPPH and ABTS methods for determining the antioxidant are *in vitro* procedures. The findings could not be extended to humans directly. However, i*n vitro* procedures could indicate the antioxidant activity of a phytochemical compound from plants.[34] The total phenol and flavonoid contents of the *E. elatior* extract were determined using the ethyl acetate fraction. The highest antioxidant activity was obtained from the n-hexane fraction when tested with DPPH or ABTS. However, all antioxidant tests revealed that the value of IC_{50} was less than 50 µg/mL, indicating that both the extract and fractions of *E. elatior* contain a strong potential antioxidant.

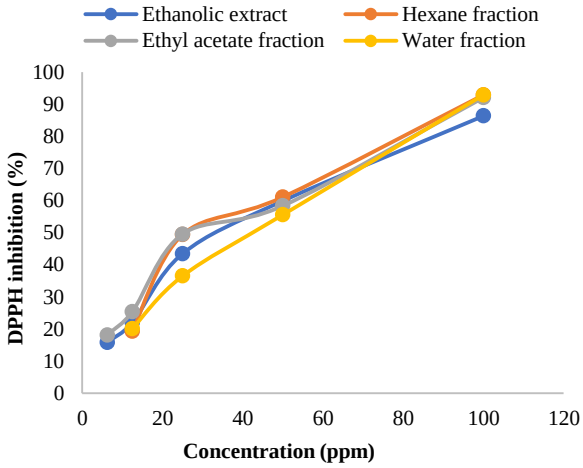

Figure 3: DPPH radical scavenging activity of water, ethyl acetate, n-hexane fractions, and ethanolic extract of *Etlingera elatior*

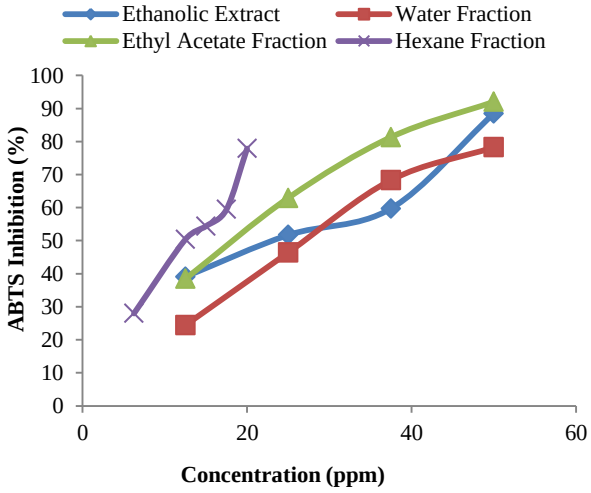

Figure 4: ABTS radical scavenging activity of water, ethyl acetate, n-hexane fractions, and ethanolic extract of *Etlingera elatior*

Table 1: Comparison between total phenolic and flavonoid contents of extract and fractions of *Etlingera elatior*

Extracts and Fractions	Total Phenol Content (TPC) % w/w GAE	Total Flavonoid Content (TFC)% w/w CE
Extract	3.70 ± 0.06	10.77 ± 0.32
Water Fraction	4.74 ± 0.13	8.65 ± 0.88
Ethyl Fraction Acetate	5.60 ± 0.02	14.66 ± 0.67
Hexane Fraction	3.44 ± 3.34	11.43 ± 0.05

Data represent mean ± SD; n = 3; level of significance: *P < 0.05.

Table 2: Comparing IC_{50} of test samples with DPPH and ABTS methods

Sample	Results of Antioxidant Activities (IC_{50})	
	DPPH (µg/mL)	ABTS (µg/mL)
Trolox (Comparison)	3.49	7.33
Water Fraction	45.31	28.29
Ethyl Acetate Fraction	40.48	18.23
Hexane Fraction	39.35	13.04
Extract	44.86	23.45

Conclusion

The ethyl acetate fraction of the *E. elatior* extract yielded the highest total phenol and flavonoid contents, while the highest antioxidant activity was obtained from the hexane fraction when tested with DPPH or ABTS assays. However, all antioxidant tests revealed that the IC_{50} value was less than 50 µg/mL, indicating that both the extract and fractions of *E. elatior* has high antioxidant activity.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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