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Antioxidant Potential of Ethanol Extracts from Zingiberaceae Plant Leaves

Addrian Maulana [1], Aufa Sabrina Thahar [2] and Khairan Khairan [3,*]

[1] Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; addrianmaulana1@gmail.com (A.M.)
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; aufasabr@gmail.com (A.S.T.);
[3] School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; khairankhairan@usk.ac.id (K.K.);

* Correspondence: khairankhairan@usk.ac.id

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Abstract

The development of disease related to oxidative stress must be addressed immediately. An approach is to identify natural antioxidant compounds in plants commonly used by communities. This study measured the potential antioxidant activity of ethanol extracts from three Zingiberaceae species leaves: ginger (*Zingiber officinale* var. Amarum), turmeric (*Curcuma domestica* Val), and temulawak (*Curcuma xanthorrhiza*). Phytochemical profiling was performed using specific reagents, FT-IR analysis, and GC-MS, while antioxidant activity was evaluated using DPPH and ABTS methods. The result showed the presence of saponins, flavonoids, steroids, alkaloids, tannins, fatty acid derivatives, and phytol. The IC_{50} values of the extracts, determined using the DPPH method, were found to be 28.75 ppm for ginger, 65.86 ppm for turmeric, and 51.41 ppm for temulawak. Using the ABTS method, the IC_{50} values were 35.4 ppm for ginger, 75.9 ppm for turmeric, and 58.9 ppm for temulawak. The strongest antioxidant activity of ethanol leaf extracts from Zingiberaceae family was found in ginger leaf extract with the lowest value of IC_{50}. These results provide preliminary evidence that Zingiberaceae leaves, which are less studied compared to their rhizomes, possess notable antioxidant potential. Further studies, including the isolation of active compounds and in vivo evaluation, are required to validate these findings and explore their possible applications in the future.

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1. Introduction

The increasing prevalence of various diseases, such as cancer, acute kidney failure, neurological diseases, and various degenerative diseases, is known to be related to oxidative stress [1]. Oxidative stress occurs when there is an imbalance between the amounts of endogenous antioxidants and free radicals in body. Free radicals are molecules that contain one or more unpaired electron that are highly reactive and unstable. Free radicals can react easily to take electron from other substances and achieve stability [2]. Antioxidants are chemical compounds that can stabilize free radicals, help prevent diseases caused by oxidative stress, and maintain optimal cellular function [3]. Antioxidants are divided into two types: endogenous and exogenous antioxidants. Endogenous antioxidants are antioxidants that can be produced naturally in the body, in either enzyme or non-enzyme form, which can combat free radicals and mitigate oxidative stress. Superoxide dismutase (SOD), which can break down superoxide radicals, catalase (CAT), which can detoxify H_2O_2, and glutathione Peroxidase (GPx), which neutralizes H_2O_2 and ROOH, are

examples of enzymatic endogenous antioxidants. Non-enzymatic endogenous antioxidants, such as glutathione (GSH), NADPH, albumin, uric acid, and bilirubin, were examples. Exogenous antioxidants are antioxidants that can be obtained from outside human body, such as in fruits, grains, and vegetables. Exogenous antioxidants include ascorbic acid (vitamin C), tocopherol (vitamin E), flavonoid and their derivatives, trace elements (selenium and zinc), carotenoids, and phenolic acids [4].

One of the primary sources of exogenous antioxidants is medicinal plants, particularly those from the Zingiberaceae family. Zingiberaceae species widely used by the community for daily purposes and as traditional medicine include ginger (Zingiber officinale var. Amarum), turmeric (Curcuma domestica Val.), and temulawak (*Curcuma xanthorrhiza*). Previous studies have investigated the use of Zingiberaceae rhizome extracts as antioxidants, but the use of Zingiberaceae leaf extracts as antioxidants is lacking. A study tested the rhizome of *Curcuma aeruginosa* Roxb. using the DPPH method and obtained a low activity result with an IC_{50} value of 171 ppm [5]. Other research was conducted on turmeric rhizome powder using Liquid Chromatography-Mass Spectrometry (LC-MS) analysis and found various antioxidants, including caffeic acid, ascorbic acid, curcumin, and quercetin [6], and research on turmeric rhizome extract as antioxidants was conducted using the DPPH method and obtained a very strong activity result with an IC_{50} value of 21.22 ppm [7]. Another study concluded that ginger contains phenolic compounds and can help repair acute kidney injury in an animal model at doses less than 200 mg/kg and consumption frequencies less than 20 times [8]. Ginger rhizome extract, as an antioxidant, was tested using the ABTS method and yielded a very strong category with an IC_{50} value of 28.83 ppm [9].

Based on these findings, we know that the rhizomes of Zingiberaceae have significant potential as sources of natural antioxidants, and further research is needed related to the antioxidant activity of Zingiberaceae leaf extracts. These findings highlight the potential of Zingiberaceae leaves, which are less studied compared to rhizomes, as promising sources of antioxidant compounds. This study aims to test the antioxidant activity of ginger leaves (Zingiber officinale var. Amarum), turmeric leaves (Curcuma domestica Val), and temulawak leaves (Curcuma xanthorrhiza) using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) methods. Both methods are based on the same principle, which is reducing free radicals and redox-active compounds, and applying an appropriate standard when evaluating antioxidant activity using a spectrophotometer. However, they have different solubility systems, radical species, and sensitivities [10]. The combined use of DPPH and ABTS assays provides complementary information, as these methods differ in solubility systems, radical species, and sensitivity. Employing both assays ensures a broader and more reliable evaluation of antioxidant activity across compounds with varying polarities. The challenges in identifying natural antioxidants lie in the fact that, while the present in vitro assays and GC-MS profiling indicated promising antioxidant constituents in the leaf extracts, further steps are required, including the isolation of active compounds, quantitative assays against reference standards, cellular antioxidant assays, and pharmacokinetic studies [11]. However, further research is necessary to isolate the active constituents and conduct additional investigations that will provide a stronger basis for future therapeutic applications.

2. Materials and Methods

2.1. Sampling Point and Plant Identification

Turmeric (*Curcuma longa*), ginger (*Zingiber officinale* Rosc.), and temulawak (*Curcuma xanthorrhiza* R.) leaves were obtained from Pako Village in Keumala District, Pidie, Aceh Province. The quantity of fresh leaves collected was 15 kg for each sample. The plants were 8–10 months old at harvest, which was conducted in the afternoon. The samples were then stored at room temperature, protected from direct sunlight. The Biosystematics Laboratory, Department of Biology, Syiah Kuala University determined leaf samples.

2.2. Extraction of Plant Samples

The leaf sample was thoroughly washed with flowing water and then dried for two days out of direct sunlight. The next step was drying using a drying cabinet at a temperature of 50°C for 8 hours. Next, the simplisia was pulverized, and ethanol extraction was performed via maceration. Ethanol was chosen as it is a versatile solvent capable of extracting compounds across a wide polarity range, and it is commonly used as the first-choice solvent in preliminary studies where prior data are limited. The maceration process used 96% ethanol, and the ratio of simplisia to ethanol is 1:10. First extraction used six parts of solvent to macerate the leaf for 3 days, and remaceration used four parts for 3 days with regular agitation. The macerate was then concentrated using a rotary evaporator [12].

2.3. Phytochemical Screening

For the saponin screening, leaf extracts were prepared by adding 10 mL of hot water to 5 g of extract in a tube test.

Next, the solution was shaken for ten seconds until a foam-like consistency was achieved. A single drop of HCl 2N was added, and the change in the foam was observed. The presence of saponin was determined by observing the persistence of foam for 10 minutes [13]. Saponin screening was performed using a foam test because its amphiphilic properties are similar to those of surfactants, which tend to stabilize foam bubbles. Acidification increases the polarity of the liquid phase, thereby strengthening the foam film [14].

The flavonoid screening was performed by adding 10 mL of methanol to 0.5 g of leaf extracts and heating them in a water bath. After boiling, five drops of concentrated HCl and 0,1 g magnesium powder were added to the solution. The color changes to yellow, orange, and red indicate a positive flavonoid result. Flavonoid detection was performed using the Shinoda test (Mg-HCl) based on the reduction of flavonoid nuclei by Mg minerals in a strong acidic condition, which can cause a yellow, orange, and red color change [15].

For terpenoid and steroid screening, 500 mg of leaf extracts were mixed with 0.5 mL of chloroform. Then, 0.5 mL of anhydrous acetic acid and 2 mL of concentrated sulfuric acid were added through the wall of the test tube. Triterpenoid was contained in the sample if a reddish-brown or purple ring forms, and contains a steroid if a green color forms. The Liebermann-Buchard reagent ($HCl-H_2SO_4$) was chosen because terpenoids and steroids undergo sulfonation reaction and formation of conjugated cations in strong acids, causing different color changes in the presence of terpenoids or steroids [13].

For alkaloid identification, leaf extracts were mixed with 1 mL of 2 N HCl and 9 mL of distilled water to a 500 mg sample, then heated in a water bath for 2 minutes and filtered. Three drops of each extract were put into nine test tubes, and then two drops of each of three different reagents were added to each extract. The reagents used were Mayer's reagent, Dragendorff's reagent, and Bouchardat's reagent. If the sample is positive for alkaloids, the following changes will occur. A yellowish-white precipitate will form after adding Mayer reagent, an orange-yellow precipitate will form after adding Dragendorff reagent, and a blackish-brown precipitate will form after adding Bouchardat reagent [13]. HCl was used in alkaloid detection to form an alkaloid salt. Meyer, Bouchardat, and Dragendorff reagents were added to form-colored complexes between alkaloids and the heavy metals in the reagents [16].

Tannin was assessed by soaking 500 mg of leaf extracts in distilled water for 15 minutes, followed by filtration. Next, every 2 mL filtrate was transferred to a test tube, and two drops of 1% FeCl3 were added. The change in color to blackish-green indicates the presence of tannin in the extract. $FeCl_3$ was added because tannins can form iron phenolate complexes that give a blackish-green color [12].

2.4. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

Identification of functional groups in leaf extracts was performed using FT-IR spectroscopy with a wavelength 4000-400cm^{-1}. Analysis was conducted in the Research Laboratory of the Chemical Engineering Department at the University of Syiah Kuala.

2.5. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

Identification of chemical substituents in leaf extract was conducted using a TRACE 1310 GC and single quadrupole MS ISQ LT with Triplus RSH autosampler, HP-5MS column, and TG-WAXMS (Thermo Scientific, Inc., United States of America). Analysis was conducted in the Instrument Laboratory of the Chemistry Department, Faculty of Mathematics and Natural Sciences, University of Syiah Kuala.

2.6. Antioxidant Analysis

2.6.1. DPPH Method

The determination of DPPH free radical scavenging capacity was carried out according to [17], as further applied in the study by [10] with slight adjustments. First, a DPPH ethanolic solution was made with a concentration of 100 mM. Sample extracts with varying concentrations (6.25, 12.5, 25, 50, 100 ppm) and a standard (ascorbic acid) with varying concentrations (3, 6, 9, 12, 15 ppm) were added to a 2 mL DPPH ethanolic solution. Mixtures were homogenized by vortexing for 1 minute and then stored in a dark place at 25°C for 30 minutes. The control was tested by dissolving 1 mL of an ethanolic DPPH solution in 4 mL of ethanol. Mixtures were homogenized by vortexing and incubated for 30 minutes at 37°C. The decrease in free radicals was measured at a wavelength of 517 nm using a UV/Vis spectrophotometer.

2.6.2. ABTS Method

Determination of ABTS radical scavenging activity was used as a modified method [18] with slight modifications. ABTS water solution (7mM) 5 mL and $K_2S_2O_8$ (2,45 mM) 5 mL were mixed, then ethanol was added until it reached 25mL in a dark place. Next, the mixture was incubated for 12 hours. The varying concentrations of sample extracts and standards were the same as those used in the DPPH assay. The sample extracts and standard were added 2

Table 1. Yield of extract and phytochemical screening result.

Plants	Yield (%)	Phytochemical component					
		S	F	Tt	St	A	T
Zingiber officinale var. Amarum	8.19	+	+	-	+	+	+
Curcuma domestica Val	7.46	+	+	-	+	+	+
Curcuma zanthorrhiza	11.95	+	+	-	+	+	+

Note: S = saponin; F = flavonoid; Tt = triterpenoid; St = steroid; A = alkaloid; T = tannin.

mL to 2 mL of the ABTS solution and homogenized by vortexing for 1 minute. Then the mixtures were stored in a dark place at a temperature of 25°C for 30 minutes. The control was examined by dissolving 1 mL of the ABTS solution in 4 mL of ethanol and homogenizing. After incubating for 30 minutes at 37°C, the mixture was measured at a wavelength of 734 nm using a UV/Vis spectrophotometer.

2.7. Calculation for IC_{50}

The inhibition percentage of DPPH and ABTS was calculated using Equation 1 [18]:

$$\%\text{Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \qquad (1)$$

where, A_{sample} was absorbance of the etanol leaf extract and $A_{control}$ is absorbance of control. After obtaining the inhibition percentage for each concentration, the equation of the line (y = a + bx) was determined using linear regression calculations, where x represents the concentration (ppm) and y represents the percentage of inhibition (%). Antioxidant activity was expressed as Inhibitory Concentration 50% (IC_{50}), which is the concentration of sample that can reduce DPPH and ABTS radicals by 50%.

3. Results and Discussion

Diseases caused by oxidative stress continue to develop and become more prevalent, making the exploration and development of natural antioxidants from plants essential. Rhizomes from the Zingiberaceae family have long been utilized ethnobotanically in traditional medicine and exhibit significant potential as a source of bioactive compounds with antioxidant properties. This study identified the antioxidant activity of ethanol extracts from the leaves of three species of the Zingiberaceae family, namely ginger (*Zingiber officinale* var. Amarum), turmeric (*Curcuma domestica* Val), and temulawak (*Curcuma zanthorrhiza*). The ethanol leaf extracts from these three species were then tested for phytochemicals using specific reagents for each compound.

As shown in Table 1, all three extracts contained secondary metabolites, including saponins, flavonoids, steroids, alkaloids, and tannins. Among these compounds, flavonoids are the primary focus because they have been proven to exhibit antioxidant activity that plays a role in preventing inflammation caused by oxidative stress and providing anticancer effects related to the oxidative stress mechanism [19].

3.1. FT-IR Analysis

FT-IR analysis was performed to identify the functional groups present in the ethanol extracts of ginger, turmeric, and temulawak leaves. This test was conducted using an FT-IR spectrometer with a wavelength range of 4000-400 cm^{-1}. Table 2 shows the results of FTIR analysis of ethanol extracts of Zingiberaceae family leaves.

The results of characterization using FT-IR, as shown in Table 2, confirmed the presence of alkenes, amines, phenols, ethers, and alcohols in the Zingiberaceae ethanol leaf extract. The detection of these groups revealed the presence of organic compounds in the ethanol extract of Zingiberaceae leaves, particularly flavonoids with antioxidant activity, characterized by the presence of alkenes, phenols, alcohols, and ketones. The functional groups alkena (C – C) and phenolic (O – H) can indicate that the extracts contain flavonoids, which are known to have antioxidant activity [20].

3.2. GC-MS Analysis

GC-MS analysis was performed to detect bioactive chemical compounds in ethanol extracts of Zingiberaceae leaves. The principle is to observe the retention time, peak, Similarity Index (SI), and percentage area of bioactive compounds [21]. Table 3 shows the results of GC-MS analysis of ethanol extracts from Zingiberaceae leaves. The compounds selected are those with the largest percentage area and SI ≥80%.

Based on the results of GC-MS analysis, many fatty acids found in the ethanol extract of Zingiberaceae leaves. These fatty acids are found in leaves as precursors of the mevalonate and shikimate pathways, which can then be converted into terpenoid, steroid, and flavonoid compounds [22]. This indicates that the Zingiberaceae leaves can synthesize flavonoids and their derivatives from fatty acids. Although fatty acid derivatives were identified in the Zingiberaceae leaf extracts, their direct contribution to the observed antioxidant activity remains

Table 2. FT-IR analysis result.

Plants	Functional Group	Detected Wavelength (cm^{-1})	Literary Wavelength (cm^{-1})	Component Class	Intensity
Zingiber officinale var. Amarum	C – C	1643.35	1638-1648	Alkena	Strong
	N – H	1585.49	1580-1650	Amine	Moderate
	O – H	1379.1	1310-1390	Phenol	Moderate
	C – O	1244.09	1200-1275	Eter	Strong
	C – C	719.45	665-730	Alkena	Strong
Curcuma domestica Val	N – H	3385.07	3300-3400	Amine	Moderate
	N – H	1585.49	1580-1650	Amine	Moderate
	O – H	1396.46	1330-1420	Alcohol	Moderate
	C – O	1076.28	1050-1085	Alcohol	Strong
	C – C	931.62	960-980	Alkena	Strong
Curcuma zanthorrhiza	C – H	2958.8	2840-3000	Alkena	Moderate
	O – H	1377.17	1310-1390	Phenol	Moderate
	C – O	1220.94	1200-1225	Eter	Strong

Table 3. GC-MS analysis result.

Plants	Compound Name	% Area	Similarity Index (SI)
Zingiber officinale var. Amarum	Hexadecanoic acid, methyl ester	34.81	87.5
	Phytol	29.71	85.7
	Methyl 10-trans,12-cis octadecadienoate	8.14	84.8
Curcuma domestica Val	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl, methyl ester	12.46	80.2
	12,15-Octadecadiynoic acid, methyl ester	11.06	81.6
	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	4.46	84.1
	10-Octadecenoic acid, methyl ester	1.04	82.3
Curcuma xanthorrhiza	Trans-13-Octadecenoic acid, methyl ester	8.41	92.1
	Hexadecanoic acid, methyl ester	5.16	90.6
	Clionasterol acetate	5.10	83.4
	Sitosterol	3.60	86.8
	Phytol	2.47	87.5
	Methyl stearate	2.18	86.1
	9,12-Octadecadienoic acid, methyl ester	1.53	87.7
	Trans-13-Octadecenoic acid, methyl ester	1.03	86.5
	Nonacos-1-ene	0.87	81.3
	Benzene, 4-ethenyl-1,2-dimethyl-	0.66	87.8
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.45	80.5

uncertain. Previous studies, however, have suggested that unsaturated fatty acids such as oleic and linoleic acids can correlate with radical scavenging capacity, as demonstrated in Prunus seed oils where fatty acid composition was linked to antioxidant and anti-inflammatory activities [23]. These findings indicate that the fatty acids detected in the present study may contribute, at least in part, to the antioxidant potential of the leaf extracts, although further experimental validation is required. Phytol is an acyclic unsaturated diterpene alcohol derived from chlorophyll. Phytol is a precursor to the exogenous antioxidant vitamin E. Phytol is also known to have antioxidant and anti-inflammatory effects and to be cytotoxic [24]. Sitosterol can contribute as an antioxidant due to its ability to neutralize free radicals and reduce damage [25].

3.3. Antioxidant activities

Antioxidant activity was tested using two methods: DPPH and ABTS. Both methods are often used to test antioxidants from plants. The DPPH method works by capturing or accepting electrons from donor compounds, exhibiting strong absorption at a wavelength of 515-520 nm. In contrast, the ABTS method is used to determine the ability of antioxidants to react with strong oxidants, such as potassium persulfate, to produce ABTS radicals. The activity of these two methods is expressed in IC_{50} values, which are the concentrations required for a compound to produce 50% of its maximum biological effect [10]. Ascorbic acid was used as the standard. Ascorbic acid was chosen as the standard because it exhibits very strong antioxidant properties and a low IC_{50}, specifically 1 ppm and 4.64 ppm. The lower the IC_{50} value, the more effective an antioxidant is. Antioxidants

Table 4. Antioxidant activities of Zingiberaceae leaf.

Plants	DPPH Method		ABTS Method	
	IC_{50} (ppm)	**Category**	**IC_{50} (ppm)**	**Category**
Ascorbic acid	1	Very strong	4.64	Very strong
Zingiber officinale var. Amarum	28.75	Very strong	35.4	Very strong
Curcuma domestica Val	65.86	Strong	75.9	Strong
Curcuma xanthorrhiza	51.41	Strong	58.6	Strong

are classified as very strong if the IC_{50} value is less than 50 ppm, strong if the value is 50-100 ppm, moderate if the value is 101-150 ppm, weak if the value is 151-200 ppm, and considered to have no antioxidant effect if the IC_{50} value is more than 200 ppm [26]. Table 4 contains the results of antioxidant activities from Zingiberaceae ethanol leaves extracts using these two methods.

Among the ethanol extracts of Zingiberaceae leaves, ginger leaves (*Zingiber officinale* var. *Amarum*) exhibited the lowest IC_{50} values in both DPPH and ABTS assays, indicating consistently strong antioxidant activity. Although the IC_{50} values obtained with ABTS were generally higher than those from DPPH, this reflects comparatively lower antioxidant activity when measured by the ABTS method. This difference arises from the distinct characteristics of the two methods: the DPPH assay is more sensitive to free radicals in organic solvents. It thus better detects lipophilic compounds, whereas the ABTS assay is more responsive to hydrophilic antioxidants in aqueous systems. The higher IC_{50} values observed with ABTS therefore suggest that the leaf extracts may contain a greater proportion of lipophilic constituents, which are more effectively captured by the DPPH assay. Ginger leaves exhibited very strong antioxidant activity, which can be attributed to the percentage of phytol found in the GC-MS result and functional groups such as alkenes and phenols detected in the FT-IR result. Next is temulawak leaves (*Curcuma xanthorrhiza*), which have an IC_{50} value that falls into the strong category in both test results. The highest IC_{50} value is exhibited by turmeric leaves (Curcuma domestica Val), which falls into the strong category in both tests. Turmeric had the lowest antioxidant activity with the highest IC_{50} value among these three plants, according to GC-MS and FT-IR results, which show that turmeric leaf extract does not contain phytol and phenolic functional groups.

4. Conclusions

In this study, a comprehensive analysis of ethanol leaf extracts from three Zingiberaceae species, including ginger (Zingiber officinale var. Amarum), turmeric (Curcuma domestica Val.), and temulawak (Curcuma xanthorrhiza), was conducted. Phytochemical screening confirmed the presence of secondary metabolites, including saponins, flavonoids, steroids, alkaloids, and tannins, which are widely associated with antioxidant activity. FT-IR analysis confirmed the presence of functional groups, including alkenes, phenols, and alcohols, indicating the presence of bioactive compounds, particularly flavonoids, which exhibit antioxidant activity. GC-MS profiling revealed diverse bioactive constituents, including fatty acid derivatives and phytol, which are known to have antioxidant activity and anti-inflammatory effects.

Antioxidant assays using DPPH and ABTS methods showed that ethanol leaf extracts of Zingiberaceae exhibited notable free radical scavenging activity, as reflected in their IC_{50} values. Although the IC_{50} values of rhizome extracts were lower, indicating stronger antioxidant activity, the leaf extracts still demonstrated substantial potential as alternative sources of natural antioxidants. These findings underscore the importance of further investigating the bioactive properties of Zingiberaceae leaves, which are less studied than those of rhizomes. Future research should focus on isolating the active compounds present in ginger, turmeric, and temulawak leaf extracts, as well as evaluating their bioactivity through in vivo studies. Overall, this study provides preliminary evidence supporting the antioxidant potential of Zingiberaceae leaves and suggests that further investigation is needed before practical applications can be established.

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