

**Antioxidant Activity, Total Phenolic and Total Flavonoid Contents of *Etilingera elatior* (Jack) R.M. Smith from North Luwu, Indonesia**Yuri P. Utami^{1,4}, Risfah Yulianty^{2*}, Yulia Y. Djabir³, Gemini Alam²¹ Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia.² Department of Pharmacy Science and Technology, Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia.³ Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia.⁴ Faculty of Health Sciences, Almarisah Madani University, Makassar, South Sulawesi, Indonesia.

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ABSTRACT

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Etilingera elatior (Jack) R.M. Smith is an endemic plant of North Luwu, Indonesia. It is used traditionally as acidifiers in food. This study aim to evaluate the antioxidant activity, total phenolic and total flavonoid contents of the ethanol leaf extract of *E. elatior*. The dried leaves of *E. elatior* were extracted by maceration in 70% ethanol at room temperature for three days. The antioxidant activity of the extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays and ferric reducing antioxidant power (FRAP) method. The total phenolic and total flavonoid contents were determined using the Folin-Ciocalteu and Aluminium chloride colorimetric methods. The results showed that *E. elatior* leaf extract had antioxidant activity with IC₅₀ value of 42.45 ± 1.37 µg/mL compared to 2.78 ± 0.01 µg/mL for vitamin C (Positive control) for DPPH radical scavenging activity. While for the ABTS scavenging activity, the IC₅₀ was 26.46 ± 0.09 µg/mL compared to 0.15 ± 0.02 µg/mL for vitamin C. In the FRAP assay, the extract showed a ferric reducing antioxidant power of 78.52 ± 0.26 mgAAE/g extract. The total phenolic and total flavonoid contents of the extract were 27.19 ± 0,07 mgGAE/g extract and 4,60 ± 0,01 mgQE/g extract, respectively. From the results of the study, it can be concluded that the ethanol leaf extract of *E. elatior* has strong antioxidant activity which could be attributed to its high phenolic and flavonoid contents.

Keywords: *Etilingera elatior*, Antioxidants, Phenolics, Flavonoids.**Introduction**

Free radicals are atoms or molecules with unpaired electrons in their outer orbital. They are unstable and extremely reactive substances. Highly reactive radicals can set off a series of events that harm body cells.¹ Antioxidants are compounds that combat free radicals. An antioxidant prevent oxidation reactions by giving its electrons to a free radical molecule and therefore stop a chain reaction.² However, the use of synthetic antioxidants is limited due to their toxic effect, hence the need for natural antioxidants.³ Plants with high polyphenol content have been reported to be source of antioxidant chemicals. *Etilingera elatior* (Jack) R.M. Smith, also known as patikala, is a plant that may be a source of natural antioxidants. This plant belongs to Zingiberaceae tribe that are edible and has been used as medicine.⁴ The leaves contain bioactive compounds such as polyphenols, alkaloids, flavanoids, steroids, and saponins that have potential as antioxidants.⁵ Empirically, people frequently use it to flavour meals, and its blooms are also used as fragrance, shampoo, and ingredients for detergent. Its leaves are used to clean wounds and to cure ear ache.⁶

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Previously, phytochemical screening of the extract of patikala leaves have shown the presence of flavonoids, tannins, saponins, steroids and terpenoids.⁷ Furthermore, a number of studies have been carried out on the leaves of *Etilingera elatior* of Enrekang origin, including the optimization of distillation time for the content and characteristics of *Etilingera elatior* leaf essential oil. It was found out that 4 hours was the optimum distillation time for patikala essential oil with a yield of 0.15% v/v and 2-Decen-1-ol (C₁₀H₂₀O) as the main component.⁸ The plant has been shown to have antibacterial activity against *Staphylococcus aureus* with inhibition zone diameter of 8.91 mm, 8.17 mm, and 8.03 mm for the ethyl acetate, ethanol, and n-hexane extracts, respectively.⁹

Phenolics and flavonoids are secondary metabolites that are most frequently present and well distributed in plants. Certain plant components, particularly those responsible for photosynthesis, contain flavonoids.¹⁰ Flavonoids function in the human body as antioxidants, and can prevent cancer. Other benefits of flavonoids are protection of cell structures, as anti-inflammatory agents, prevention of osteoporosis, and as antiseptic.¹¹ Phenolic compounds are biologically active compounds with numerous health benefits, one as an antioxidant for the management and prevention of immune system disorders, cancer, degenerative disorders, and early aging.¹² The quality of extracts and contents of active compounds in medicinal plants varies due to numerous factors. Factors that could influence the phytochemical constituents include the nature of seedlings for propagation, climatic conditions, and the location of cultivation. The location of plant growth or cultivation has the greatest influence on the phytochemical constituents. For example, Indonesia has a tropical climate with various soil types like sandy, red, and black soils, which influence the constituents of plants grown in this region.¹³ In addition to soil type, phytochemical composition is also influenced by a variety of environmental factors, such as light, temperature, pH, and height.¹⁴

Several methods are available for the antioxidant testing of plant extracts, one of such method is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (RSA). The DPPH RSA is the most commonly used technique; it is suitable for measuring total antioxidant activity both in polar or water-soluble solvents as well as in non-polar solvent or oil. The DPPH technique is commonly used because of its simplicity, speed, accuracy, and does not require a lot of samples.¹⁵ Another method of measuring antioxidant activity is the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation radical scavenging assay.¹⁶ ABTS has the advantages of having a high level of sensitivity and ease of reproducibility.¹⁷ The Ferric Reducing Antioxidant Power (FRAP) method which measures an antioxidant capacity to convert ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) can ascertain the overall antioxidant capacity of a substance.¹⁷ The FRAP assay like the DPPH assay is also cheap, fast and the reagent used is quite simple, readily available and does not require any special technique in estimating total antioxidant activity.¹⁸ Although the antioxidant capacity of plants and their secondary metabolites have been studied extensively, but the antioxidant activity of the extract and metabolites of the leaves of *Etilingera elatior* have not been thoroughly investigated. Therefore, the present study aim to determine the antioxidant activity, total phenolic, and total flavonoid contents of the leaves of *Etilingera elatior* (Jack) R.M. Smith from Northern Luwu, Indonesia.

Materials and Methods

Materials

The materials used in the study include; Rotary evaporator (Rotavapor® R-300, Buchi Corporation, USA), UV-Vis spectrophotometer (UV-1900, Shimadzu®), Dragonlab Micropipette (Bio Red®), AlCl_3 10% (Sigma Aldrich®), DPPH (Sigma Aldrich®), Ethanol (Merck®), ABTS (Sigma Aldrich®), 2,4,6-tripyridyl-s-triazine (Sigma Aldrich®), CH_3COONa (Sigma Aldrich®), FeCl_3 (Sigma Aldrich®), quercetin (Sigma Aldrich®), FRAP (Sigma Aldrich®), Folin-Ciocalteu (FC) (Sigma Aldrich®), and $\text{K}_3\text{Fe}(\text{CN})_6$ (Sigma Aldrich®).

Plant collection, identification and drying

Fresh leaves of *Etilingera elatior* (Jack) R.M. Smith) were collected from Tallu Tulak Village, Sabbang Prefecture, North Luwu District, and South Sulawesi ($2^\circ 36' 25''\text{S}$ $120^\circ 08' 30''\text{E}$) on March 2023. The plant leaves were identified and authenticated at the Plant Determination Unit, Pharmacognosi-Phytochemistry Laboratory, Faculty of Pharmacy, Muslim University, Indonesia. Herbarium specimen was deposited and a Voucher No. 0064/C/UD-FF/UMI/IX/2023 was issued. Subsequently, the plant samples were separated based on their collection locations. Patikala (*Etilingera elatior*) leaves were sorted when they were still wet to remove soil and any other impurities. Then, the samples were washed in running water and chopped into smaller sizes. The chopped patikala leaves were air-dried for one week. The dried samples were sorted and pulverized, and their moisture content was determined before weighing.

Extraction of Powdered leaves samples

Powdered patikala leaves (500 g) were extracted by maceration in 70% ethanol (5 L) at room temperature for 72 h with stirring every 8 h. The extract was filtered, the residue was again macerated four more times, and then the filtrates obtained were combined and concentrated using a rotary evaporator to obtain a concentrated extract. The percentage yield of the extract was calculated.⁷

Qualitative test for phenolic compounds and flavonoids

Test for Phenolic compounds

Two milligrams (2 mg) of patikala leaf extract was mixed with 8 mL of warm distilled water, then filtered, and the filtrate was collected and placed in a reaction tube. Three drops of FeCl_3 (1%) was added. The presence of phenolic compounds is indicated by the formation of a dark brown, dark blue, or dark green colour.¹⁹

Test for flavonoids

Thirty milligrams (30 mg) of patikala leaf extract was weighed and placed in a reaction tube, and then a small amount of magnesium metal powder and a few drops of concentrated HCl were added. The presence of flavonoids is indicated by the formation of yellow, red, or orange colour.¹⁹

Quantitative estimation of the total phenolic content

The total phenolic content was estimated using the Folin-Ciocalteu (FC) reagent. The plant extract (0.5 mL) and FC reagent (0.5 mL) were mixed and then incubated for five minutes at 22°C . Two milliliters (2 mL) of 20% Na_2CO_3 was added to the reaction mixture. After another 90 minutes of incubation at 22°C , the absorbance of the reaction mixture was measured at 768 nm. Gallic acid was used as a reference standard for the determination of the total phenolic content, and the result was expressed as milligrams gallic acid equivalent per gram of the extract mgGAE/g.²⁰

Quantitative estimation of the total flavonoid content

The total flavonoid content was estimated using the aluminium chloride (AlCl_3) colorimetric assay. The test solution containing 0.3 mL of 5% sodium nitrite (NaNO_2), 0.5 mL of distilled water, and 0.5 mL of plant extract was incubated for 5 min at 25°C . After the incubation, 3 mL of 10% AlCl_3 was added. The reaction mixture was mixed with 2 mL of 1 M NaOH, and the absorbance was measured at 432 nm. Quercetin was used as the reference standard, and the result was expressed as milligrams quercetin equivalent per gram of the extract mgQE/g.²⁰

Determination of Antioxidant Activity

DPPH radical scavenging assay

The ability of patikala extract to scavenge DPPH radical was determined using previous reported method. Briefly, 0.1 mL of the patikala extract at varying concentrations (10 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$, and 100 $\mu\text{g/mL}$) was mixed with 1.5 mL of the ethanoic solution of 0.1 mM DPPH. The mixture was incubated at room-temperature for 30 min in the dark. The absorbance of the reaction mixture was measured at 517 nm using a spectrophotometer. Vitamin C was used as the reference standard under the same condition, but at concentrations of 0.25 $\mu\text{g/mL}$, 0.5 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$, 2 $\mu\text{g/mL}$, and 4 $\mu\text{g/mL}$. Finally, the half maximal inhibitory concentration (IC_{50}) was calculated.²¹

ABTS radical scavenging assay

ABTS radical cation was made by mixing 7 mM ABTS and 2.45 mM potassium persulfate in equal amounts. The mixture was incubated at room-temperature for 12 h in the dark. After incubation, the mixture produced an ABTS radical solution with a maximum absorbance at 730 nm. Patikala leaf extract solutions were prepared in the following concentrations: 20, 40, 60, 80, and 120 $\mu\text{g/mL}$. As a positive control, various concentrations of vitamin C (0.25 $\mu\text{g/mL}$, 0.5 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$, 2 $\mu\text{g/mL}$, and 4 $\mu\text{g/mL}$) were made. The experiment involved combining 2.7 mL of the ABTS radical solution with 300 μL of either the patikala leaf extract solution or the vitamin C solution. After an incubation period of 30 min at 30°C , the absorbance of the reaction mixture was measured at 730 nm. Each experiment was done in triplicate.²²

FRAP assay

A solution of Patikala leaf extract was prepared by dissolving 5 mg of the extract in 5 mL of 96% ethanol. To 1 mL of the extract solution were added 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% $\text{K}_3\text{Fe}(\text{CN})_6$. The mixture was then incubated for 20 min at 50°C . After the incubation period, 1 mL of trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, 1 mL of the supernatant, 0.5 mL of 0.1% FeCl_3 , and 1 mL of distilled water were mixed in a reaction tube. The reaction mixture was allowed to stand for 10 min at room temperature, after which the absorbance was measured at 720 nm against a blank solution of oxalate. The calibration curve was prepared using different concentrations of ascorbic acid solutions. Results were expressed in

terms of milligrams ascorbic acid equivalent per gram of extract (mgAAE/g).²¹

Results and Discussion

The study investigated the total phenolic and total flavonoid contents of the extracts of patikala (*Etilingera elatior*) leaf obtained from Tulak Tallu Village, Sabbang District, North Luwu Regency, and South Sulawesi Province. The antioxidant capacity of these extracts was evaluated using three different methods; DPPH, ABTS, and FRAP assays. The maceration method was used to obtain the extracts due to its simplicity and use of basic equipment,⁹ while 70% ethanol was used as the extraction solvent because it can extract more secondary metabolites compared to other organic solvents.²³ The ethanol extract gave a yield of 14.09%.

Phytochemical screening is done to reveal the class of secondary metabolites present in plants.²⁴ In this study, only flavonoids and phenolic compounds were tested, and the extract showed the presence of flavonoids and phenolic compounds (Table 1).

Total Phenolic Content of *Etilingera elatior* leaf extract

The Folin-Ciocalteu (FC) reagent was used to determine the total phenolic content in the extract. This method is based on the formation of a molybdenum-tungsten complex (a complex mixture of heteropolyphosphotungstatemolybdate in the presence of sodium carbonate) with a blue hue by the reduction of F-C reagent by phenolates or phenolic groups. The intensity of the blue colour is proportional to the amount of reactive phenolic compounds in the sample. Proton dissociation of phenolic compounds into phenolate ions is induced by the FC reagent only in a basic environment. The amount of phenolate ions that degrade heteropoly acid (phosphomolybdate-phosphotungstate) into a molybdenum-tungsten complex increases with the concentration of phenolic compounds, giving a strong blue colour.³³ Gallic acid is a phenol and a phenolic compound derivative of hydroxybenzoic acid, which is a member of the simple phenolic acid family, it is also readily available, stable and very reactive with the Folin-Ciocalteu reagent.³⁴ Hence, it was used as the reference standard in this assay.

The molybdenum-tungsten complex formed from the reaction of phenolic compound with FC reagent absorbs within a wavelength range of 600-800 nm. In the present study, the wavelength of maximum absorption of complex formed from reaction of standard solution of gallic acid with Folin-Ciocalteu reagent was found to be 768 nm, and so this wavelength was selected for measurement. The linear regression equation obtained from the gallic acid calibration curve was $y = 0.0364x + 0.0157$ with a coefficient of determination; $R^2 = 0.9993$ (Figure 1). The average total phenolic content of patikala leaf extract calculated from the regression equation was $27.19 \pm 0,07$ mgGAE/g extract (Table 2).

Total Flavonoid Content of *Etilingera elatior* leaf extract

The study employed aluminium chloride ($AlCl_3$) colorimetric assay to assess the total flavonoid content with quercetin as the reference standard. The $AlCl_3$ method is based on the formation of a stable yellow coloured complex between aluminium and the C-4 keto group

and the C-3 or C-5 hydroxyl groups found in flavones and flavonols. The orthohydroxyl groups on the A- or B-ring of flavonoid compounds generate a stable acid complex when aluminum chloride is added. Since quercetin is one of the flavonoid molecules that can react with $AlCl_3$ to produce a stable complexes, it was selected as the reference standard.³² The use of quercetin was further motivated by the fact that it is a flavonoid that belongs to the flavonol group and has hydroxyl groups at the nearby C-3 and C-5 atoms and a keto group at the C-4 atom.³⁵

The aluminium chloride-quercetin complex absorbs maximally between 400-500 nm.

Table 1: Phytochemical constituents of *Etilingera elatior* leaves

Phytochemical	Inference
Flavonoids	+
Phenolics	+

Note. + (positive) means present

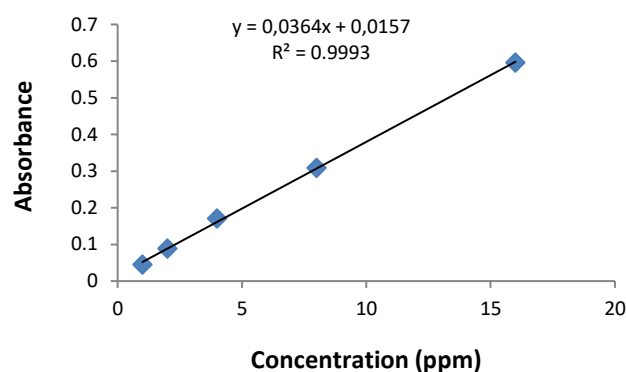


Figure 1: Gallic acid calibration curve for determination of total phenol content

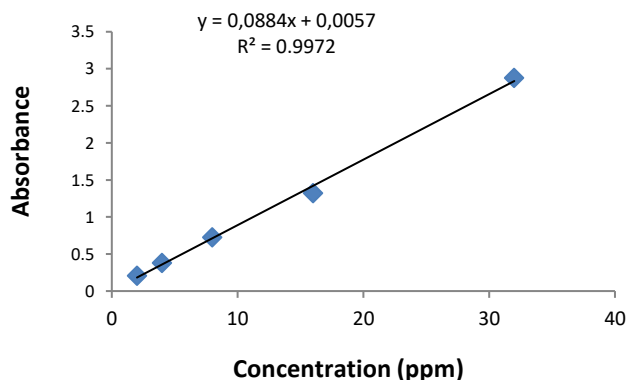


Figure 2: Quercetin calibration curve for determination of total flavonoid content

Table 2: Total phenol content of patikala leaf extract

Replicate	Abs	Dilution Factor	Sample weight (g)	Volume of solution (mL)	x (μ g/mL)	X (mg/mL)	Total Phenolic Content (mgGAE/g)	Mean Total Phenolic Content (mgGAE/g extract)	Total Phenolic Content (mgGAE/g extract) \pm SDV
1	0.509	10	0.05	10	13.56	0.01355	27.10	27.19	27.19 \pm 0.07
2	0.232	10	0.05	10	13.61	0.01361	27.22		
3	0.231	10	0.05	10	13.63	0.01363	27.26		

The wavelength of maximum absorption in this study was determined to be 432 nm. The standard quercetin calibration curve was linear with a regression equation of $y = 0.0884x + 0.0057$ and a coefficient of determination of $R^2 = 0.9972$ (Figure 2). The total flavonoid content of patikala leaf extracts was estimated from the regression equation, and was found to be 4.60 ± 0.01 mgQE/g extract (Table 3).

Antioxidant Activity of *Etilingera elatior* leaf extract DPPH radical scavenging activity

The DPPH radical is a stable free radical that is commonly used to assess the antioxidant capacity of samples. The DPPH assay is simple, fast, sensitive, and only require a small amount of sample. The DPPH assay is based on the principle of hydrogen atom donation from the test antioxidant substance to DPPH radical, resulting in a non-radical compound; phenyl picryl hydrazine, as indicated by a colour change.²⁵ In the present study, the DPPH radical scavenging activity of patikala leaf extract was estimated in reference to vitamin C standard which is a known antioxidant compound. Vitamin C scavenges and neutralizes reactive oxygen species, such as hydrogen peroxide.²⁶ Ethanol extract of patikala leaves are known to contain bioactive substances, including steroids, polyphenols, alkaloids, flavonoids, saponins, and essential oils, which may function as antioxidants and neutralize free radicals.⁵ The ethanol extract of patikala leaves used in this study exhibited antioxidant activity, as shown by the decrease in DPPH absorbance, which can be attributed to the ability of ethanol extract of patikala leaves to scavenge DPPH free radicals. Furthermore, by contributing electrons to neutralize DPPH radicals, the antioxidants in the ethanol

extract of patikala leaves cause the colour of the DPPH solution to change from purple to yellow or to colourless.²⁷ From the antioxidant activity results, the mean IC_{50} value for vitamin C was found to be 2.78 ± 0.01 μ g/mL (Table 4), compared to patikala leaves extract with mean IC_{50} value of 42.45 ± 1.37 μ g/mL (Table 5). Compounds are considered to have very strong antioxidant activity if their IC_{50} values are less than 50 μ g/mL, strong if IC_{50} is 50-100 μ g/mL, moderate if IC_{50} is 101-150 μ g/mL, and weak if the IC_{50} value of the sample is above 150 μ g/mL.^{25,28} The low IC_{50} values (<50 μ g/mL) obtained for the ethanol extract of patikala leaves indicates that the plant has very strong antioxidant activity.

ABTS radical scavenging activity

ABTS technique measures the ability of an antioxidant to directly react with the ABTS cation radical by measuring the decrease in absorbance of the coloured ABTS cation as it is being scavenged by the antioxidant. When reduced by an antioxidant, the nitrogen-centred radical ABTS is transformed from a blue-green coloured radical to a colourless non-radical. The ABTS radical is incredibly light-sensitive, hence a 12- to 16-hour incubation in the dark is necessitated in the assay procedure.¹⁶ The result of the antioxidant activity measured by the ABTS scavenging assay showed mean IC_{50} value for the positive control (Vitamin C) as 0.15 ± 0.02 μ g/mL (Table 6), while the ethanol extract of patikala leaves had mean IC_{50} value of 26.46 ± 0.09 μ g/mL (Table 7). This result agrees with that obtained for the DPPH radical scavenging assay which indicated that the ethanol extract of patikala leaves has very strong antioxidant activity.

Table 3: Total flavonoid content of patikala leaf extract

Replicate	Abs	Sample weight (g)	Volume of solution (mL)	Measurable Flavonoids (mg/mL)	mg equivalent quersetin/g extract	Mean mg equivalent quersetin/g extract	mg equivalent quersetin/g extract \pm SDV
1	2.043	0.05	10	23.05	4.61		
2	2.040	0.05	10	23.01	4.60	4.60	4.60 \pm 0,01
3	2.033	0.05	10	22.93	4.59		

Table 4: Antioxidant activity of Vitamin C (positive control) based on DPPH radical scavenging assay

Replicate	Concentration (μ g/mL)					IC_{50} (μ g/mL)	Mean (μ g/mL)	IC_{50}
	0.25	0.50	1.00	2.00	4.00			
	Radical scavenging activity (%)							
1	30.38	33.40	36.79	43.21	59.81	2.76		
2	30.19	33.40	36.79	43.40	59.43	2.78	2.78 \pm 0.01	
3	30.19	32.45	36.23	43.40	59.43	2.79		

Table 5: Antioxidant activity of patikala leaf extracts based on DPPH radical scavenging assay

Replicate	Concentration (μ g/mL)					IC_{50} (μ g/mL)	Mean IC_{50} (μ g/mL)
	10	20	40	80	100		
	Radical scavenging activity (%)						
1	18.54	40.09	44.22	77.18	93.58	43.84	
2	18.54	40.23	44.22	77.18	93.58	43.81	42.45 \pm 1.37
3	18.40	40.23	58.77	77.32	93.58	39.71	

Table 6: Antioxidant activity of Vitamin C (positive control) based on ABTS radical scavenging assay

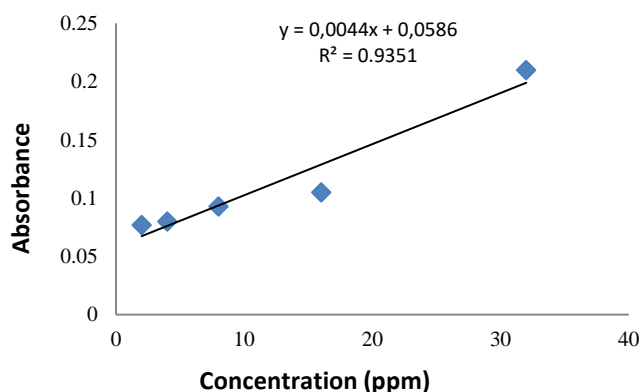
Replicate	Concentration (μ g/mL)					IC_{50} (μ g/mL)	Mean IC_{50} (μ g/mL)
	0.25	0.50	1.00	2.00	4.00		
	Radical scavenging activity (%)						
1	50.21	51.93	53.21	57.35	66.33	0.18	
2	50.36	52.21	53.21	57.20	66.90	0.16	0.15 \pm 0.02
3	50.50	52.07	53.78	57.20	66.76	0.11	

Table 7: Antioxidant activity of patikala leaf extract based on ABTS radical scavenging assay

Replicate	Concentration ($\mu\text{g/mL}$)					IC ₅₀ ($\mu\text{g/mL}$)	Mean IC ₅₀ ($\mu\text{g/mL}$)
	20	40	60	80	120		
1	42.83	62.94	65.91	69.58	92.31	26.29	
2	42.48	62.94	66.08	69.76	92.31	26.53	26.46 \pm 0.09
3	42.48	42.94	65.91	69.76	92.13	26.57	

Table 8: Antioxidant activity of patikala leaf extract based on FRAP assay

Replicate	Abs	Antioxidant Activity		Dilution Factor	Calculated antioxidant activity (mg/L)	Sample weight (g)	Volume of solution (mL)	mg ascorbic acid equivalent/g extract (mgAAE/g extract) \pm SDV	
		Ascorbic Acid (mg/L)	Acid					mg ascorbic acid equivalent/g extract	mg ascorbic acid equivalent/g extract
1	0.231	39.18		10	391.82	0.05	0.01	78.36	
2	0.232	39.41		10	394.09	0.05	0.01	78.82	78.52 \pm 0.26
3	0.231	39.18		10	391.82	0.05	0.01	78.36	

**Figure 3:** Ascorbic acid calibration curve for determination of FRAP

Ferric Reducing Antioxidant Power

The FRAP assay is an easy and straightforward approach in assessing the antioxidant activity of extracts. It is also cost-effectiveness, and does not require the use of sophisticated equipment.²⁹ The principle of the FRAP assay is based on the ability of an antioxidant compound to reduce Fe^{3+} to Fe^{2+} . A compound with reducing capacity has the potential as an antioxidant because it donates electrons or hydrogen atoms, thereby stabilizing radicals.³⁰ In the FRAP reagent, FeCl_3 is added to form a green to blue-green coloured complex. The addition of phosphate buffer is to maintain the effective pH between 6.6 - 7.4. It is known that this complex is stable at acidic pH, hence pH 6.6 is used.³¹ In this study, Vitamin C served as the reference standard because of its ability to inhibit chain reactions by capturing free radicals and acting as a secondary antioxidant.²⁹ The FRAP value as a measure of the antioxidant activity of the extract was obtained from a linear regression equation of the vitamin C standard curve (Figure 3). The FRAP value was expressed in milligrams of ascorbic acid equivalent per gram of extract (mgAAE/g). Ascorbic acid equivalent (AAE) is a commonly used reference for calculating the quantity of vitamin C present in a substance. The ethanol extract of patikala leaves gave a FRAP value of 78.52 ± 0.26 mg AAE/g extract which suggests that 78.52 mg of ascorbic acid is present per gram of the extract (Table 8).

Conclusion

The findings from the present study indicate that the ethanol extract of patikala leaves exhibits antioxidant activity with IC₅₀ values of 42.45 ± 1.37 $\mu\text{g/mL}$ and 26.46 ± 0.09 $\mu\text{g/mL}$ for DPPH radical scavenging

activity and ABTS radical scavenging activity, respectively. The extract displayed a high ferric reducing antioxidant power with a mean FRAP value of 78.52 ± 0.26 mg AAE/g extract. Furthermore, the extract has total phenolic content of 27.19 ± 0.07 mgGAE/g extract and a flavonoid content of 4.60 ± 0.01 mgQE/g extract. The presence of flavonoids and phenolic compounds in the ethanol extract of patikala leaves could contribute significantly to its strong antioxidant activity.

Conflict 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.

References

- Badarinath AV, Rao KM, Chetty CMS, Ramkanth S, Rajan TV, Gnanaprakash K. A Review on In-Vitro Antioxidant Methods: Comparisons, Correlations and Considerations. *Int J PharmTech Res.* 2010; 2(2):1276-1285.
- Sitorus E, Momuat LI, Katja DG. Antioxidant Activity of Seruhan Plant (*Peperomia pellucida* [L.] Kunth). *J Sci.* 2013; 13(1):80-85.
- Aditya M and Ariyanti PR. Benefits of Gambir (*Uncaria gambir* Roxb) as an antioxidant. *J Major.* 2016; 5(3):129-133.
- Jabbar A, Wahyuni W, Malaka M, Apriliani A. Antioxidant Activity of Ethanol Extract of Fruit, Leaves, Stems and Rhizomes on Wualae (*Etilingera elatior* (Jack) R.M Smith) Plants. *J Farm Galen.* 2019; 5(2):189.
- Handayani V and Ahmad AR. Phytochemical Laboratory, Faculty of Pharmacy, Indonesian Muslim University, Makassar; Sudir, M.; Phytochemical Laboratory, Faculty of Pharmacy, Indonesian Muslim University, Makassar. Antioxidant Activity Test of Methanol Extract of Patikala Flowers and Leaves (*Etilingera elatior* (Jack) R.M.Sm) Using the DPPH Method. *Pharm S Res.* 2014; 1(2):86-93.

6. Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z. Pharmacological Activity, Phytochemical Analysis and Toxicity of Methanol Extract of *Etilingera elatior* (Torch Ginger) Flowers. Asian Pac J Trop Med. 2010; 3(10):769–774.
7. Utami YP. Measurement of Simplicia Parameters and Ethanol Extract of Patikala Leaves (*Etilingera elatior* (Jack) R.M. Sm) from Enrekang Regency, South Sulawesi. J of Pharmacy and Pharmacology Magazine.2020; 24(1):6.
8. Utami YP, Kristiyanti AMD, Imrawati. Optimization of Distillation Time on the Content and Characteristics of the Essential Oil of Patikala Leaves (*Etilingera elatior* (Jack) R.M.Smith). J Katalisator 2022; 7(2):205–212.
9. Utami YP, Ismail I, Nisa M, Oktaviani I. Antibacterial Activity Test of *Etilingera elatior* (Jack) R.M. Sm Leaf Extract against Bacteria *Staphylococcus aureus* and *Escherichia coli* Diffusion Method. Int J Res Pharm Pharm Sci. 2023; 8(3):1–4.
10. Kumar K, Kamboj R, Rao N, Yadav J. Status and Role of Coccinellids in Insect Pest Management. In: Karem Ghoneim (Ed), Advances in Agricultural Entomology, AkiNik Pub, Vol. 7, 2019. 15–33 p.
11. Munteanu IG and Apetrei C. Analytical Methods Used In Determining Antioxidant Activity: A Review. Int J Mol Sci. 2021; 22(7):3380.
12. Hapsari BW, Manikharda, Setyaningsih W. Methodologies in the Analysis of Phenolic Compounds in Roselle (*Hibiscus sabdariffa* L.): Composition, Biological Activity, and Beneficial Effects on Human Health. Horticult. 2021; 7(2):35.
13. Yuniarti R, Nadia S, Alamanda A, Zubir M, Syahputra RA, Nizam M. Characterization, Phytochemical Screenings and Antioxidant Activity Test of Kratom Leaf Ethanol Extract (*Mitragyna speciosa* Korth) Using DPPH Method. J Phy Conf Ser. 2020; 1462:012026.
14. Sholekah FF. Differences in Altitude on The Content of Flavonoids and Beta Carotene in Karika Fruit (*Carica pubescens*) Dieng Wonosobo Area. Proc Natl Semin Biol Biol Educ Yogyakarta. 2017; 75-82.
15. Susana I, Ridhay A, Bahri S. Study of Antioxidant Activity of Kecombrang Stem Extract (*Etilingera elatior*) Based on Solvent Polarity Level. J of Covalent Chemistry Research. 2018; 4(1):16–23.
16. Setiawan F, Yunita O, Kurniawan A. Testing the Antioxidant Activity of Ethanol Extract of Secang Wood (*Caesalpinia sappan*) Using the DPPH, ABTS, and Frap Methods. J of Science and Health. 2018; 2:82–89.
17. Serlahwaty D and Sevian AN. Antioxidant Activity Test of 96% Ethanol Extract Combination of Strawberries and Tomatoes Using the ABTS Method. Proceedings of the 50th National Seminar on Indonesian Medicinal Plants. 2016; 3:322–330.
18. Aminah A, Muflihunna A, Abidin Z. Antioxidant Activity Test of Ethyl Acetate Fraction of Wungu (*Graptophyllum pictum* (Linn) Griff) Leaves Using the FRAP Method (Ferric Reducing Antioxidant Power). J Ilm -Syifaa. 2016; 8:39–44.
19. Indrisari M, Aksa R, Khair N, Agiel FMB, Harmawati H, Muslimin L. Acute Safety Assessment of Ethanol Leaves Extract of Cemba (*Acacia rugata* L.) on The Kidney in Male Wistar Rats. Trop J Nat Prod Res. 2023; 7(7):3360–3363.
20. Thao TTP, Tu PTC, Men TT. A Comparative Study on Polyphenol, Flavonoid Content, Antioxidant and Antiinflammatory Capacity of Different Solvent Extract from *Portulaca oleracea* in Carrageenan-Induced Paw Edema in Mice. Trop J Nat Prod Res. 2023; 7(10):4152-4159.
21. Kamari FE, Chlouchi A, Hamzaoui NE, Harmouzi A, Lhilali I, Amrani Jihane E, El-Mouhdi K, El Omari H, Abdellaoui A. Chemical Composition, Antioxidant and Antimicrobial Activities of The Essential Oil of *Origanum majorana* Growing in Middle Atlas of Morocco. Trop J Nat Prod Res. 2023; 7(10):4232-4237.
22. Saralieva EN, Petkova NT, Ivanov Van G, Aneva IY, Georgiev VG, Nikolova KT. Polyphenolic Compounds, Triterpenes, Carlina Oxide, Antioxidant Activity And Carbohydrate Profile Of Different Vegetal Parts Of *Carlina vulgaris* L., *Carlina acanthifolia* All. and *Carlina corymbosa*. Trop J Nat Prod Res. 2023; 7(10):4242-4248.
23. Hasanah N and Novian DR. Analysis of Ethanol Extract of Yellow Pumpkin (*Cucurbita moschata* D.).Parapemikir J Ilm Farm. 2020; 9(1):54.
24. Santoso U. Antioksidan pangan; Ugm Press, 2021.
25. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang C-M. Determination of Antioxidants By DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. Mol Basel Switz. 2022; 27(4):1326.
26. Ingrid HM and Santoso H. Extraction of Antioxidants and Active Compounds from Kiwi Fruit (*Actinidia deliciosa*). Unpar J Engr Sci . 2014; 2:43.
27. Herni K, Anas S, Ajeng D, Iskandar Y. Cytotoxicity of Quercetin and Quercetin-3-O-Rhamnoside of *Etilingera elatior* (Jack) R.M.Sm. Leaves against HeLa Cervical Cancer Cells. J Appl Pharm Sci. 2021; 11(05):085–090.
28. Ulfa SM. Department of Chemistry, Faculty of Science and Technology, State Islamic University (Uin) Maulana Malik Ibrahim Malang. 2016.
29. Maryam S, Baits M, Nadia A. Measurement of the Antioxidant Activity of Ethanol Extract of Moringa Leaves (*Moringa oleifera* Lam.) Using the FRAP Method (Ferric Reducing Antioxidant Power). J Fitofarmaka Indones. 2016, 2(2):115–118.
30. Maulana KA, Naid T, Dharmawat DT, Pratama M. Analysis of Antioxidant Activity of Jackfruit Seed Extract (*Artocarpus heterophyllus* Lam) Using the FRAP (Ferric Reducing Antioxidant Power) Method. J Bionat. 2019; 20(1):27-33.
31. Maulida Z. Antioxidant Activity of Flavanoid Isolates from the Ethanol Extract of Butterfly Pea Flowers (*Clitoria ternatea*) with Frap Method. Benzema Pharm Sci J. 12 2023.
32. Chang C-C, Yang M-H, Wen H-M, Chern J-C. Estimation of Total Flavonoid Content in Propolis By Two Complementary Colometric Methods. J Food Drug Anal. 2002; 10(3): Article 3.
33. Kupina S, Fields C, Roman MC, Brunelle SL. Determination Of Total Phenolic Content Using The Folin-C Assay: Single-Laboratory Validation, First Action 2017.13. J AOAC Int. 2018, 101(5):1466–1472.
34. Royani A, Hanafi M, Julistiono H, Manaf A. The Total Phenolic And Flavonoid Contents of *Aloe vera* and *Morinda citrifolia* Extracts as Antibacterial Material against

- Pseudomonas aeruginosa*. Mater J Today Proc. 2023, 72:2796–2802.
35. Da Silva LAL, Pezzini BR, Soares L. Spectrophotometric Determination of The Total Flavonoid Content In *Ocimum basilicum* L. (Lamiaceae) Leaves. Pharmacogn Mag. 2015; 11(41):96–101.