Activity-guided Isolation of Antioxidant Phenolic Compound from *Etlingera elatior* Leaves

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Abstract
Thorch ginger (Etlingera elatior) plant is one of Zingiberaceae family used as natural medicine such as antimicrobe, analgetic, anti-inflammatory and antioxidant. The chemical contents of thorch ginger are tannins, phenolic compounds, essential oil, saponins, flavonoids and terpenoid/steroids. Antioxidant activity-guided isolation and purification process were used to identify the DPPH (1,1 diphenyl-2-picrylhydrazyl) radical-scavenging component of Etlingera elatior. Dry leaves were macerated with ethanol 70% and then fractionated by liquid-liquid extraction with different solvents and tested its DPPH radical-scavenging activity.

The ethyl acetate fraction showed the highest Total Phenolic Content (TPC) and DPPH radical-scavenging activity which was column chromatographed to obtain phenolic compound as antioxidant active component. Good correlation was observed between TPC and antioxidant activity among extracts. The UV-VIS and IR spectrum of isolate showed the phenolic compound to be promising as antioxidant.

Keywords: Thorch ginger (Etlingera elatior), antioxidant, DPPH, phenolic compound.

Introduction

Degenerative diseases can caused by free radicals formed through radiation and oxidation processes. It can cause the damage of cells and continue with less functioning cells and tissues so that the organ function becomes greatly reduced.

The free radical is unstable and highly reactive which tends to react with other molecules to achieve stability. Radical with this high reactivity can initiate a chain reaction in one formation causing an abnormal compound and initiate a chain reaction which can damage important cells in the body. The free radical can be overcome with the use of antioxidants[1].

Antioxidants are substances that the body need to neutralize free radicals and prevent damage caused by free radicals to normal cells, proteins and fats. Antioxidants have the ability to donate electrons to stabilize free radicals[2,3].

Thorch ginger (*Etlingera elatior*) as shown in fig. 1 is one of the zingiberaceae family which is the original plant of Indonesia, is also believed to be a neutralizer of cholesterol, as well as antimicrobial[4].

(a) **(b)**

Figure 1: Macroscopic of flower (a) a leave (b) of *Etlingera elatior*

The antioxidant content in plants acts as a scavenger and helps to convert free radicals. The natural antioxidants are found in all parts of the plant such as berocarotenoids, vitamins, flavonoids and phenols. The natural antioxidants have interest in the nutritional potential and therapeutic effects[1]. Several studies have been conducted on the antioxidant and other activities of *E. elatior* plant.[5-8]

In a continuation of our study, here we report an activity-guided isolation and purification procedure to isolate the antioxidant activity from *E. elatior*.

Material and Methods

Chemical and Reagent: DPPH (1,1 diphenyl-2-picrylhydrazyl) was purchased from Sigma Aldrich USA, Follin Chiocalteu's phenol reagent was purchased from Merck, Germany. Ethanol, methanol, ethyl acetate and n-Hexane as eluents from CV. Tri Putra Jaya Abadi. All chemicals and reagents were of analytical grade.

Plant: Leaves of *E. elatior* commonly used as spices, mouth wash and medicinal purposes in Indonesia were planted at local garden in Bandung City, West Java, Indinesia. Leaves were harvested after 3 months of growth. This plant was identified and confirmed by Herbarium Jatinangor, Biology Departement of Universitas Padjadjaran, Indonesia.

The study was conducted in several stages including preparation of materials, extraction and fractionation, total Phenolic Content and DPPH radical-scavenging activity assay, isolation and identification of isolates.

Sample Preparation: Leaves of *E. elatior* were washed, cleaned in running tap water, cuts into small pieces and dried to constant weight using oven at 40^0C. The sample was pounded into small size using grinder machine; it was stored in an air tight container till when needed.

Extraction and Fractionation: The dried leaves powder of *E. elatior* (1000g) was extracted sequently by maceration method in ethanol 70% (3x 2000 mL) at room temperature. The filtrate was concentrated in vacuo, obtained 100 g extract, then extracted with MeOH: H_2O (8:2), nHexane and etOAc successively. The EtOAc fraction (5 g) thus obtained was subjected to vacuum chromatographed over silica gel 60H with gradient eluent nHexane, EtOAc and MeOH, then separated into 20 fractions. The 11th to 17th fractions (Fraction A) showed the yellow spot after sprayed with DPPH 0,2% in methanol. Fraction A (3 g) was subjected to colomn chromatography over silica gel with formic acid-EtOAc-H2O (0.2:8:0.2) as eluent, obtain 30 fractions.

Fraction B (fraction 10,11 and12) showed yellow spot with DPPH 0,2% in methanol. The final purification of sample B (80 mg) was achieved by preparative TLC silica gel 60 F254 (Merck KgaA 64271, Darmstadt Germany) solvent formic acid-EtOAc-H2O (0.2:8:0.,2) to yield phenolic antioxidant active compound (11 mg).

DPPH Radical Scavenging Assay: Determine scavenging effect of different concentration compounds on DPPH, 1,5 mL of samples, after standing in dark for 30 min measured against methanol with absorbance at 515 nm. Controls containing methanol instead of the quercetin as antioxidant solution and blanks containing methanol instead of DPPH solution were also made.

The inhibition of the DPPH radical by the samples was calculated according to the following formula:

DPPH scavenging activity (%) :
(1 - (Abs. of sample-Abs. of blank)/Abs. of control).

The percentage of scavenging activity was plotted against the sample concentration to obtain the IC50 defined as the concentration of sample necessary to cause 50% inhibition.[3,9]

Determination of Total Phenolic Content (TPC): Total phenolic content was estimated by the Folin–Ciocalteu method. 5 mL of diluted sample were added to 5 ml of 1:10 diluted Folin–Ciocalteu reagent. After 5 min, 4,0 mL of saturated sodium carbonate (0,7 g/l) was added. After 15 min of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (10–500 mg/l) was used for the standard calibration curve. The results were expressed as gallic acid equivalent (GAE)/mg dry weight of sample and calculated as mean value ± SD (n = 3)[10].

Identification and Characterization of Isolates: Identification and characterization of isolates was performed by ultraviolet-visible spectrophotometry and infrared spectrophotometry. Spectral data was compared with the literature.[11]

Results and Discussion

The total phenolic content and DPPH radical-scavenging activities were tested on extract and their fraction of *Etlingera elatio*r leaves. Total Phenolic Content (TPC) was obtained from absorbance of the extract and fractions treated with Follin-Chiocalteu reagent (Table 1). The TPC of extract and fractions of *E. ealatior* was found to be maximum in ethyl acetate fraction than others.

Table 1
Precentage Yield and Phenolic Content Value of Extract and Fractions of *E. ealatior*

Sample *E.elatior* leaves	%yield [a]	Phenolic (%GAE)[b]
Extract	11,08	3,39±0,07
nHexane Fraction	0,24	0,34±0,01
EtOAc Fraction	0,55	0,67±0,03
MeOH Fraction	4,98	O,45±0,01

Note : [a] %yield extract or fraction to simplicia
[b] As gallic acid equivalent present in corresponding extract/fractions calculated using a standard graph

The extract and fractions were further tested for their DPPH radical-scavenging activity. It was found that ethyl acetate fraction showed the maximum actuvity than others (table 2). A good correlation between TPC and DPPH radical-scavenging activity among sample was observed. The result suggested that the phenolic compound contributed significantly to the antioxidant capacity of *E. ealatior*. Hence, ethyl acetate fraction of leaves of *E. elatior* was selected for the isolation af antioxidant.

Ethyl acetate fraction of leaves of *E. elatior* was evaporated under reduced pressure and the residue thus obtained was subjected to activity-guided fractionation on silica gel column and eluted with solvent of increasing polarities to isolate pure compound.

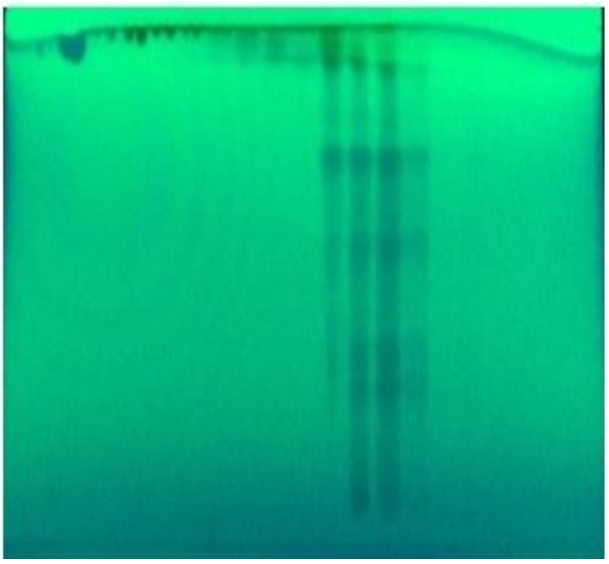
Active fractions 11-16th (sampel A), 3 g, were obtained from vacuum chromatography over silica gel 60 H (Merck, Germany) with gradual eluation of n Hexane-EtOAc-MeOH (fig. 2). The active compounds were show with yellow spot after sprayed with DPPH 0,2% in methanol.

Active fractions of sampel A were isolated with column chromatographed over silica gel 60 ; 0.2-0.5 mm (Merck, Germany) on elution with Formic Acid:EtOAc:H_2O (0.2:8:0.2) and obtained 80 mg yellow compound from 10-12th fraction (Sampel B) (fig. 3).

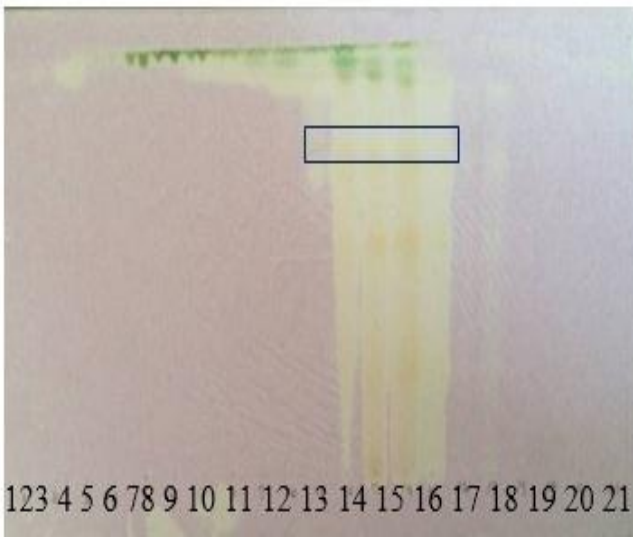
Table 2
Antioxidant activities of Extract and Fractions of *E. elatior* against DPPH

Sample *E.elatior* leaves	IC_{50} (μgmL^{-1})[a]
Extract	52,05
nHexane Fraction	263,93
EtOAc Fraction	14,27
MeOH Fraction	35,31
Quercetin [a]	10,35

Note: IC_{50}, concentration of antioxidant needed to reduce the original amount of radical by 50%
[a] positive control



(a)



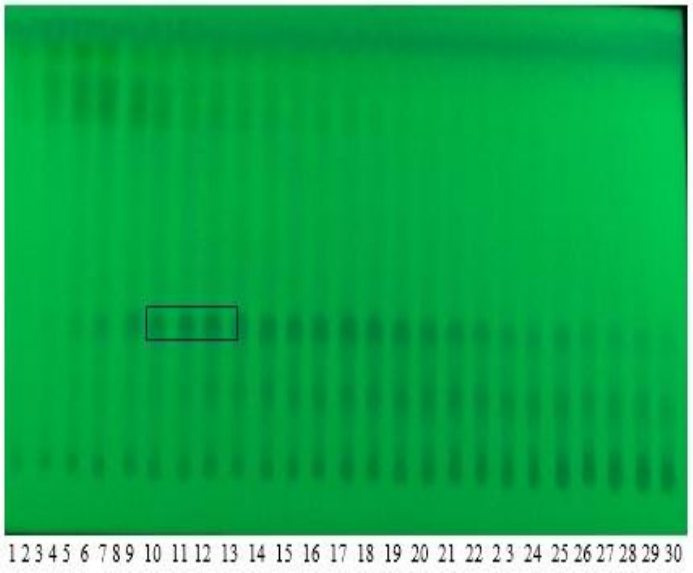
(b)

Figure 2: The thin-layer chromatograms of extracts and fractions, phases of pre-coated silica gel GF254, eluent of formic acid : EtOAc : H_2O (1:8:1), (a) UV light λ 254 nm (b) DPPH 0,2%

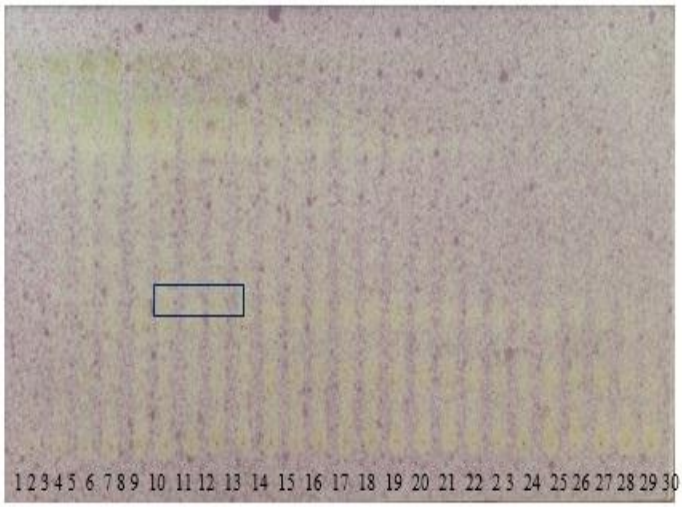
The final purification was achieved by preparative chromatographed with formic acid:EtOAc:H_2O (0.2:8:0.2) as eluent to yield phenolic antioxidant active compound (11mg).

The isolate was characterized as phenolic antioxidant active compound. TLC showed yellow spot with DPPH 0,2% reagent (antioxidant) and black spot with $FeCl_3$ 10% reagent (phenolic compound) (fig. 4).

In the ultraviolet spectrum isolate showed maximum absorbance at 349 nm and identification with infrared spectrophotometry showed the presence of the functional group –OH, C-H aromatic, C=C aromatiic, C-H and $–CH_2$ (fig. 5).

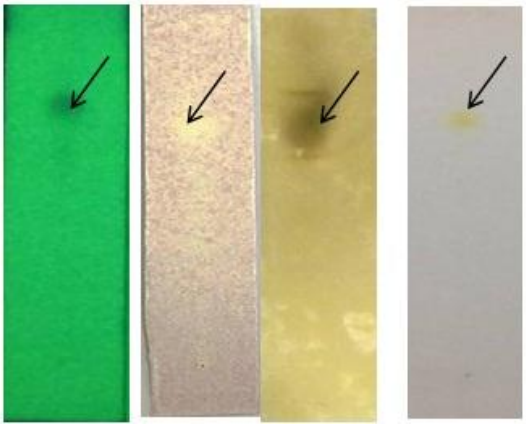


(a)



(b)

Figure 3: The thin-layer chromatograms of extracts and fractions, phases of pre-coated silica gel GF254, eluent of formic acid : EtOAc : H_2O (0.2:8:0.2), (a) UV light λ 254 nm (b) DPPH 0,2%



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

Figure 4: The thin-layer chromatograms of isolate, phases of pre-coated silica gel GF254, eluent toluene-acetone-formic acid (3:5:1), (a) UV light λ 254 nm, (b) DPPH 0,2%, (c) $FeCl_3$ 10% and (d) H_2SO_4 10%

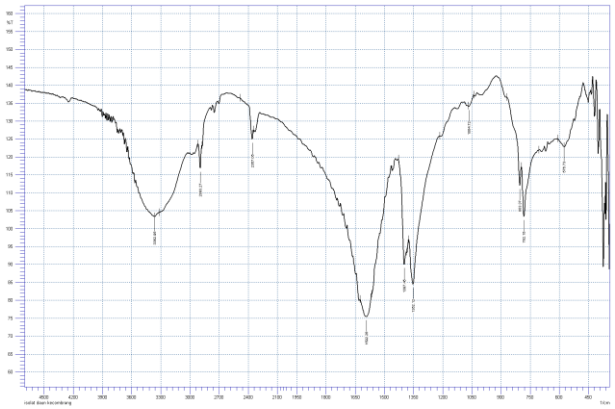


Figure 5: IR Spectrum of Isolate

Conclusion

The DPPH mediated in vitro studies revealed that phenolic compound is responsible for the free radical scavenging activity of *Etlingera elatior*.

Acknowledgement

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