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Phytochemical Analysis And Antioxidant Activity Of Leaves Extracts Of Endemic Plant Jahe Balikpapan (Etlingera Balikpapanensis A.D. Poulsen)

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Abstract: Jahe Balikpapan (*Etlingera balikpapanensis* A.D.Poulsen) is an endemic plant which can be found in East Kalimantan, Indonesia and potentially to be used as a medicinal plant. This research was held to screen phytochemicals and antioxidant activity of different extract (ethanol, hexane, and ethyl acetate) of *E. balikpapanensis*. The phytochemical test was held towards alkaloids, phenolics, flavonoids, tannins, saponin, steroids, and triterpenoid. Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically by using Folin-Ciocalteu's reagent and aluminum chloride (AlCl₃) method, respectively. Antioxidant activity was evaluated by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The screening of phytochemical revealed the presence of alkaloids, phenolics, flavonoids, and steroids. Ethyl acetate extract showed the highest TPC (109.8 ± 0.011 mg GAE/gr) followed by ethanol extract (57.6 ± 0.004 mg GAE/gr) whereas hexane extract showed the least TPC (29.0 ± 0.009 mg GAE/gr). Hexane extract showed the highest TFC (268.4 ± 0.05 mg CE/gr) followed by ethanol extract (200.4 ± 0.02 mg CE/gr) whereas ethyl acetate extract showed the least TFC (141.8 ± 0.03 mg CE/gr). The highest antioxidant activity was observed in ethyl acetate extract (IC₅₀ = 7.59 µg mL⁻¹) followed by ethanol extract (IC₅₀ = 74.80 µg mL⁻¹) and hexane extract (IC₅₀ = 189.89 µg mL⁻¹). This result exhibited that the leaves extract of *E. balikpapanensis* is very potential to be used as a medicinal plant and as a natural antioxidant.

Keywords: Antioxidant Activity, Endemic Plant, Jahe Balikpapan (*Etlingera balikpapanensis*)

1 INTRODUCTION

Since ancient times, Plants have been widely recognized as an important source of novel therapeutic compounds for the treatment of various diseases and had been reported in traditional medicine system. Nowadays, medicinal plant utilization in traditional medicine has been increasing, especially for health matter. According to the World Health Organization (WHO), the majority of the world population depends on traditional medicine for health care, so that medicinal plant explorations were continuously held to meet community needs. The interest enhancement toward medicinal plants and its active substance has been happened because of its great potency as medicine and has no negative side effect. Some researches around the world about medicinal plants, revealed that it plays a role as an antioxidant [1], [2], [3], [4], [5], antibacterial [6], [7], antihypertensive [8], and

anticancer [9]. Exploration in 2006 by a botanist, Axel Dalberg found new species from Zingiberaceae i.e. *Etlingera* which is named *Etlingera balikpapanensis*. This plant type has been found as endemic in Balikpapan region, particularly in the protected forest of Sungai Wain Balikpapan, East Kalimantan, Indonesia. Local community mentioned this plant as Jahe Raksasa (or giant ginger) and named as Jahe Balikpapan. *Etlingera* type is traditionally utilized by the local community at Kalimantan for various necessities, either raw consumed or used as mixture ingredient of food flavoring. Flower part can be consumed and aromatic. *Etlingera* is plentiful used, especially on Kecombrang (*E. elatior*) which is used for treating headache, stomachache, skin irritation and air fresher. Leaf, stem, rhizome and flower extract of *E. balikpapanensis* was reported by Jaafar [10] that comprised of essential oil as a bioactive compound. Leaf and flower extract of this species was utilized to eliminate mosquito larvae of *Aedes aegypti* [11]. The phytochemical compound which contained in flower are glycosides, flavonoids, phenolic [12], while leaves extract has high flavonoids and total phenolic content [13]. Young flower bud extract was reported as antimicrobial, cytotoxic and antitumor [14] and antibacterial against *E. coli* and *S. aureus* [15]. *E. elatior* flower was utilized previously in mixture ingredient of jelly candy [16] and the extract has a very high antioxidant activity [12], [17].

Jahe Balikpapan is found only and grown as endemic in Balikpapan region, East Kalimantan, only a few published elsewhere either for phytochemical compound or its potency as a medicinal plant. This species was estimated has great potential as medicinal plant nevertheless, till nowadays the scientific knowledge about its utilization as well as its relative

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i.e. *E. elatior* are not available yet. To best of our knowledge, this is the first study to evaluate the phytochemical and antioxidant activity of *E. balikpapanensis* leaves extracts that can be used as a new source of medicinal plant. Several known chemical substances and beneficial as traditional medicine are phenolic, flavonoids, alkaloids, tannin, steroids, triterpenoid, etc. To find out whether this plant is potential as medicine, then chemical analysis of phytochemical content and antioxidant activity assay from *E. balikpapanensis* is necessary. Hence, the present study was performed to investigate the potency of *E. balikpapanensis* from leaves extract as a medicinal plant based on its phytochemical, total phenolic, total flavonoids content and antioxidant activity.

2 MATERIAL AND METHODS

2.1 Sample Preparation

Fresh leaves of *E. balikpapanensis* were collected from protected forest of Sungai Wain Balikpapan, East Kalimantan. It was washed thoroughly with tap water, sliced, air-dried at room temperature (no sunlight exposure) for \pm 6 days. The dried sample refined by using the blender. Refined leaves were weighed approximately 500 g then macerated with ethanol 95%, placed on a shaker for 5 days while occasionally stirred. The extract was filtered pass-through Whatman filter paper no.1 then the solvent was evaporated by rotary evaporator until we obtained ethanol extract in paste shape. The crude extract was weighed and obtained 42.65 g. The crude extract was extracted successively with hexane and ethyl acetate. All extracts were processed by phytochemical test to find out the secondary metabolite compound. The antioxidant activity assay was held with free radical immersion method DPPH (2,2-diphenyl-1-picrylhydrazyl).

2.2 Phytochemical Analysis

One gram of leaves extract was dissolved in 100 mL ethanol 95%, the solution was used to preliminary phytochemical screening following Harborne [18] and Kokate [19] standard methods.

2.2.1 Alkaloids

Approximately 1 ml of extract solution was mixed with 3-5 drops of sulfuric acid 2 M and shaken till it developed two layers. The acid layer (top layer) was collected then placed in a test tube, dripped 1 pipette of Dragendorff's reagent (a mixture of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ in nitrate acid and KI solution). Presence of alkaloids was indicated with the formation of orange to brownish red precipitation.

2.2.2 Phenolic

A-1 mL extract solution was added with a few drops of FeCl_3 1%. The formation of green, red, purple, red, or blue-black color indicated the presence of phenolic.

2.2.3. Flavonoids

One mL extract solution was mixed with 3 mL of boiled water and incubated for 5 minutes. After that, it was added with 0.05 mg of Mg powder and 1 mL of concentrated HCl then it was shaken. Immediate development of a red, yellow or orange color will indicate the presence of flavonoids.

2.2.4 Tannins

A-1 mL of extract solution was stirred with 5 mL of distilled water. The formation of a blue, blue-black or blue-green color or precipitation on the addition of reagent FeCl_3 (5%) indicated the presence of tannins.

2.2.5. Saponin

About 1 mL of extract solution was mixed with 3 mL of boiled water and shaken vigorously. If a foam was produced and it was stable for 1-2 minutes and persisted on warming, its evidence for the presence of saponin.

2.2.6. Steroids-triterpenoid

About 1 mL extract solution was mixed with Liebermann-Burchard's reagent acetic acid (CH_3COOH): concentrated H_2SO_4 (19:1 v/v). The Solution was shaken gently and left for a few minutes. The presence of triterpenoid was indicated the formation of red or purple colors, presence of steroids was indicated by the formation of a green or blue colors.

2.3 Determination of Total Phenolic Content (TPC)

The total phenolic content of different crude extracts leaves of *E. Balikpapanensis* was determined spectrophotometrically using Folin-Ciocalteu reagent [20] with slight modifications. A-1 mg crude extract was dissolved in 10 mL DMSO and used as an extract sample. As a standard solution of gallic acid, one mg of gallic acid was dissolved in 10 mL DMSO. Folin-ciocalteu reagents: to one mL Folin-ciocalteu was added with 9 mL sodium carbonate 7.5% (7.5 mg of sodium carbonate dissolved in 100 mL of distilled water). To 1 mL sample was added with 0.4 mL of distilled water, 0.25 mL Folin-ciocalteu reagents, and 1.25 mL of sodium carbonate. The solution was incubated for one hour in the darkroom. Before sample testing, a standard calibration curve of the gallic acid solution was prepared (0, 20, 40, 60, 80, 100 $\mu\text{g mL}^{-1}$). The absorbance was measured at 760 nm using UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The TPC of crude extracts was determined from extrapolation of the calibration curve and it was expressed as milligrams of gallic acid equivalents (GAE) per gram crude extracts.

2.4 Determination of Total Flavonoid Content (TFC)

The total flavonoid content of plant extract was determined by using Aluminum chloride colorimetric technique (ACCT) method [20]. In brief, 1 mg of different crude extract was dissolved in 10 mL DMSO and used an extract solution. 5% NaNO_2 solution (5 mg in 100 mL of distilled water), 1M NaOH solution (4 mg in 100 mL of distilled water), and 10% AlCl_3 solution (10 mg in 100 mL of methanol) was prepared. The test was performed on 0.1 mL of extract solution added with 0.7 mL of distilled water, 0.1 mL NaNO_2 5%, 0.1 mL AlCl_3 10% and 0.5 mL 1M NaOH and then incubated for 10 minutes at room temperature in a darkness. The absorbance was measured at 510 nm using UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan) against a blank. The standard curve was prepared using catechin by dissolving in DMSO followed by serial dilution to 2, 4, 6, 8, 10 $\mu\text{g mL}^{-1}$. The TFC in the plant extracts was expressed as mg catechin equivalents (CE)/g extract.

2.5 Determination of Antioxidant Activity

Antioxidant activity of the different crude extract was held using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) as described by Manurung [3], [20]. Three mg of different crude extracts such as ethanol, hexane, and ethyl acetate dissolved with 1 mL ethanol and used as a stock solution. Five concentration (6.25, 12.25, 25, 50, 100 µg/mL) of dilution were prepared from different crude extract. Thirty-three µL of each concentration was placed in a separate test tube and added with 467 µL of ethanol and 500 µL of 27% DPPH solution. After that, all of the mixed solutions were placed at room temperature in darkness and incubated for 20 minutes. The control was prepared in the same way without adding any crude extract. The absorbance was measured using a UV-VIS spectrophotometer at 517 nm. The antioxidant activity percentage was calculated using the formula:

$$\% \text{ Antioxidant activity} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{bs Control}} \times 100\%$$

The IC₅₀ value was calculated by linear regression ($y = a + bx$) where the extract concentration as absis and the percent of antioxidant activity as ordinat.

2.6 Data Analysis

Phytochemicals compound was analyzed by a descriptive method and presented in table form. TPC, TFC, and antioxidant activity assay were analyzed using regression linear equation and least-square method.

3 RESULT AND DISCUSSION

3.1 Phytochemical Analysis

The phytochemical screening of different crude extract of leaves *E. balikpapanensis* revealed the presence of some secondary metabolites as shown in the table below.

Table 1. Phytochemical analysis of different extract of *E. balikpapanensis*

Phytochemical	Extract		
	Ethanol	Hexane	Ethyl acetate
Alkaloids	+	+	+
Phenolic	+	-	-
Flavonoids	+	+	+
Saponin	-	-	-
Steroids	+	+	+
Triterpenoid	-	-	-

Abbreviation: + indicate presence, - indicate absence of phytochemicals

Ethanol extract revealed the presence of alkaloids, phenolic, flavonoids, and steroids. Hexane extract and ethyl acetate extract showed the presence of alkaloids, flavonoids, and steroids. The phytochemical compounds (alkaloids, phenolic, flavonoids, steroids) detected are known to have medicinal importance. This secondary metabolite also was reported as one of the medicinal plants which have a lot of biological and therapeutic trait. Hence this species was expected to has many medicinal purposes. The presence of secondary metabolite compound in plants is influenced by several environmental factors, drought stress, light intensity, temperature, salinity,

etc. Phenolic and flavonoids content will be higher on intense drought stress condition [20], [21].

Soobrattee [22] reported that the phenolic compounds have redox properties and act as an antioxidant. Flavonoids have several functions as an antioxidant, antibacterial, anti-inflammation, anti-allergy and anti-mutagenic [23], [24]. Alkaloid had been reported as cytotoxicity [25], antimalarial [26], and anti-inflammatory [27]. Steroids and triterpenoid could be effective as an analgesic and also possess antibacterial and insecticidal properties [28], [29]. In the medical field, alkaloids were used to promote nerve system, improve blood pressure, reduce pain and prevent microbial infection. The new finding suggested that alkaloids extract of *Gnetum africanum* could be used as the new neuroprotective compound source [30].

Presence of phytochemical compound extracted from *E. balikpapanensis* is biologically significant, as was achieved within this research, had a contribution on its medicinal value, therefore leaves extract of *E. balikpapanensis* had high potency and was useful as the medicinal source. Then phytochemical and pharmacology investigation from the active compound of *E. balikpapanensis* should be continued, which is regarded from its various traditional utilization and was potential to be used at therapeutic application.

3.2 Total Phenolic and Flavonoid Content

Quantitative phytochemical result of ethanol extract, hexane extract, and ethyl acetate extract indicated various total phenolic and total flavonoids content. Total phenolic and total flavonoids content from leaves extract of *E. balikpapanensis* was presented in table 2.

Table 2. Total phenolic and total flavonoid content from ethanol, hexane, and ethyl acetate extract of leaves *E. balikpapanensis*

Extract	Total phenolic content (mg GAE/g)	Flavonoids content (mg CE/g)
Ethanol	57.6 ± 0.004	200.4 ± 0.02
Hexane	29.0 ± 0.009	268.4 ± 0.05
Ethyl acetate	109.8 ± 0.011	141.8 ± 0.03

The total phenolic content of the different crude extract calculated from the calibration curve, where we obtained linear regression $y = 0.01x + 0.030$ with R² value = 0.994. The TPC of crude extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram crude extracts. The TPC of leaf *E. balikpapanensis* was different when extracted with different solvents. The highest TPC was obtained from ethyl acetate (109.8), followed by ethanol (57.6), and the lowest from hexane (29.0). The previous research showed that the total phenolic of *Goniothalamus umbrosus*, *Goniothalamus velutinus* and *Ficus carica* was different when extracted with different solvents [24], [31], [32].

The phenolic compound is considered at the major class of antioxidant which isolated from plants. This kind chemical substance was known as free radical terminator which is commonly identified as a phenolic compound that has positive correlation towards antiradical activity [33]. The phenolic compound isolated from plants was known as free

radical scavenger and a major antioxidant compound [34] Basically, antioxidant mechanism of polyphenols compound was based on activities such as donating hydrogen and chelating metal ion [35].

Total flavonoids content was calculated from catechin calibration curve, where we obtained regression equation $y = 0.001x + 0.006$ with R^2 value = 0.993. Total flavonoids content of plant was stated in CE (catechin equivalent) or defined as an equivalent amount of mg catechin per gram sample [36]. Result of flavonoids content indicated that hexane extract has the highest flavonoids content (268.4 mg CE/g extract), followed by ethanol extract (200.4 mg CE/g extract) and the lowest was ethyl acetate extract (141.8 mg CE/g extract). The TFC hexane extract of *E. balikpapanensis* (268.4 mg CE/g) as compared to TFC of the same genus *E. elatior*, (244.83 mg RE/g) it is found that hexane extract of *E. balikpapanensis* has a higher value. A study by Abdelwahab [37] stated that essential oil of *E. elatior* leaves has flavonoids content about 244.83 mg RE/g, yet it was processed using rutin standard solution. Rutin and catechin are commonly used as a standard solution because they found in plants and one kind of flavonol glycoside [18]. Result of the quantitative assay (TPC and TFC) that had been held on three samples (ethanol, hexane, ethyl acetate extract) was indicated that each extract has secondary metabolite compound which is potential as an antioxidant. Leaves extract of *E. balikpapanensis* has a higher TFC than TPC. The previous study showed [20] that the total flavonoids content and antioxidant activity were higher in leaves methanolic extract of *F. deltoidea* than fruit and stem extract. Phenolic and flavonoids distribution at plant organ are influenced by genetic factor and environmental stress such as water deficit, salinity, soil fertility, etc. Secondary metabolite content (such as phenolic and flavonoids) and antioxidant activity in *F. deltoidea* were influenced by the type, plant organ age, where a plant reached old age, its flavonoids and antioxidant activity were simply higher [3], [38].

3.3 Antioxidant Activity

Antioxidant activity result with free radical inhibition method DPPH (2,2-diphenyl-1-picrylhydrazyl) from ethanol extract, hexane extract, and ethyl acetate extract of *E. balikpapanensis* leave was presented in figure 1.

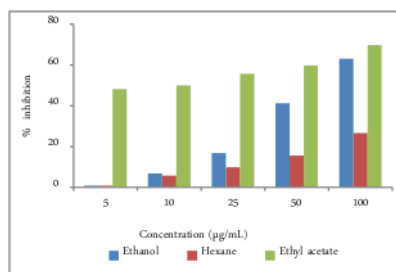


Figure 1. Antioxidant potential from different crude extract from *E. balikpapanensis* against DPPH

According to figure 1, the higher sample concentration then it was easy to induce a higher inhibition percentage (%). This happened because of the higher concentration on the sample, it stimulated the higher antioxidant content which affected on free radical inhibition level. Ethyl acetate fraction showed the

highest antioxidant activity, followed by ethanol extract and the lowest was hexane extract. This finding was similar to the other researchers who found that antioxidant activity of alcoholic hydro extract and ethyl acetate was higher than hexane and chloroform from *Ficus carica* [32]. Variation of antioxidant potential could be polyphenols of plant crude extract.

The IC_{50} of ethanol, hexane and ethyl acetate extract of *E. balikpapanensis* leaves was calculated to determine its antioxidant activity value. Comparison of IC_{50} value from three type leaves extract was presented in figure 2. The highest antioxidant potency was measured from ethyl acetate extract after compared with methanol and hexane extract. Antioxidant activity assay was sorted from the highest was: ethyl acetate > ethanol > hexane. Ethyl acetate fraction showed very high antioxidant potency, followed by ethanol extract and the lowest was hexane fraction. Variation of antioxidant activity could be influenced by polyphenols content from the extract. The phenolic compound was included in flavonoids which has the highest antioxidant potency at DPPH assay [32], [39].

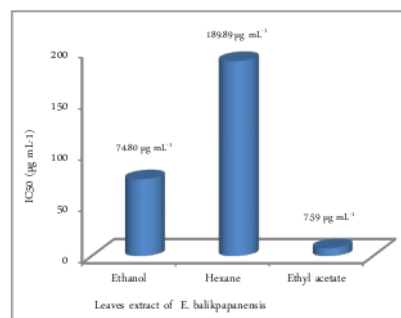


Figure 2. IC_{50} value from ethanol, hexane, and ethyl acetate leaves extract of *E. balikpapanensis*

To show antioxidant activity, we recently used IC_{50} (inhibition concentration), where IC_{50} was defined as the value of extract concentration which inhibits radical activity DPPH about 50%. This value was calculated from the linear regression equation which stated the correlation between tested extract concentration and radical interception percentage. The IC_{50} value showed the lower of its number then related to the higher antioxidant activity of extract [40]

According to picture 2, the antioxidant activity from leaves extracts of Jahe Balikpapan using DPPH method (2,2-diphenyl-1-picrylhydrazyl) had the lowest value from ethyl acetate fraction about 7.59 µg mL⁻¹, followed by ethanol extract about 74.80 µg mL⁻¹ and the last position was η-hexane fraction about 189.89 µg mL⁻¹. A compound specifically was mentioned as a very high antioxidant if IC_{50} value < 50 ppm, high antioxidant if IC_{50} value ranging from 50 - 100 ppm, medium antioxidant if IC_{50} value 100 - 150 ppm, low antioxidant if IC_{50} value 150 - 200 ppm, [40]. After considering previous range, we declared that ethyl acetate fraction was a very high antioxidant, followed by ethanol extract as a high antioxidant and the hexane was a low antioxidant. The previous study by Ningtyas [41] used water extract of *E. elatior* leaves, whom it showed that antioxidant activity was 2.44 µg mL⁻¹ by DPPH method. Chan [42] reported that methanol extract of *E. elatior* leaves had antioxidant activity about 3.75

$\mu\text{g mL}^{-1}$. Abdelwahab [37] stated that antioxidant activity from the essential oil of *E. elatior* was $995.1 \mu\text{g mL}^{-1}$. After compared with previous results, noticed by similar genus namely *Etilingera*, ethyl acetate fraction of *E. balikpapanensis* leaves has lower antioxidant activity than *E. elatior*, yet it was higher than essential oil of *E. elatior* about $7.59 \mu\text{g mL}^{-1}$.

The study by Handayani [43] using the same genus, i.e. *E. elatior* leaves, while they obtained from ethanol extract, it indicated that potential alkaloids and flavonoids are beneficial as an antioxidant. On the other hand, antioxidant activity from methanol extract of *E. elatior* leaves (IC_{50}) was $30.65 \mu\text{g mL}^{-1}$ and was considered as a very strong antioxidant. After we compared the phytochemical test with antioxidant activity assay of ethyl acetate extract, surprisingly, it was pretty much higher than ethanol extract and hexane extract. Ethyl acetate extract indicated few secondary metabolite compounds, such as alkaloids, steroids, and flavonoids. According to Harborne [18] the secondary metabolite compounds such as alkaloids, phenolic, and flavonoids have an antioxidant effect. This could have happened because that compound has excess hydrogen atom, therefore it could donor its atoms against free radical whom deficit one or more electrons.

4 CONCLUSION

Phytochemical analysis showed that the leaves extract of *E. balikpapanensis* had alkaloids, phenolic, flavonoids, and steroids content. Ethyl acetate and ethanol extract had very high and high antioxidant activity, subsequently. Then leaves extract of this species is very potential to be used as a medicinal plant and natural antioxidant. Advanced study is strongly recommended to isolate major phenolic compound as well as other bioactive compounds and perform several bioactivity tests such as antimicrobial, anticancer, or certain assay against diseases which are mediated by free radical.

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