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The antioxidant potential of kecombrang (*Etlingera elatior*)

INDAH SOLIHAH[1*], NIRWAN SYARIF[1], RIANA SARI PUSPITA RASYID[2], AND TRI SUCIATI[2]
[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Ogan Ilir, South Sumatera, Indonesia; [2]Department of Medicine, Faculty of Medicine, Sriwijaya University, Ogan Ilir, South Sumatera, Indonesia

Kata kunci:
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ABSTRAK: Kecombrang (*Etlingera elatior*) merupakan salah satu tanaman asli Indonesia. *E.elatior* digunakan sebagai bumbu dapur, makanan, atau obat-obatan. Kapasitas antioksidan rimpang, daun, dan bunga *E.elatior* belum pernah dilaporkan. Penelitian ini bertujuan untuk mengetahui kapasitas antioksidan rimpang, daun, dan bunga *E.elatior* dalam bentuk ekstrak dan fraksi. rimpang, daun, dan bunga *E.elatior* dimaserasi dengan etanol 96% untuk menghasilkan ekstrak kasar. Ekstrak kasar difraksinasi dengan n-heksana, etil asetat, dan etanol 96% untuk menghasilkan fraksi n-heksana, etil asetat, dan etanol. Total senyawa fenolik ditentukan secara spektrofotometri menggunakan kompleks warna follin-ciocalteau. Efek antioksidan ekstrak dan fraksi *E.elatior* dievaluasi menggunakan aktivitas penangkal DPPH. Total senyawa fenolik terbesar dari *E.elatior* terdapat pada fraksi etanol bunga (298,47mgGAE/100g), dan terendah terdapat pada ekstrak kasar rimpang (61,80mgGAE/100g). Kapasitas antioksidan yang sangat kuat (nilai IC50 <50ppm) terdapat pada ekstrak kasar, fraksi n-heksana rimpang, dan fraksi etanol daun. Kapasitas antioksidan yang kuat (nilai IC50: 50-100 ppm) terdapat pada ekstrak kasar dan semua fraksi bunga; fraksibetil asetat dan fraksi etanol rimpang; ekstrak kasar, fraksi n-heksana, dan fraksi etil asetat daun. Kesimpulan dari penelitian ini adalah tergantung pada jenis pelarut, dan jenis senyawa antioksidan yang diekstraksi maka kapasitas antioksidan akan bervariasi.

Keywords:
DPPH,
AEAC,
Follin-ciocalteaeu,
QEAC,
Fitofarmakologi

ABSTRACT: Kecombrang (*Etlingera elatior*) is one of the native plants of Indonesia. *E.elatior* is used as a condiment, food, medicine, and ornament. The antioxidant properties of rhizomes, leaves, and flowers of *E.elatior* has not been reported. This study aimed to explore the antioxidant capacity of the rhizome, leaves, and flowers of *E.elatior* in extracts and fractions. The rhizome, leaves, and flower of *E.elatior* were macerated by ethanol 96% to generate crude extract. The crude extract was fractionated by n-hexane, ethyl acetate, and ethanol 96% to generate n-hexane, ethyl acetate, and ethanol fraction. The total phenolic compound was determined spectrophotometrically using the follin-ciocalteau color complex. The antioxidant effect of extracts and fractions of *E.elatior* was evaluated using DPPH scavenging activity. The largest total phenolic compound of *E.elatior* was in the ethanolic fraction of flower (298.47mgGAE/100g), and the lowest was in a crude extract of rhizome (61.80mgGAE/100g). The very strong antioxidant capacity (IC_{50} value <50ppm) was in crude extract, n-hexane fraction of rhizome, and an ethanolic fraction of leaves. The strong antioxidant capacity (IC_{50} value: 50-100 ppm) was crude extract and all fractions of flower; ethyl acetate and an ethanolic fraction of rhizome; crude extract, n-hexane, and ethyl acetate fraction of leaf. The conclusion of this study is that, depending on the type of solvent, the type of antioxidant compounds being extracted and antioxidant capacity also vary.

1 INTRODUCTION

Exposure to free radicals is increasing and becoming a threat to humans nowadays. Several factors can cause free radicals, such as smoke, dust, pollution, and eating unbalanced nutrition fast food. Free radicals could also damage the biomolecules in human body cells, such as protein, lipid, and nucleic acid. Besides, free radicals are one of the factors that contribute to several diseases, such as cancer and heart disease [1]. Antioxidants can be used to neutralize the free radicals and inhibit the oxidative stress process [2]. Many plants which contain phenolics are found to be a source of antioxidants. One of the mechanisms of phenolic as antioxidants is to scavenge the free radicals, which are influenced by

* **Corresponding Author:** indahsolihah@mipa.unsri.ac.id

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the reduction of potential and dissociation bonding energy between oxygen and hydrogen in phytochemicals [1].

One of the medicinal plants that all parts have phenolic content is *Etlingera elatior* (Jack) Smith or torch ginger. The rhizome of *E.elatior* contains diarylheptanoids and dimethoxy-curcumin [3]. The inflorescences of *E.elatior* contain phenolics such as flavonoids, anthocyanin, and tannin [4]. The leaves of *E.elatior* contain phenolics, flavonoid, and glycoside-flavonoid such as quercetin 3-glucoside, kaempferol 3-glucuronide, caffeoylquinic acid, chlorogenic acid, quercetin 3-glucuronide, isoquercitrin, quercitrin, quercetin 3-rhamnoside, and catechin [5-6].

Etlingera elatior (Jack) Smith or torch ginger is native to Indonesia and Malaysia. The indigenous communities consume young shoots, inflorescences, and fruits as a condiment, cooked as vegetables, or eaten raw [7]. Previous studies reported some pharmacological action of *E.elatior* such as antioxidant [5], antibacterial [8], antifungal [9], tyrosinase inhibitor [10], cytotoxic [11], and hepatoprotective activity [12]. In this current study, the total phenolic content and antioxidant capacity of rhizomes, flowers, and leaf extracts and fractions of *E.elatior* will be evaluated using DPPH radicals. To the best of our knowledge, this is the first report of antioxidant capacity comparison of several parts of *E.elatior* in several polarity ranges of extraction solvent.

2 MATERIALS AND METHODS

Chemicals

The reagents and chemicals used in this study were ethanol 96%, n-hexane, methanol, ethyl acetate, Folin-Ciocalteu, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl).

Plant material

Etlingera elatior was collected from Musi Rawas, South Sumatra. The plant was separated into leaves, flowers, and rhizomes. All parts were washed and dried. The dried leaves, flowers, and rhizomes were ground into powdered form.

Extraction and fractionation procedure

The 250 mg of dried powder of each part was macerated with 96% of ethanol with ratio 1:10 to powder. The maceration process was kept in the dark bottle at 25-27°C for 48 hours and shaken occasionally. The macerates were filtered and then evaporated with a rotary evaporator at 60°C to generate a thick-crude extract.

The partial crude extracts were fractionated with n-hexane, ethyl acetate, and ethanol 96%. The fractionation procedure used liquid-liquid fractionation with a separating funnel. All the fractions were evaporated with a rotary evaporator at 60°C to generate a thick-fractions.

Total phenolic content determination

Total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu colour complex. Gallic acid was used for the calibration curve. Gallic acid was made in a concentration series of 50, 75, 100, 125, and 150 μg/ml in methanol. The *E.elatior* extracts and fractions were made at a concentration of 1000 μg/ml in methanol. The 0.5 mL of standard and samples were added with 4 mL sodium carbonate 1 M and 2 mL of Folin-Ciocalteu for 15 min. The absorbances of mixtures were measured at 775 nm. The total phenolic content was expressed as gallic acid equivalent in mg/100g extract or fraction.

Antioxidant assay

The scavenging ability of *E.elatior* to DPPH radicals was measured according to a previous study [13]. A solution of 0,1 mM DPPH was prepared in methanol p.a, then 3,8 mL of 0,1 mM DPPH (Ab) added to 0,2 mL solution of extracts and fractions (20, 40, 60, 80, and 100 μg/ml) or standard (ascorbic acid and quercetin). The mixtures (As) were incubated at room temperature for 20 minutes, and then their absorbances were obtained at 515 nm. The scavenging activity is expressed as percent inhibition was calculated by equation (1), and the half-maximum inhibitory concentration (IC_{50}) value was calculated from the regression of percent inhibition vs test sample concentration (μg/ml) relationship.

$$\text{Inhibition (\%)} = \frac{\text{Ab}-\text{As}}{Ab} \times 100\% \quad (1)$$

Free radical scavenging is also expressed as quercetin equivalent antioxidant capacity (QEAC) in mg Q/100g (Eq.2) and ascorbic acid equivalent antioxidant capacity (AEAC) in mg AA/100g (Eq.3) as follows:

$$\text{QEAC (mg Q/100g)} = \frac{\text{IC50 quercetin}}{\text{IC50 sample}} \times 100\% \quad (2)$$

$$\text{AEAC (mg AA/100g)} = \frac{\text{IC50 ascorbic acid}}{\text{IC50 sample}} \times 100\% \quad (3)$$

The IC_{50} value for the calculation above of quercetin was 0.0159 mg/ml, and ascorbic acid was 0.011027mg/ml

Statistical analysis

Results were expressed as mean ± standard deviation and analyzed using one-way ANOVA followed by Duncan's *post-hoc* test. The *p*-value < 0.05 was regarded as significant.

3 RESULTS

Extraction and fractionation yield and total phenolic content of *Etlingera elatior* are mentioned in Table 1. The total phenolic of *E.elatior* was measured using the Folin-Ciocalteu test. The total phenolic content was determined by the standard curve method using gallic acid (y = 0.07x + 0.0566), where the y value is the absorbance and the x value is the concentration of a gallic acid solution (μg/ml), expressed as: mg/g GAE.

Table 1. The yield and total phenolic content of extracts and fractions of *E.elatior*

Sample		Yield (%)	Total phenol (mg GAE/100 g)
Rhizome	Crude extract	23.54	61.800±6.616^{f}
	N-Hexane fraction	5.30	246.633±0.819^{b}
	Ethyl acetate fraction	6.47	175.867±3.289^{cd}
	Ethanol fraction	7.36	188.246±1.014^{c}
Flower	Crude extract	19.04	233.833±8.086^{b}
	N-Hexane fraction	5.66	183.467±0.633^{cd}
	Ethyl acetate fraction	5.78	230.330±5.598^{b}
	Ethanol fraction	5.67	298.470±14.583^{a}
Leaf	Crude extract	25.64	155.080±2.241^{d}
	N-Hexane fraction	6.76	252.433±25.744^{b}
	Ethyl acetate fraction	7.59	182.667±4.914^{cd}
	Ethanol fraction	6.66	116.780±6.510^{e}

Note: numbers in the same column followed by the different superscript letters (^{a,b,c}) are significantly different ($p<0.05$)

The total phenolic content of *E.elatior* was in the range of 61.800-298.470 mg GAE/100g. The flower ethanolic fraction had the highest total phenolic content (298.470±14.583mg GAE/100g). The rhizome n-hexane fraction, flower crude extract, and leaf n-hexane fraction had no significant difference of total phenolic content, closely followed by rhizome ethyl acetate fraction, flower n-hexane fraction, and leaf ethyl acetate fraction. The flower crude extract had higher total phenolic content than leaf ethanol fraction, and the lowest was obtained by rhizome crude extract (61.800±6.616mg GAE/100g).

The antioxidant capacity of *E.elatior* crude extracts and fractions was evaluated using the DPPH-scavenging activity. The inhibition of DPPH radical formation was shown in Figure 1 for quercetin, ascorbic acid, and *E.elatior* crude extracts and fractions. The DPPH scavenging ability of rhizome crude extract 100 ppm (90.263%) was the highest, and leave ethyl acetate fraction 20 ppm (14.073%) was the lowest. The IC_{50} was obtained by the regression of the test sample concentration-percent inhibition relationship, and the QEAC and AEAC were calculated.

The antioxidant activities of *E.elatior* extracts and fractions are expressed as IC_{50}, QEAC, and AEAC values. They are shown in table 2.

The best antioxidant was obtained in rhizome crude extract with the lowest IC_{50} value (25.430 μg/mL) and the highest QEAC (62.527 mg Q/100g) and AEAC (43.364 mg AA/100g) value. Based on the IC_{50} value, there are two category of antioxidant capacity of *E.elatior*: very strong (IC_{50} < 50 μg/mL) and strong (IC_{50} = 50-100 μg/mL) [14]. The very strong antioxidant capacity was obtained in leaf ethanol fraction, rhizome crude extract, and n-hexane fraction. The strong antioxidant capacity was obtained in flower crude extract and fractions, rhizome ethyl acetate and ethanol fraction, leaf crude extract, n-hexane, and ethyl acetate fractions.

4 DISCUSSION

The yield of crude extracts of leaf > rhizome > flower of *Etlingera elatior* had a higher yield than the fractions. However, the yield of fractions of each part is varied; the yield of rhizome fractions is ethanol > ethyl acetate > n-hexane, the yield of flower fractions is ethyl acetate > ethanol > n-hexane, and the yield of leaf fractions is ethyl acetate > n-hexane > ethanol. The crude leaf of *E.elatior* had the highest yield because containing many phytochemical compounds such as flavonoid (quercetin, isoquercitrin, quercitrin, and catechin), glycoside (quercetin 3-glucoside, quercetin 3-rhamnoside, quercetin 3-glucuronide, kaempferol 3-glucuronide), phenolic (5-O-caffeoylquinic acid methyl ester, 5-O-caffeoylquinic acid, 3-O-caffeoylquinic acid), terpenoids, saponins, tannins, carbohydrate, and essential oil (β-pinene, 1-dodecane, (E)-farnesene, and (E)-caryophyllene [15]. The reports answered t]hat the

ethyl acetate fraction yield in *E.elatior* leaf was the largest among other leaf fractions; the semipolar compounds are higher than polar and non-polar compounds. The major constituent of *E.elatior* leaf, besides phenolic and flavonoid, is also the essential oils. The rhizome of *E.elatior* contains diarylheptanoid, labdane diterpenoids, demethoxy-curcumin, rutin, quercetin, quercitrin [16], steroids (5α, 8α-epidioxyergosta-6,22-dien-3β-ol, stigmast-4-en-3-one, stigmast-4-en-6β-ol-3-one, stigmast-4-ene-3,6-dion,), 16-hydroxylabda-8(17),11,13-trien-16,15-olide, 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and also essential oil (1,1-dodecane-diol diacetate, (E)-β-farnesene, (E)-5-dodecane, β-pinene, and cyclododecane) [6]. The flower of *E.elatior* contains phenolic, flavonoid, anthocyanin, tannin, and essential oil (dodecanol, dodecanal, and α-pinene).

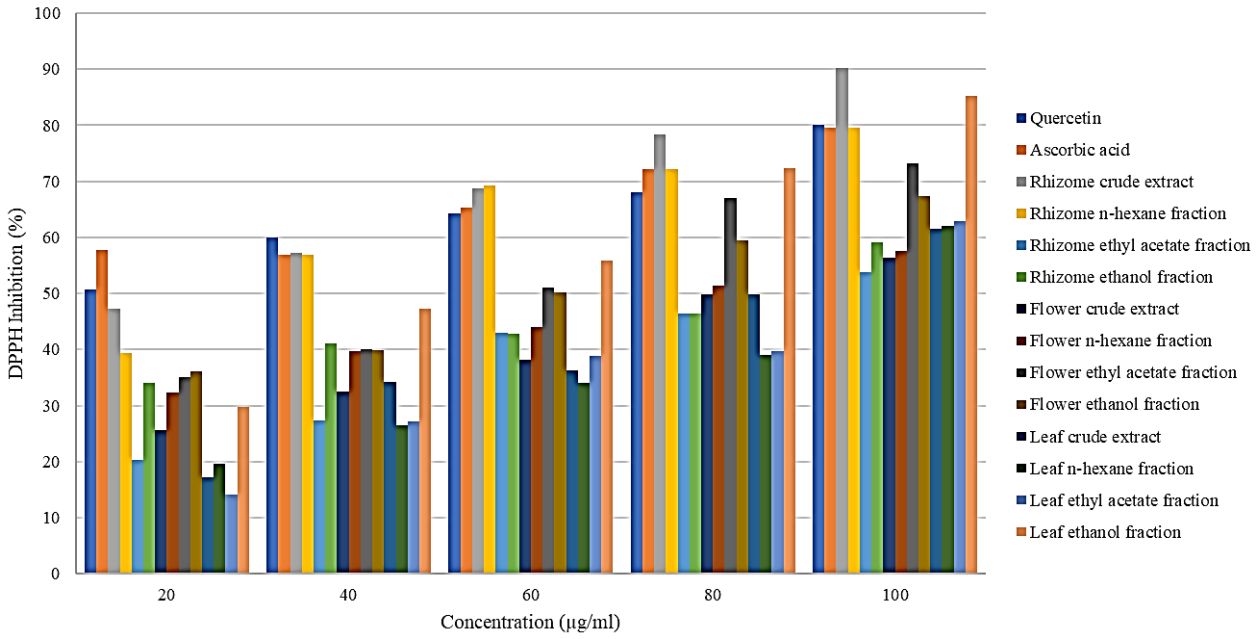

Figure 1. DPPH inhibition of *Etlingera elatior* crude extracts and fractions

Table 2. The IC_{50}, QEAC, and AEAC of *E.elatior* extracts and fractions

	Sample	IC_{50} (μg/mL)	QEAC (mg Q/100 g)	AEAC (mg AA/100g)
Rhizome	Crude extract	25.430±0.115^{a}	62.527±0.491^{a}	43.364±0.341^{a}
	N-Hexane fraction	31.330±0.057^{b}	50.751±0.162^{b}	35.196±0.113^{b}
	Ethyl acetate fraction	87.580±0.115^{i}	18.155±0.041^{i}	12.591±0.029^{i}
	Ethanol fraction	78.990±0.057^{g}	20.129±0.025^{g}	13.960±0.018^{g}
Flower	Crude extract	84.050±0.577^{h}	18.919±0.225^{h}	13.121±0.156^{h}
	N-Hexane fraction	76.010±0.577^{f}	20.921±0.158^{f}	14.509±0.191^{f}
	Ethyl acetate fraction	53.730±0.173^{d}	29.593±0.165^{d}	20.523±0.115^{d}
	Ethanol fraction	58.560±0.173^{e}	27.152±0.139^{e}	18.830±0.096^{e}
Leaf	Crude extract	79.590±0.115^{g}	19.977±0.051^{g}	13.855±0.035^{g}
	N-Hexane fraction	87.830±0.208^{i}	18.103±0.074^{i}	12.555±0.051^{i}
	Ethyl acetate fraction	83.953±0.260^{h}	18.939±0.102^{h}	13.135±0.071^{h}
	Ethanol fraction	47.763±0.233^{c}	33.291±0.282^{c}	23.088±0.196^{c}

Note: numbers in the same column followed by the different superscript letters (^{a,b,c}) are significantly different ($p<0.05$)

The total phenolic compound of *E.elatior* crude extracts and fractions are as follows: flower ethanol fraction>leaf n-hexane fraction>rhizome n-hexane fraction>flower crude extract>flower ethyl acetate fraction>rhizome ethanol fraction>flower n-hexane fraction>leaf ethyl acetate fraction>rhizome ethyl acetate fraction>leaf crude extract>leaf ethanol fraction>rhizome crude extract. The total phenolic

content of fractions is higher than crude extract in each part of *E.elatior* because the crude extract generally contains ballast compounds such as carbohydrates, resins, and chlorophyll. The previous studies report that the methanol extract of *E.elatior* inflorescences contains 361 mg GAE/100g [4], and the methanol extract of *E.elatior* contain 3550 and 7656 mg GAE/100g for fresh and dried leaf respectively [5,13].

The DPPH scavenging activity of *E.elatior* crude extracts and fractions expressed as IC_{50}(μg/ml), QEAC(mg Q/100g), and AEAC(mg AA/100g) value. The power of antioxidant capacity of *E.elatior* is as follows: rhizome crude extract>rhizome n-hexane fraction>leaf ethanol fraction>flower ethyl acetate fraction>flower ethanol fraction>flower n-hexane fraction>rhizome ethanol fraction>leaf crude extract>leaf ethyl acetate fraction>flower crude extract>rhizome ethyl acetate fraction> leaf n-hexane fraction. The studies before revealed that methanol leaf extract of *E.elatior* had very weak antioxidant capacity (IC_{50} = 232.90 μg/ml) [13], methanol rhizome extract of *E.elatior* also had very weak antioxidant capacity (IC_{50} = 586.38 μg/ml) [17], methanol and ethyl acetate extract of flower of *E.elatior* had very strong (IC_{50} = 21.14 μg/ml) and strong (IC_{50} = 68.24 μg/ml) antioxidant capacity respectively [18].

5 CONCLUSION

The conclusion of this study is that depending on the type of solvent, the types of antioxidant compounds extracted, and the antioxidant capacity also differ. Further research is needed to investigate the individual or major polyphenol groups and other bioactive compounds contained in each part of *E.elatior* and their contribution to health.

CONFLICT OF INTERESTS

Authors declare no conflict of interests

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