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Research article

Antioxidant Activity, Total Phenolic and Total Flavonoid Content of *Etlingera maingayi* (Baker) R.M.Sm.

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Abstract

Etlingera maingayi (Baker) R.M.Sm. is a rare plant species native to southern Thailand. It is infrequently utilized, with its primary use being for edible flowers. This study aimed to investigate and evaluate the antioxidant properties of the aerial parts of *E. maingayi*. Dried plant materials were extracted using ethanol, followed by solvent removal via rotary evaporation. Antioxidant activities were assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays, and the ferric reducing antioxidant power (FRAP) assay. The chemical constituents of the extract were quantified using the Folin–Ciocalteu method for total phenolic content and the aluminum chloride colorimetric method for total flavonoid content. The *E. maingayi* extract exhibited antioxidant activity with an IC_{50} value of 0.56 ± 0.01 mg/mL in the DPPH assay, compared with Trolox as a positive control (IC_{50}= 0.036 ± 0.003 mg/mL). In the ABTS assay, the extract showed an IC_{50} value of 4.53 ± 0.59 mg/mL, compared with Trolox (IC_{50}= 0.28 ± 0.06 mg/mL). Furthermore, the extract demonstrated ferric reducing antioxidant power (FRAP) at 150.54 ± 1.79 mg $FeSO_4$/g extract. The total phenolic and flavonoid contents were 83.76 ± 3.36 mg gallic acid equivalents (GAE)/g extract and 65.11 ± 6.84 mg catechin equivalents (CE)/g extract, respectively. These results indicate that the ethanolic extract of the aerial parts of *E. maingayi* possesses significant antioxidant activity and is rich in polyphenolic compounds. Therefore, this species may serve as a potential natural source of antioxidants for future applications.

Keywords: Antioxidant properties, Ethanolic extract, Polyphenol, Zingiberaceae

Introduction

Etlingera is a genus belonging to the Zingiberaceae family, primarily distributed in Southeast Asia, with approximately 150–200 species reported worldwide (Poulsen, 2006; Poulsen, 2012). Many species in this genus are used as vegetables, food ingredients, and in traditional medicine (Chongkraijak *et al*., 2013; Saudah *et al*., 2022; Shahid-Ud-Daula and Mohammad, 2019).

Several species of *Etlingera* have demonstrated antioxidant properties, which vary depending on the plant parts used and the solvent extraction methods applied (Mendez *et al*., 2023; Utami *et al*., 2024). For example, ethanolic extracts from the leaves of *E. elatior* (Jack) R.M.Sm., and *E. philippinensis* (Ridl.) R.M.Sm. exhibited strong antioxidant activity (Barbosa and Minguillan, 2021; Utami *et al*., 2024), while methanolic extracts of *E. coccinea* (Blume) S. Sakai & Nagam. also showed high antioxidant capacity (Shahid-Ud-Daula *et al*., 2015). Furthermore, the potent antioxidant properties of *E. elatior* leaf extract have led to its development into a skin-whitening cosmetic cream (Whangsomnuek *et al*., 2019). This study focuses on *E. maingayi* (Baker) R.M.Sm., a species found in Peninsular Malaysia and Thailand, specifically in the southern region of Thailand. To date, there have been no reports on the use of its leaves or rhizomes; only the flowers are known to be consumed as a vegetable in Thailand (Khaw, 2001; Saudah *et al*., 2022; Sirirugsa, 1998). A previous study from Malaysia reported that methanolic extracts of *E. maingayi* exhibited low antioxidant activity (Chan *et al*., 2007).

However, no studies have investigated the antioxidant activity of *E. maingayi* collected from Thailand. Since the production of secondary metabolites in plants is influenced by environmental conditions (Pant *et al*., 2021), this study aimed to investigate and evaluate the antioxidant properties of *E. maingayi* from southern Thailand.

Materials and Methods

1. Plant collection and extraction

Samples of *E. maingayi* were collected from Srinagarindra district, Phatthalung province, Thailand (25 March 2022, 7°30'28"N, 99°55'11"E) (**Figure 1**). The aerial part (leaf and pseudostem) of this plant was prepared by hot air oven and incubated at 60 ˚C for 48 hours. One hundred grams of dried plant material was ground into coarse powder. The dried plant powder was extracted in 5 replicates by ethanol (1:5 w/v) at room temperature for 24 hours and concentrated using a rotary evaporator. The crude extract was stored at -20 ˚C until use.

Figure 1 The morphological characters and habitat of *E. maingayi* in southern Thailand. A) Aerial part (leaves and pseudostem), and B) Rhizome and inflorescence

2. DPPH free radical scavenging assay

Antioxidant scavenging capacity of the *E. maingayi* was investigated by the DPPH (1,1-diphenyl-2-picryhydrazyl) method, which was a modified method from Alam *et al*. (2013). The crude extract of *E. maingayi* was dissolved in ethanol at various concentrations. The extract was mixed with 0.2 mM DPPH solution in methanol. Trolox was used as a positive control and treated under the same conditions as the samples. The reaction mixtures were incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm after incubation by using a microplate reader. The percentage of scavenging activity was calculated as follows: % Inhibition = $[(A_{(blank)}-A_{(sample)})/A_{(blank)}] \times 100$. The results were analyzed by half maximal inhibitory concentration (IC_{50} value) for the scavenging DPPH radical effect from the curve plotting between % inhibition and concentration. The DPPH assay was performed in triplicate (n=3).

3. ABTS radical scavenging assay

Free scavenging activity of $ABTS^{\bullet+}$ of *E. maingayi* extract was determined by modifying the ABTS radical cation decolorization method (Alam *et al*., 2013). The various concentration of plant extracts was mixed with 7 mM ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) in water and 2.45 mM potassium persulfate (1:1) and were stored in the dark at room temperature for 12–16 hours before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to obtain an absorbance of 2.2±0.05 at 734 nm. These plant extract solutions and Trolox (positive control) were combined with 950 µl of $ABTS^{\bullet+}$ solution and incubated for 30 min at room temperature. After 30 min incubation, the absorbance of the final product was measured at 734 nm by a microplate reader. The percentage of scavenging activity was calculated as follows: ABTS+ radical scavenging activity (%) = $[(A_{(blank)}-A_{(sample)})/A_{(blank)}] \times 100$. The results were analyzed by half maximal inhibitory concentration (IC_{50} value) for the scavenging ABTS radical effect from the curve plotting between % inhibition and concentration. The ABTS assay was implemented in triplicate (n=3).

4. Ferric ion reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) of *E. maingayi* extract was observed FRAP assay method as described by Benzie and Strain (1996). The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer, 10 mM TPTZ, and 20 mM ferric chloride in the proportions 10:1:1 at 37 °C. The reagent (150 µl) was mixed with 50 µl of the sample and incubated for 30 min at room temperature. These reactions were measured in absorbance at 593 nm. Trolox was used as a standard. Relative antioxidant activity (FRAP value) was obtained by comparing the absorbance change in the test mixture with a standard curve, and the results were expressed as µM Trolox equivalent per gram sample. This experiment was performed in triplicate (n=3).

5. Determination of total phenolic content

The total phenolic content was determined by modifying the Folin-Ciocalteau method (Jia *et al.*, 1999). The plant extract was mixed with 50% Folin–Ciocalteu reagent and incubated at room temperature for 10 min. After 10 min, Na_2CO_3 (7%) was added and incubated at 45 °C for 15 min; the absorbance was measured at 765 nm by a microplate reader. Gallic acid was used as a standard, and the content was expressed as mg of gallic acid equivalent per gram extract (mg GAE/g of extract).

6. Determination of total flavonoid content

The total flavonoid content was investigated by the aluminum chloride colorimetric method, using catechin as a standard (Jia *et al.*, 1999). The reaction mixture contained plant extract, 5% $NaNO_2$, and 10% $AlCl_3 \cdot 6H_2O$, and

was incubated for 20 min at room temperature. After 20 min, 1M NaOH was added, and the absorbance was measured at 532 nm. The result was expressed as mg of catechin equivalent per gram extract (mg CEE/g of extract).

Results and Discussion

The aerial part of *E. maingayi* from ethanolic extract exhibited the DPPH free radical scavenging capacity at an IC_{50} value of 0.56±0.01 mg/ml, while Trolox showed the IC_{50} of 0.036±0.003 mg/ml. For ABTS radical scavenging activity, the crude extract showed the IC_{50} value at 4.53±0.59 mg/ml compared with Trolox as a positive control (IC_{50} = 0.28±0.06 mg/ml) (**Table 1**). The FRAP assay revealed high antioxidant capacity, with a value of 150.54±1.79 mg $FeSO_4$/g extract (**Table 1**).

Table 1 Antioxidant activity of ethanolic extract from aerial part of *Etlingera maingayi*

Plant extract and chemical	Antioxidant activity		
	DPPH IC_{50} (mg/ml)	ABTS IC_{50} (mg/ml)	FRAP (mg $FeSO_4$/g extract)
E. maingayi extract	0.56 ± 0.01	4.53 ± 0.59	150.54 ± 1.79
Trolox	0.036 ± 0.003	0.28 ± 0.06	-

* Each value is expressed as mean ± SD (n=3)

The total phenolic and flavonoid contents of the *E. maingayi* ethanolic extract were 83.76 ± 3.36 mg GAE/g extract and 65.11 ± 6.84 mg CE/g extract, respectively (**Table 2**). In a previous study, methanolic extracts from the leaves of *E. maingayi* collected in Malaysia were reported to exhibit low antioxidant activity and a low total phenolic content (11.1 ± 0.93 mg GAE/g) (Chan *et al*., 2007). In contrast, our results, based on ethanolic extracts from the aerial parts, revealed a total phenolic content more than 7.5 times higher (83.76 ± 3.36 mg GAE/g) (**Table 2**). Environmental conditions are known to influence the production of secondary metabolites in plants (Pant *et al*., 2021). The tropical rainforest climate of Srinagarindra District, Phatthalung Province, Thailand, may play a key role in enhancing the biosynthesis of phenolic and flavonoid compounds in *E. maingayi*. Additionally, the choice of extraction solvent could significantly impact the yield of antioxidant compounds. Ethanol may be more effective than methanol in extracting certain antioxidant constituents. Strong antioxidant activities have been reported in ethanolic extracts of *E. fimbriobracteata* and *E. philippinensis* from the Philippines (Mendez *et al*., 2023), and *E. elatior* from North Luwu, Indonesia (Utami *et al*., 2024).

The ethanolic extract from the aerial parts of *E. maingayi* appears to be highly effective for isolating natural antioxidant compounds. The presence of compounds with a range of polarities in the extract likely contributes to its strong antioxidant potential, which is associated with its high phenolic and flavonoid content. Phenolic compounds are well-known for their antioxidant activity, primarily due to their hydroxyl groups, which allow them to scavenge free radicals and directly contribute to antioxidant action (Hall and Cuppett, 1997; Duh *et al*., 1999). The antioxidant properties are closely linked to both the concentration and structure of phenolic and flavonoid compounds (Barhé and Tchonya, 2016). These compounds can donate protons to free radicals, stabilizing them and preventing oxidative damage (Zeb, 2020).

In summary, the findings of this study suggest that the ethanolic extract from the aerial parts of *E. maingayi* contains a high concentration of polyphenols and exhibits strong antioxidant potential. Therefore, *E. maingayi* may serve as a promising natural source of antioxidants for future applications. However, this study did not identify the individual phenolic compounds responsible for the observed activity. Future research should focus on the isolation and characterization of specific antioxidant constituents present in the ethanolic extract to better understand their individual contributions and potential uses.

Table 2 Total phenolics and flavonoid contents from ethanolic extract of *Etlingera maingayi*

Plant extract	Total phenolic contents (mg gallic acid equivalent (GAE)/g of extract)	Total flavonoid contents (mg catechin equivalent (CE)/g of extract)
E. maingayi extract	83.76 ± 3.36	65.11 ± 6.84

* Each value was expressed in teams of mean ± SD (n=3)

Conclusion

The ethanolic extract of *Etlingera maingayi* demonstrated strong antioxidant potential, suggesting that this species may serve as a promising natural source of antioxidant compounds. The aerial parts of the plant were found to be suitable for extraction using ethanol, highlighting the effectiveness of this method for obtaining bioactive constituents. However, further studies are necessary to isolate and characterize the individual bioactive compounds responsible for the antioxidant activity, to better understand their mechanisms and potential applications.

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