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ORIGINAL ARTICLE **OPEN ACCESS**

Antioxidant activity and phenolic content of the leaves and rhizomes of *Etlingera philippinensis* (Zingiberaceae)

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ABSTRACT
In order to broaden scientific investigation of Philippine endemic Zingiberaceae plants, the total antioxidant activity and total phenolic content of the crude methanol leaf and rhizome extracts of Etlingera philippinensis were determined for the first time. Phosphomolybdenum assay and Folin-Ciocalteu method were employed in the study. Total antioxidant activity, expressed as milligram ascorbic acid equivalents per gram sample (mg AAE/g sample), of the leaf extracts (0.79 mg AAE/g sample) had significantly higher values than the rhizome (0.55 mg AAE/g sample). A significantly higher total phenolic content, expressed as mg gallic acid equivalents per gram sample (mg GAE/ g sample), was also observed in the leaves (0.55 mg GAE/g sample) of E. philippinensis than the rhizomes (0.35 mg GAE/g sample). Based on correlation analysis, a significant positive correlation was observed between the total antioxidant activity and total phenolic content (r=0.918, p<0.001). Results indicate that phenolic compounds have profound contribution to the antioxidant activities of E. philippinensis extracts. Interestingly, results suggest that methanol extract of the leaves and rhizomes of E. philippinensis is a potential natural source of antioxidant compounds.
Keywords: *Etlingera philippinensis*, antioxidant activity, phenolic content

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INTRODUCTION

In recent years, there has been an increasing interest in natural plant-derived products as sources of active compounds with antioxidant properties because of their low toxicities and strong bioactivities [17]. Plants have been recognized as potential source of natural antioxidants [18]. For plants' own survival, they produce antioxidants such as carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, and tocotrienols. Widely used antioxidants include ascorbic acid, beta-carotene, and alpha tocopherols [15].

Antioxidants are compounds that are able to scavenge free radicals or prevent their generation [4]. Antioxidants have the potential for lowering risk for cancer, heart disease and hypertension [20]. Aside from its role as a health benefactor, antioxidants are also added in food to prevent early food deterioration caused by free radicals formed during food's exposure to environmental factors such as to air, light and temperature [10].

The genus *Etlingera,* under the Zingiberaceae family, are tall forest plants with larger species that grows up to six (6) meters in height [12]. *Etlingera* species have bracts and flowers which are of varying shades of pink and red colors which make them very attractive [14]. Some of *Etlingera* species have traditional and commercial uses [5] as foods, condiments, medicines, and as ornamental plants [17]. The Philippine endemic *E. philippinensis* has been shown to contain important secondary metabolites which can be associated to the antioxidant activity of the plant [2]. Moreover, the ethanol and water extracts of *E. philippinensis* leaves and rhizomes were found to possess 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [3].

Past studies on antioxidant activities were mainly confined to the rhizomes. Although some ginger leaves have been used as food flavoring, little or few research has been done on its antioxidant activity [8, 11, 21]. In the present study, both the leaves and rhizomes were studied for its total antioxidant activity and phenolic content. To the best of our knowledge, there are no scientific reports on the total phenolic

content and the antioxidant activity determination of the methanol extracts of the leaves and rhizomes of *E. philippinensis* using phosphomolybdenum assay. Hence, this study was conducted.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used include absolute methanol (99.8% assay), standard gallic acid, dibasic phosphate, monobasic sodium phosphate, potassium ferrocyanide, trichloroacetic acid, ferric chloride, ferrous sulfate, Folin-ciocalteu reagent, sodium carbonate, ammonium molybdate, and sulfuric acid.

Plant materials

Healthy and mature rhizomes and leaves of *E. philippinensis* were collected from Kibawe, Bukidnon, Philippines (7°33'33.8976"N, 24°55'58.7964"E). The collected samples were placed in clean net bags and were washed thoroughly. The plant sample was identified and authenticated by Dr. Florfe M. Acma of the Center of Biodiversity Research and Extension in Mindanao, Central Mindanao University, University Town, Musuan, Bukidnon.

The plant leaf and rhizome samples were oven-dried at 40 °C until the loss of drying was less than 10%, powdered using a blender, and stored inside airtight plastic container prior its extraction.

Preparation of plant extracts

Methanol extract preparation. Solvent extraction was performed using the method of Wijekoon, Bhat, and Karim [19] with slight modifications such as the amount of sample, solvent and extraction time. Accurately weighed oven-dried sample powder of leaves and rhizomes, respectively, were mixed with 450 mL of absolute methanol and was extracted using magnetic stirrer and hotplate at 1150 rpm for three (3) hours at room temperature. Extracts were filtered using Whatman No. 1 filter paper. The residues that remained in the filter paper were subjected for re-extraction for two more times by placing the residue again to same flask following the same procedure. Residues were re-extracted with 300mL absolute methanol on second extraction and with 250 mL absolute methanol on the third extraction. The filtrates collected after three consecutive extractions were pooled. Filtrates were stored in 1000-mL Erlenmeyer flasks covered with aluminum foil to prevent exposure to light. Solvent removal was done using rotary evaporator at 40°C. The concentrated extracts of the leaves and rhizomes were stored in screw-capped vials covered with aluminum foil at -20°C

Determination of the Total Antioxidant Activity

A 300 mg/L solution of ascorbic acid was prepared by dissolving 0.0150 g of the standard gallic acid in 50 mL absolute methanol. Various concentrations (0, 10, 70, 100, 140, 170, and 200 mg/L) were prepared from the stock solution of ascorbic acid as working standards for the calibration curve. Stock sample solutions (1000 mg/L) of the methanol extracts from the leaves and rhizomes were prepared separately by dissolving 0.1000 g of the extracts in absolute methanol and diluted to 100 mL. In this method, 500 mg/L of test solution was used in the succeeding steps.

The total antioxidant activity of the extracts obtained from the leaves and rhizomes of *E. philippinensis* was determined using the method previously described by Prieto, Pineda, and Aguilar [16] with slight modifications. In an eppendorf tube containing 200 µL of 500 mg/L sample solution, 600 µL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The reaction mixture was incubated at 95°C for 90 min, allowed to cool at room temperature, and centrifuged at 11000 rpm for three (3) minutes. Total antioxidant activity, expressed as milligram ascorbic acid equivalent per gram sample (mg AAE/g sample), was calculated using the equation of the line obtained from the calibration curve using equation 1.

$$\text{Total Antioxidant activity (mg AAE/g sample)} = A/B \quad (1)$$

where A and B are the ascorbic acid concentration in the test solution determined from the calibration curve, mg AAE/L, and concentration of the test solution, g/L, respectively.

Determination of the Total Phenolic Content

A 300 mg/L solution of gallic acid was prepared by dissolving 0.0150 g of the standard gallic acid in 50 mL absolute methanol. Various concentrations (0, 30, 45, 60, 75, 90, and 120 mg/L) were prepared from the stock solution of gallic acid as working standards for the calibration curve. A stock sample solution (1000 mg/L) of the methanol extracts from the leaves and rhizomes were prepared by dissolving 0.1000 g of the extracts with 100 mL absolute methanol. A 500 mg/L of stock solution was used in the succeeding analysis.

The total phenolic content of the extracts obtained from the leaves and rhizomes of *E. philippinensis* was determined using the method previously described by Ainsworth and Gillespie [1] with slight

modifications. In an eppendorf tube containing 200µL of 500 mg/L sample solution, 200 µL of 10% Folin-Ciocalteu reagent and 800µL 10% sodium carbonate were added. The reaction mixture was then allowed to stand for two (2) hours prior absorbance measurement at 750 nm. After incubation at room temperature, the reaction mixture in the Eppendorf tube was centrifuged for two (2) minutes and were transferred to microplates. Similar procedure was done on the working standards for the calibration curve and the blank (absolute methanol). The absorbance readings of these mixtures were then determined at 750 nm using a microplate reader.

Total phenolic content, expressed as milligram gallic acid equivalent per gram sample (mg GAE/g sample) was calculated using equation 2.

Total Antioxidant activity (mg GAE/g sample) = A/B (2)

where A and B are gallic acid concentration in the test solution determined from the calibration curve, mg GAE/L, and concentration of the test solution, g/L, respectively.

RESULTS AND DISCUSSIONS

Total Antioxidant Activity

The total antioxidant activity of the methanol extracts of the leaves and rhizomes of *E. philippinensis* was determined employing the Phosphomolybdenum method using ascorbic acid as the standard. Table 1 and Figure 1 present the total antioxidant activity expressed in mg AAE/g sample.

Table 1. Mean total antioxidant activity of the methanol extracts of the leaves and rhizomes of *E. philippinensis*

Plant Part	Mean Total Antioxidant Activity, mg AAE/ g sample (%RSD)
Leaves	0.79 (4.13)
Rhizomes	0.55 (8.75)

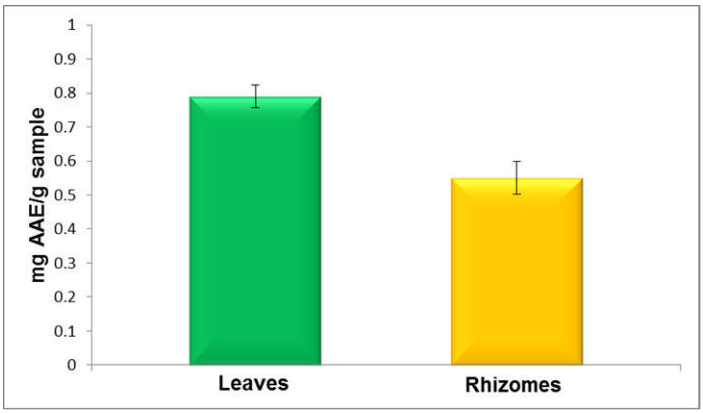

Figure 1. Graphical presentation of the total antioxidant activity of the methanol extracts of the leaves and rhizomes of *E. philippinensis*. Error bars are standard deviation (n=5).

As depicted in Figure 1, the level of total antioxidant activity of the leaves (0.79 mg AAE/g sample) is higher than in rhizomes (0.55 mg AAE/g sample). Results of t-Test revealed that the total antioxidant activity of the methanol extracts of the leaves and rhizomes have significant difference. Hence, total antioxidant activity of the leaves is significantly higher ($p<0.001$) than the rhizomes of *E. philippinensis*. Previous study revealed that methanol extracts of *E. coccinea* showed the highest total antioxidant activity in leaves (254.25 mg AE/g DW), followed by stems (116 mg AE/g DW), and rhizomes (107.25 mg AE/g DW) [17].

The reducing properties are generally associated with the presence of reductones [6]. Antioxidant action of plant extracts is based on breaking of the free radical chain by donating a hydrogen atom [7]. The total antioxidant activity of plant extracts might be due to the presence of polyphenols which acts in a similar fashion as reductones by donating electrons and reacting with free radicals converting them to a more stable product and subsequently terminating free radical chain reaction [7].

Total Phenolic Content

The level of phenolic compounds in the methanol extracts of the leaves and rhizomes of *E. philippinensis* are presented in Table 2 and Figure 2. Total phenolic content, expressed as mg GAE/ g sample, of the

leaves and rhizomes of *E. philippinensis* are 0.55 and 0.35, respectively. Total phenolic content of the leaves was higher than the rhizomes.

Table 2. Mean total phenolic content in the methanol extracts of leaf and rhizomes of *E. philippinensis*

Plant Part	Mean Total Phenolic Content, mg GAE/ g sample (%RSD)
Leaves	0.55 (12.70)
Rhizomes	0.35 (10.51)

Results of t-Test revealed significant difference on the total phenolic content between the methanol extracts of the leaves and rhizomes of *E. philippinensis*. The total phenolic content of the leaves of *E. philippinensis* was significantly higher ($p<0.001$) than in rhizomes. This was similarly observed in other reported studies. For instance, leaves of *E. sayapensis* had the highest total phenolic content as compared to other plant parts [13]. Generally, leaves of wild and cultivated *Etlingera* species contain the most antioxidants by having the highest total phenolic content [4]. The outstanding total phenolic content of the leaves of both *E. maingayi* and *E. elatior* were seven to eight times higher than those of rhizomes [5]. In addition, the leaves *of E. coccinea* had significantly ($p<0.05$) higher total phenolic content than those of stems and rhizomes [17].

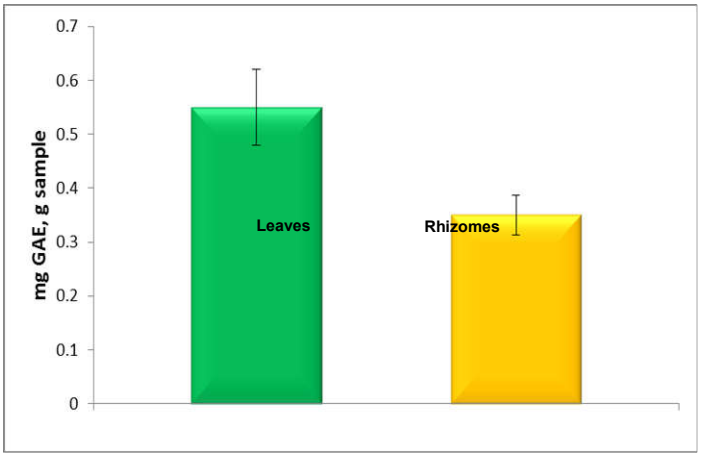

Figure 2. Graphical presentation of the total phenolic content of the methanol extracts of the leaves and rhizomes of *E. philippinensis*. Error bars are standard deviation (n=5).

Phytochemical screening of the leaves of *E. philippinensis* revealed the presence of flavonoids and higher DPPH radical scavenging activity in leaves than in rhizomes [3]. Metabolomics study of the leaves of *E. philippinensis* revealed the presence of chlorogenic acid [3]. Herrmann [9] reported that much greater concentrations of flavones and flavonols in leaves are observed for all types of plants which are exposed to sunlight. This implies that the formation of these compounds depends on light conditions. Only trace amounts can be found in unexposed parts such as the rhizomes [9]. High levels of these secondary metabolites accumulate in the vacuoles of epidermal cells and protect the underlying tissues by absorbing ultraviolet wavelengths [9]. These may explain why leaves of *E. philippinensis* had significantly higher phenolic content than the rhizomes.

Correlation between Antioxidant Activity and Total Phenolic Content

Antioxidants include a wide range of polyphenols, reducing agents, and nucleophiles that vary in their solubility and localization, redox potential, specificity and mechanism of action [17]. In this study, the contribution of the compounds in the methanol extracts of *E. philippinensis* extracts to the antioxidant activity was determined by Pearson's correlation coefficient. Correlation analysis results are summarized in Table 3.

Table 3. Pearson's correlation coefficients between total phenolic content and total antioxidant activity

	Total antioxidant activity	Total phenolic content
Total antioxidant activity	1	0.918**
Total Phenolic content	0.918**	1

**Correlation is significant at 0.01 level (2-tailed)

A significant positive correlation was observed between the total antioxidant activity and total phenolic content ($r=0.918$, $p<0.001$). Results indicated that phenolic compounds significantly contribute to the antioxidant activities of *E. philippinensis.* Shahid-Ud-Daula *et al*. [17] reported the contribution of polyphenolic compounds in the methanol extracts of *E. coccinea* to the total antioxidant activity and significant correlation was observed between the total antioxidant activity and total phenolic content.

CONCLUSION

The total antioxidant activity and total phenolic content of the leaf extracts were significantly higher than the rhizome methanolic extracts. Significant positive correlation was also observed between the total antioxidant activity and total phenolic content. Results of this study suggest that methanol extracts of *E. philippinensis* could be considered as a potential natural source of antioxidants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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