

PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *Etlingera dostseiana* Naive, Demayo & Alejandro, 2020 (Zingiberaceae)

Noe P. Mendez^{1*}, Florfe M. Acma^{1,2}, Angie Rose A. Villafranca-Tuba³,
Rainer A. Mendez⁴

¹Department of Biology, College of Arts and Sciences, Central Mindanao University,
University Town, Maramag, 8714 Bukidnon, Philippines

²Center for Biodiversity Research and Extension in Mindanao (CEBREM),
Central Mindanao University, University Town, Maramag, 8714 Bukidnon, Philippines

³Natural Science Research Center, Central Mindanao University, University Town,
Maramag, 8714 Bukidnon, Philippines

⁴Soil and Plant Analysis Laboratory, College of Agriculture, Central Mindanao University,
University Town, Maramag, 8714 Bukidnon, Philippines

*Corresponding author; E-mail: npolomendez@gmail.com

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ABSTRACT. The ginger species (Zingiberaceae family) have attract a lot of scientific attention because of their perceived beneficial effects on health. Despite this, for the numerous species of the family, scientific reports are quite scarce and warrant further analyses. The most of until now analyzed ginger species are allochthonous in the Philippines. Thus, this study was carried out to determine the total phenolic content and antioxidant activity of the ethanolic extracts of the leaves and rhizomes of *Etlingera dostseiana* Naive, Demayo & Alejandro, a Philippine endemic ginger species. Data recorded for total phenolic content (mg GAE/g dried sample) revealed that the leaves (15.44 ± 0.80) have a greater amount of phenolics than the rhizomes (0.59 ± 0.03). The total antioxidant activity (mg AAE/g dried sample) was also observed to be higher in leaves (14.24 ± 0.25) than in the rhizomes (0.91 ± 0.04), as well as the reducing power (mg GRPE/g dried sample) – 10.33 ± 1.13 in leaves versus 0.21 ± 0.06 in rhizomes. Based on the correlation analysis, a perfect positive linear relationship was observed among the total phenolic content, total antioxidant activity, and reducing power ($r=1$, $p<0.001$). These imply that the high contents of phenolic compounds contribute to the antioxidant activity of extracts of *E. dostseiana*. It can be regarded that *E. dostseiana* could be a candidate as a natural plant source of antioxidant compounds.

Keywords: *Etlingera dostseiana*, Philippine endemic plant, total phenolic content, total antioxidant activity, reducing power.

INTRODUCTION

The phenolic compounds are good electron donors because their hydroxyl group can directly contribute to antioxidant actions (BENDARY *et al.*, 2013). The antioxidants delay or

inhibit oxidative damage to a target molecule (YAMAGISHI and MATSUI, 2011), and their main characteristic is the ability to neutralize free radicals (MAHDI-POUR *et al.*, 2012). In the Philippines, recent studies on the determination of total phenolic content and antioxidant activity were done on some species of Zingiberaceae (MABINI and BARBOSA, 2018; REDONDO and BARBOSA, 2018; BARBOSA and NUEVA, 2019) to validate the claims of the local people in Mindanao on their ethnomedicinal uses (MENDEZ *et al.*, 2017; ACMA and MENDEZ, 2018a,b; ACMA *et al.*, 2020).

The Zingiberaceae plant family is comprised of over 1,500 species distributed in at least 53 genera (KRESS *et al.*, 2002; LAMB *et al.*, 2013; CHRISTENHUSZ and BYNG, 2016). Nineteen genera and 142 species of Zingiberaceae have been recorded so far in the Philippines. Among these, *Etilingera* Giseke is the second largest genus with 17 species (PELSER *et al.*, 2011 onwards). Members of this genus are distinguished from the others by having a tube formed by the base of the filament, and staminode-derived labellum above the point of insertion of the corolla lobes (POULSEN, 2003).

Etilingera dostseiana Naive, Demayo & Alejandro was recently collected and described by NAIVE *et al.* (2020) at Mt. Kiamo, locality Bukidnon, at elevations from 1,000 to 1,400 m asl. The species is unique among the other *Etilingera* species by the presence of raised stilt roots, fusiform inflorescence, peduncle which is not embedded in the ground, and papery sterile and fertile bracts (NAIVE *et al.*, 2020). This species was also collected and reported in 2018 at Mt. Malambo, Marilog District, Davao City (ACMA *et al.*, 2020). It grew near the populations of *Mitrastemon yamamotoi* Makino and *Plagiostachys albiflora* Ridl., the two species also newly recorded in the Philippines (AMOROSO *et al.*, 2018; ACMA *et al.*, 2019). *Etilingera dostseiana* is known as pinoon by the local people in Marilog District. Its fruits are edible. The boiled leaves and pseudostems are used traditionally to cure cough and fever (Jason Batawan, pers. comm. 2018). To validate these claims, this study was carried out to determine the total phenolic content and antioxidant activity of the ethanolic extracts of leaves and rhizomes of *E. dostseiana*. Since this plant has been used in medicine by the local people in Marilog District, it is assumed that through screening of its bioactivity, positive results would be acquired and could be used for further scientific investigations.

MATERIALS AND METHODS

Entry Protocol

A letter was submitted to the Barangay Captain of Datu Salumay in Marilog District asking permission to allow the first author to conduct botanical fieldwork and collect samples at Mt. Malambo, using the gratuitous permit with WGP number XI2017-03 issued by the Department of Environment and Natural Resources (DENR) Region XI2017 to Dr. Victor B. Amoroso on March 2021. A Prior Informed Consent (PIC) was also personally handed and signed by the Officer-in-Charge of the barangay before the collecting of plants.

Place and Duration of the Study

This study was conducted from September 2021 to January 2022. Samples and voucher specimens were collected from Mt. Malambo, Marilog District, Davao City (07°29'26.87" N, 125°15'22.23" E). Determination of total phenolic content (TPC), total antioxidant activity (TAA), and reducing power (RP) of *E. dostseiana* was carried out at the Natural Science Laboratory of Natural Science Research Center (NSRC), Central Mindanao

University, University Town, Musuan, Bukidnon after necessary permits obtained from concerned authorities.

Sample Collection, Preparation, and Extraction

The leaves and rhizomes of *E. dostseiana* were collected and placed separately inside plastic cellophane bags with wet tissue paper to prevent dehydration. These samples were brought to Central Mindanao University for further processing. Leaves and rhizomes were washed, and earthy matter were removed before air-drying. Dried samples were powdered and stored until used.



Figure 1. *E. dostseiana* leaves (a) and rhizomes (b) (Photographs: N.P. Mendez, 2021).

Extracts were prepared following the method of PADDA and PICHA (2008) with some modifications. The dried leaf and rhizome powders were extracted with absolute ethanol in a ratio of 1 g: 25.0 mL, at room temperature (25°C). The mixtures were shaken for 1 hour in an orbital shaker at 300 rpm and centrifuged at 5,000 rpm for 5 minutes. The resulting supernatants were collected in separate 15 mL conical tubes. Extracts were stored at 2–8°C and used in the succeeding analyses. The ethanolic leaf and rhizome extracts of *E. dostseiana* were subjected to the total phenolic content (TPC), total antioxidant activity (TAA), and reducing power activity (RP) determination.

Total phenolic content

The TPC of the extracts was determined using the method described by AINSWORTH and GILLESPIE (2007) with some modifications. Briefly, 200 µL of the extracts and 200 µL of 10% Folin-Ciocalteu reagent were transferred in a 2-mL centrifuge tube. The reaction mixture was set aside for five minutes and added with 800 µL of 10% sodium carbonate. The mixture was set aside at room temperature for 30 minutes and centrifuged at 11,000 rpm for three minutes, and 200 µL of the resulting solution was transferred into the assigned microplate wells. The absorbance was determined at 750 nm using a microplate reader (Molecular Devices Spectramax® 250). Gallic acid (100 ppm) was used as the standard solution with a concentration range of 0 to 200 ppm. The results were derived from a calibration curve ($y = 0.0546x + 0.0658$, $R^2 = 0.9946$) of gallic acid (0–200 mg/mL). The TPC was determined and expressed as milligram gallic acid equivalent per gram sample (mg GAE/g sample) by interpolating sample absorbance against the standard calibration curve using the formula below:

$$\text{Total Phenolic Content: } \left(\frac{\text{mg GAE}}{\text{g dried sample}} = \text{AB} \right) \quad (1)$$

where: A = gallic acid concentration of the sample solution determined from the calibration curve (mg GAE/L) and B = the concentration of test solution (g/L, gram dried sample per L solution).

Total antioxidant activity

The TAA was determined using the phosphomolybdenum method described by PRIETO *et al.* (1999) with slight modifications. Briefly, 50 μ L of extracts were placed in centrifuge tubes and diluted with 200 μ L (1 : 1 ethanol : water). The solution was then added with 600 μ L of reagent solution (prepared by mixing equal amounts of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95°C for 90 minutes. The samples were allowed to cool at room temperature (25°C) and centrifuged at 11,000 rpm for three minutes. The absorbance of the supernatant was measured at 695 nm against a blank using a microplate reader (Molecular Devices Spectramax® 250). A calibration curve was also prepared using 0 to 300 ppm ascorbic acid as standard. The results for total antioxidant activity were calculated from a calibration graph which was linear over the calibration range with an R^2 value of 0.9963 ($y = 0.0149x + 0.0465$) of L-ascorbic acid (0–100 mg/mL). TAA was determined by interpolating sample absorbance against the standard curve. The TAA was calculated using the equation as follows:

$$\text{Total Antioxidant Activity: } \left(\frac{\text{mg}}{\text{g dried sample}} \right) = \frac{A}{B} \quad (2)$$

where: A = ascorbic acid concentration of the solution determined from the calibration curve (mg AAE/L); B = concentration of the test solution (g/L, gram dried sample per L solution).

Reducing power activity

The RP was determined by adapting the method described by MURUGAN and IYER (2012) with some modifications. In a centrifuge tube containing 1 mL of extracts, 200 μ L of 0.2 M phosphate buffer (pH 6.6) and 200 μ L of 1% (w/v) solution of potassium ferricyanide were added. The mixture was incubated at 50°C for 30 minutes. After cooling to room temperature (25°C), 200 μ L of 1% (w/v) trichloroacetic acid was added. The mixture was centrifuged for 3 minutes at 11,000 rpm. An aliquot of 200 μ L of the supernatant was transferred to a 96-well plate and 20 μ L of 1% (w/v) solution of ferric chloride was added. The absorbance was measured at 620 nm using a microplate spectrophotometer (Molecular Devices Spectramax® 250). Standard gallic acid with concentrations ranging from 0 to 300 ppm was used to establish a calibration curve. Sample concentration was determined by interpolating sample absorbance against the standard curve. The results were derived from a calibration curve ($y = 0.0505x + 0.5037$, $R^2 = 0.9975$) of gallic acid (0–1000 mg/mL). The reducing power, expressed as milligram gallic acid reducing power equivalent per gram sample (mg GRPE/g sample) was calculated as follows:

$$\text{Reducing power activity: } \left(\frac{\text{mg}}{\text{g dried sample}} \right) = \frac{A}{B} \quad (3)$$

where: A = gallic acid concentration of the test solution determined from the calibration curve (mg GRPE/L); B = concentration of the test solution (g/L, gram dried sample per L solution)

Statistical analysis

The TPC, TAA, and RP analyses were done in triplicates. The determination for each parameter was carried out in three trials per replicate. The data gathered among the TPC,

TAA, and RP of *E. dostseiana* were correlated using Pearson's correlation at 0.001 level of significance.

RESULTS AND DISCUSSION

Total phenolic content

The TPC in ethanolic extracts of leaves and rhizomes was measured by the Folin-Ciocalteu reagent in each extract. The ethanolic extracts of *E. dostseiana* revealed that the leaves (15.44 ± 0.80 mg GAE/g dried sample) exhibited higher phenolic content than the rhizomes (0.59 ± 0.03 mg GAE/g dried sample) (Table 1).

Table 1. Mean total phenolic content extraction yield of leaves and rhizomes of *E. dostseiana*.

Plant parts	Total phenolic content (mg GAE/g dried sample)
Leaves	15.44 ± 0.80
Rhizomes	0.59 ± 0.03

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity (SOBRATTEE *et al.*, 2005). The total phenolic compounds also play an effective role in stabilizing lipid peroxidation (YEN *et al.*, 1993), and contribute to antioxidant activity due to the arrangement of functional groups (hydroxyl) about its nuclear structure for hydrogen donation in order to stabilize radical molecules (SOBRATTEE *et al.*, 2008; ALAM *et al.*, 2018). The quantification of TPC in ethanolic extracts of leaves and rhizomes of *E. dostseiana* was determined by employing the Folin-Ciocalteu method, which is convenient, simple, and reproducible (CIRILLO and LEMMA, 2012; DANCIU *et al.*, 2015).

CHAN *et al.* (2008) reported that out of the 26 studied ginger species, the *Etlingera* species had the highest phenolic content and radical activity compared to other Zingiberaceae species. The values of phenolic content in this study varied slightly compared to the studies on other ginger species in the Philippines, *viz.*, MABINI and BARBOSA (2018), REDONDO and BARBOSA (2018), and BARBOSA and NUEVA (2019). The mean value of TPC in leaves (15.44 ± 0.80 mg GAE/g sample) in our study is relatively higher than earlier determined in the studies of MABINI and BARBOSA (2018) with 0.55 mg GAE/g sample on methanolic extracts of *E. philippinensis* (Ridl.) R.M.Sm., REDONDO and BARBOSA (2018) with 1.95 mg GAE/g sample on ethanolic extracts of *Hedychium coronarium* Koenig, and BARBOSA and NUEVA (2019) with 1.67 mg GAE/g sample on methanolic extracts of *Hornstedtia conoidea* Ridl.

On the other hand, the mean value of TPC in rhizomes (0.59 ± 0.03 mg GAE/g sample) of *E. dostseiana* is higher than those registered in the study of MABINI and BARBOSA (2018) with 0.35 mg GAE/g sample on *E. philippinensis*, but lower than in the studies of BARBOSA and NUEVA (2019) with 1.28 mg GAE/g sample on *H. conoidea* and REDONDO, and BARBOSA (2018) with 1.48 mg GAE/g sample on *H. coronarium*. These slight variations in the mean values of TPC might be due to the presence of different amounts of sugars, carotenoids, ascorbic acid, age of plants, geographical variation, or methods of extraction, which may alter the amount of phenolics (BURRI *et al.*, 2017).

Total antioxidant activity and reducing power activity

The TAA of the ethanolic extracts of *E. dostseiana* revealed that the mean value for the leaves (14.24 ± 0.25 mg AAE/g dried sample) is higher compared to the rhizomes (0.91 ± 0.04 mg AAE/g dried sample) (Table 2). Meanwhile, the mean value for the RP of the

ethanolic extracts revealed that the leaves of *E. dostseiana* (10.33 ± 1.13 mg GRPE/g dried sample) is higher than in the rhizomes (0.21 ± 0.06 mg GRPE/g dried sample) (Table 2).

Table 2. Mean total antioxidant activity and reducing power extraction yield of leaves and rhizomes of *E. dostseiana*.

Antioxidant activity		
Plant parts	Total antioxidant activity (mg AAE/g dried sample)	Reducing power (mg GRPE/g dried sample)
Leaves	14.24 ± 0.25	10.33 ± 1.13
Rhizomes	0.91 ± 0.04	0.21 ± 0.06

The mean value of TAA of the leaves extract (14.24 ± 0.25 mg TAA/g sample) of *E. dostseiana* is higher compared to the studies of MABINI and BARBOSA (2018) with 0.79 mg TAA/g sample of *E. philippinensis*, REDONDO and BARBOSA (2018) with 2.94 mg TAA/g sample of *H. coronarium*, and BARBOSA and NUEVA (2019) with 4.67 mg TAA/g sample of *H. conoidea*. The mean value of TAA of the rhizomes extract (0.91 ± 0.04 mg TAA/g sample), it is also higher than registered in the study of MABINI and BARBOSA (2018) with 0.55 mg TAA/g sample of *E. philippinensis*, but lower than in the studies of REDONDO and BARBOSA (2018) with 1.02 mg TAA/g sample of *H. coronarium*, and BARBOSA and NUEVA (2019) with 2.03 mg TAA/g sample of *H. conoidea*.

The last several decades have seen increased research attention to potential phytochemicals from plants for therapeutic uses because many of them have been demonstrated to have antioxidant activities (KAIRUPAN *et al.*, 2019). The total antioxidant activity of plant extracts might be due to the presence of polyphenols, which acts as reductants by donating electrons and reacting with the free radicals converting them to a more stable product and subsequently terminating the free radical chain reaction (GORDON, 1990).

Correlation analysis

The contribution of the compounds in the ethanol extracts of *E. dostseiana* extracts to the antioxidant activity was determined by Pearson's correlation coefficient. The results of the correlation analysis are summarized below (Table 3).

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

Assays	Correlation		
	TPC	TAA	RP
Total phenolic content	1	1**	1**
Total antioxidant activity	1**	1	1**
Reducing power	1**	1**	1

**Correlation is significant at 0.001 level.

A perfect positive linear relationship was observed among the TPC, TAA, and RP ($r=1$, $p<0.001$). Results obviously indicate that phenolic compounds significantly contribute to the antioxidant activities of *E. dostseiana*.

CONCLUSIONS

This paper represents the first laboratory study of the new Philippine endemic plant species *Etilingera dostseiana* after its discovery in 2020. The study revealed that it contained phenolic compounds of good quantity. The recorded total phenolic content, total antioxidant activity, and reducing power of *E. dostseiana* were significantly higher in leaves than in the rhizomes extracts. Based on the correlation analysis, a perfect positive linear relationship was observed among the total phenolic content, total antioxidant activity and reducing power activity ($r=1$, $p<0.001$). These imply that the high contents of phenolic compounds also mean high potential antioxidant activity of extracts of *E. dostseiana* of which leaves are more recommended.

As this is the first report of the total phenolic content and antioxidant activity of *E. dostseiana*, this calls for thorough phytochemical analyses to be done to identify the active phenolic and antioxidant components of this Philippine endemic species.

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