Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [10] September 2018 : 97-105
©2018 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Antioxidant Activity and Phenolic Content of the Ethanol Extracts of *Hedychium coronarium* (Zingiberaceae) in Mindanao, Philippines

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ABSTRACT
There is continued search for natural sources of antioxidants from plants. Hedychium coronarium, belonging to the Zingiberaceae family, were collected in Bukidnon, Mindanao, Philippines and screened for antioxidant activity and total phenolic content. The oven dried leaves and rhizomes of H. coronarium were extracted with 95% ethanol. Evaluation of reducing power and total antioxidant activity were done using reducing power assay and phosphomolybdenum method, respectively. Analysis of total phenolic content was done using Folin-Ciocalteau method. Higher reducing power, total antioxidant activity and total phenolic content were observed in the ethanol extracts of the leaves of H. coronarium than in rhizomes. The concentration of the ethanol extracts of the leaves and rhizomes that reduced 50% of the initial ferric ions concentration (EC_{50}) were 479.27 mg/L and 166.86 mg/L, respectively. The total antioxidant activity was found to be 2.94 mg ascorbic acid equivalents (AAE) per gram sample in leaves and 1.02 mg AAE/ g sample in rhizomes. The total phenolic content of the leaves and rhizomes were found to be 1.95 gallic acid equivalent (GAE) per gram sample and 1.48 mg GAE/ g sample, respectively. The total antioxidant activity and total phenolic content between leaves and rhizomes were significantly different, hence, were plant-part dependent. An inverse relationship was observed between the EC_{50} with the total phenolic content and total antioxidant activity. A significant positive correlation was observed between total phenolic content and total antioxidant activity. The findings imply that the ethanol extracts of the leaves of H. coronarium which has higher total phenolic content exhibited higher reducing power and total antioxidant activity than the rhizomes. Thus, the phenolic compounds present in the ethanol extracts of H. coronarium have a pronounced contribution to its antioxidant activity. Hence, the leaves of H. coronarium can be considered as a potential source of natural antioxidants.
Keywords: *Zingiberaceae, reducing power, total phenolic content, antioxidant activity, Hedychium coronarium*

Received 12.05.2018 Revised 30.06.2018 Accepted 09.07.2018

INTRODUCTION

Philippines is an archipelago with a tropical rainforest climate suitable to support life in many ecosystems. In fact, the land is considered to be one of the mega-biodiverse countries of the world and preserves about five percent of the world's flora [1]. Plants provide food and serve as a haven for therapeutic and medicinal compounds that are crucial for the survival of humans. An ingenious way of utilizing plants with medicinal value is through development of valuable potent herbal drugs for treatment of presently protracted diseases. One of the most timely and pressing concerns nowadays involves the search from natural sources for cure of chronic and degenerative diseases caused by free radicals and reactive oxygen species (ROS) [2].

It was verified that free radicals and ROS were connected to the manifestation of some serious diseases, including Parkinson´s and Alzheimer´s disease, atherosclerosis, heart attacks, cancer, diabetes, cardiovascular disease, neurodegenerative diseases and chronic fatigue syndrome [3].

Plants generally produce several secondary metabolites which account for their antioxidant activity [3]. Antioxidants inhibit the formation of damaging ROS [4], inhibit the peroxidation of biological molecules by chelating transition metals that generate hydroxyl radicals [5], act as efficient scavengers of free

radicals and interrupt oxidative chain reactions [6]. Phenolic compounds are responsible for antioxidant activities of reducing ferric ions [7].

Gingers (Zingiberaceae) are worth exploring due to their wide availability on tropical and subtropical regions such as Philippines. At present, most studies concerning the antioxidant activity of gingers were confined to its rhizomes and essential oil [7].

Hedychium coronarium is an erect herb belonging to the family Zingiberaceae [8]. This plant contains numerous active chemical constituents accounting for its pharmacological activities. The rhizome of the plant is used in the treatment of diabetes, cold, body aches [9], fever, tonsillitis [10], bruises, rheumatic pain and demonstrated anti-inflammatory, antimicrobial [11], anti-cancerous [12], antioxidant, anti-hypertensive, diuretic and anti-malarial activities [8]. Previous reports on the extensive medicinal applications of *H. coronarium* and availability of this plant in Bukidnon warrant further investigations. Moreover, there are limited literatures documenting the variation on reducing power, total antioxidant activity and total phenolic content between different plant parts of *H. coronarium*. Hence, this study was conducted.

This study aimed to assess the antioxidant activity and total phenolic content of the ethanol extracts of leaves and rhizomes of *H. coronarium*.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents that were used in this study include ethanol (AR grade), gallic acid standard, ascorbic acid standard, dibasic phosphate (Na_2HPO_4), monobasic sodium phosphate (NaH_2PO_4), potassium ferricyanide, trichloroacetic acid, sulfuric acid, ammonium molybdate, ferric chloride, folin-ciocalteau reagent and sodium carbonate.

Plant materials

H. coronarium was collected in June 2018 from Dabong-dabong, Mailag, Valencia City, Bukidnon (7.973188° N, 125.116587° E). Plant samples were identified by Dr. Florfe M. Acma of the Center of Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University, University Town, Musuan, Bukidnon.

The leaf and rhizomes samples were cut into small pieces and were subjected to oven drying at 40 °C until the loss of drying was less than 10%. The dried samples were ground to a fine powder using an Osterizer (Oster 10-Speed Blender) and were stored in ziplock bags covered with aluminum foil (to prevent direct exposure to light) until further analysis.

Preparation of plant extracts

Solvent extraction was carried out using the method described by [13] with slight modifications in the amount of sample, solvent and extraction time. For the extraction of leaves, 140 g of accurately weighed oven-dried sample powder were mixed with 500 mL of ethanol (95%) and extracted using the magnetic stirrer and hotplate at 1150 rpm for three hours at room temperature. Extracts were filtered using Whatman No. 1 filter paper. The remaining residue on the filter paper was transferred back into the same flask and was re-extracted for two more times following the same procedure but using 300 mL of ethanol instead of 500 mL. The same procedure was followed for the extraction of rhizomes but the amount of sample and the amount of solvent used for the first and for re-extraction were different. The amount of sample extracted for rhizomes was 200 g and 500 mL of the solvent was used for the first extraction and 400 mL was used for the second and third extractions.

Filtrates collected from all the three successive extractions were pooled and collected in one Liter Erlenmeyer flask covered with aluminum foil and stored at -4° C in a refrigerator until the next step. The solvent in the filtrate was removed by rotary evaporator at 40° C. The sample was then stored at -20 °C in a freezer in thimble vials with screw caps covered with aluminum foil until analysis.

Reducing Power Assay

The reducing power of the rhizomes and leaves extract of the plant sample was determined by a method adapted for a 96-well plate assay as reported by [14]. Six extract solutions with concentrations of 0, 100, 250, 500, 750 and 1000 µg/mL were prepared from the stock solution. In an eppendorf containing one mL of the extract, 200 µL of 0.2 M phosphate buffer (pH 6.6) and 200 µL of 1% (w/v) solution of potassium ferricyanide was added. The mixture was incubated at 50 °C for 30 minutes. Then 1% (w/v) trichloroacetic acid (200 µL) was added after cooling the solution to room temperature. The mixture was then centrifuged for 3 minutes at 11000 rpm. An aliquot (200 µL) of the supernatant of the solution was transferred to a 96-well plate and 20 µL of 0.1% (w/v) solution of ferric chloride was added. The absorbance of the solution was measured at 620 nm using a micro-plate spectrophotometer reader.

The same procedure was done for the control (ethanol without the extract). The percent reducing power of the ethanolic extracts of the leaves and rhizomes of *H. coronarium* was calculated using equation 1 below as previously presented by [14].

$$\text{Reducing Power (\%)} = [1-(A_{control} / A_{sample})] \times 100 \quad (1)$$

where $A_{control}$ and A_{sample} are the absorbance values of the control and test sample, respectively.

The concentration of the extracts providing 50% of reducing power or half-maximal response, EC_{50} value, was determined. The percent reducing power was plotted against the corresponding concentration of the extract solutions. A curve was obtained from the data. The concentrations of the extracts were plotted on a logarithmic scale to expand the x-axis by converting the concentrations to log values. The percent reducing power was plotted against the log of the concentration. The log EC_{50} was extracted from the equation of the line and converted to EC_{50} by taking the antilog. The same procedure was done for the gallic acid standard.

Total Antioxidant Activity

The total antioxidant activity of the extracts was determined by adapting the method previously described by [15] with several modifications such as using eppendorf tubes as the reaction vessel and centrifugation after the reaction. In an eppendorf tube containing 200 µL of the extracts, 600 µL of a reagent solution (prepared by mixing equal amount of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The eppendorf tubes were then covered with aluminum foil tightly. The reaction mixture was incubated at 95 °C in an oven for 90 minutes. The mixture was allowed to cool to room temperature, centrifuged at 11000 rpm for three minutes and 200 µL was transferred into a well of a 96-well plate. The absorbance of the mixture was measured at 695 nm against a blank (ethanol in place of the extracts).

The same procedure was done for the ascorbic acid standard. The total antioxidant activity, expressed in mg ascorbic acid equivalents (AAE) per gram sample, was calculated using equation 2 obtained from the linear regression analysis of the calibration curve.

$$\text{AAE (mg/g sample)} = A/B \quad (2)$$

where A is the ascorbic acid concentration of the test solution determined from the calibration curve (mg AAE/L) and B is the concentration of the test solution (g/L).

Total Phenolic Content

The total phenolic content of the extracts was determined by adapting the method previously described by [16] with slight modifications such as the use of eppendorf tubes as reaction vessels and centrifugation after the reaction. In an eppendorf tube, 200 µL of the test solution was added with 200 µL of 10% folin-ciocalteu reagent. After mixing, the reaction mixture was set aside for five minutes and was added with 800 µL of 10% sodium carbonate. The mixture was incubated at room temperature for 90 minutes, centrifuged at 11000 rpm for three minutes and 200 µL of the resulting solution was transferred into a well of a 96-well plate. The absorbance of the solution was determined at 750 nm using a micro-plate spectrophotometer reader.

The same procedure was applied for the working standards and the blank (ethanol). The total phenolic content, expressed as gallic acid equivalent (GAE) per gram sample, was calculated using equation 3 which was derived using linear regression analysis of the calibration curve.

$$\text{GAE (mg/g sample)} = A/B \quad (3)$$

where A is the gallic acid concentration of the test solution determined from the calibration curve (mg GAE/L) and B is the concentration of the test solution (g/L).

RESULTS AND DISCUSSION

Reducing Power

Reducing power assay evaluates the ability of the potential antioxidants to reduce oxidized chemical entities. In the method, the antioxidant compounds present in the sample forms a colored complex with the other reagents in the reaction mixture due to reduction of Fe^{3+} to Fe^{2+}. Substances with reducing potential react with potassium ferricyanide to form potassium ferrocyanide which forms a ferric-ferrous complex upon addition of ferric chloride [17]. The effective concentration refers to the concentration of a sample that produces 50% activity or half-maximal response. The EC_{50} of the reducing power of the extracts refers to the concentration of the extracts at which 50% of the initial amount of Fe^{3+} ions was reduced to Fe^{2+} ions.

Summary of percent reducing power at varied extract concentrations and EC_{50} values of the ethanolic extracts of the leaves and rhizomes of *H. coronarium* are presented in Table 1 and Figure 1, respectively.

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Graphical presentation of EC_{50} values of ethanolic extracts of the leaves and rhizomes of *H. coronarium* is shown in Figure 2.

Table 1. Mean EC_{50} for the reducing power of ethanolic extracts of the leaves and rhizomes of *H. coronarium*

Plant Part	EC_{50} (RSD)
Leaves	166.86 (10.88) mg/L
Rhizomes	479.27 (13.30) mg/L
Gallic Acid Standard	< 15 mg/L

Values are reported as grand mean (RSD) (n=5).

Reducing power is an assay that is based on the principle of increase in the absorbance of the reaction mixture which indicates an increase in the antioxidant activity [18]. It can be observed from Table 1 and Figure 1 that the reducing power of ethanolic extracts of the leaves of *H. coronarium* increases as the concentration of the extracts increases. It may imply that the concentration of antioxidants involved in the reaction also increases along with the concentration of the extract solution.

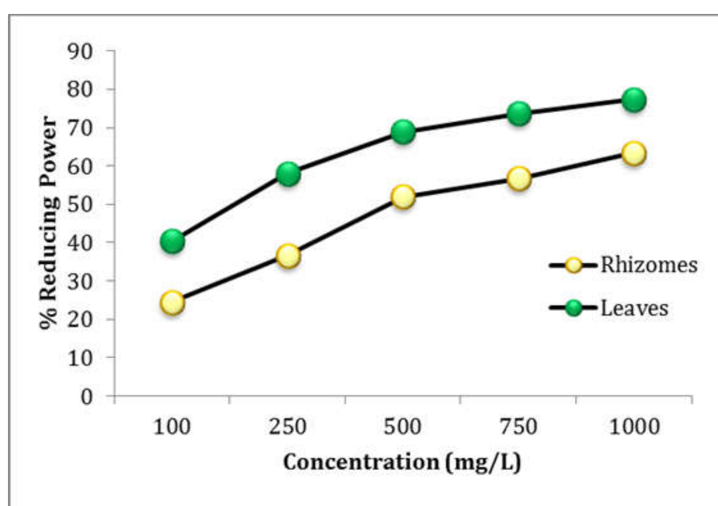

Figure 1. Graphical presentation of the reducing power of ethanolic extracts of the leaves and rhizomes of *H. coronarium* at varied extract concentrations.

Similar trend can be observed from the reducing power of the ethanolic extracts of the rhizomes (Table 1 and Figure 1). The reducing power also increases as the concentration of ethanolic rhizome extracts increases. The findings suggest that the ethanolic extracts of both rhizomes and leaves of *H. coronarium* contain antioxidant compounds capable to function as reductants in redox reactions. This particular property of antioxidants is specifically relevant in reducing oxidized intermediates of lipid peroxidation processes via electron donation [19].

The EC_{50} of gallic acid standard (< 15 mg/L) is lower than both ethanolic extracts of rhizomes and leaves of *H. coronarium* as shown in Table 1. This implies that gallic acid is a more effective reducing agent than both ethanolic extracts of rhizomes and leaves of *H. coronarium*. This can be attributed to the standard which was in pure form while the extracts used are in crude form. The compounds that are responsible for the antioxidant activity of *H. coronarium* were not isolated so the concentrations of the antioxidant compounds in the extracts were expected to be minute. Similar result was observed with the antioxidant activity in terms of free radical scavenging activity of several ginger species in comparison to the antioxidant activity of the ascorbic acid standard [20].

It can be observed from Figure 2 that there is a variation between EC_{50} values between the ethanolic extracts of the leaves and rhizomes of *H. coronarium*. Ethanolic leaf extracts has EC_{50} value (166.86 mg/L) that is lower than that of the rhizomes (479.27 mg/L). Lower EC_{50} value implies stronger reducing power of the extract since smaller concentration of the extracts was needed to reduce 50% of the initial concentration of Fe^{3+} cations to Fe^{2+}. Hence, the ethanolic leaf extracts of *H. coronarium* has higher antioxidant activity in terms of reducing power than the rhizomes. Thus, it can be deduced that the leaves of *H. coronarium* contains more antioxidant compounds that are generally more effective in acting as reducing agents than the rhizomes.

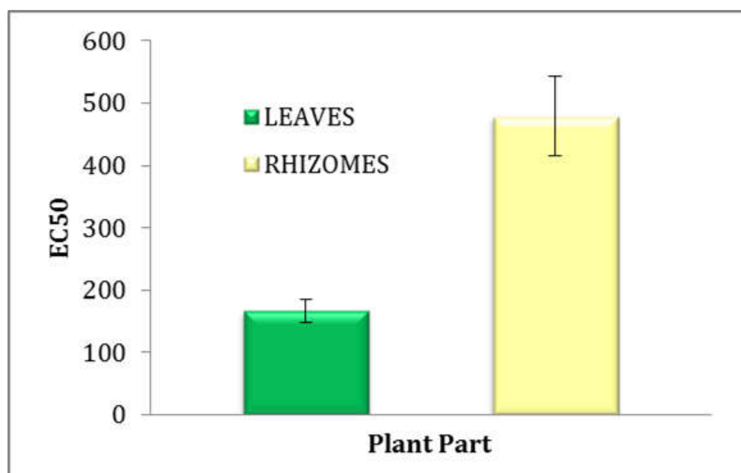

Figure 2. EC_{50} values for the reducing power of the ethanolic extracts of the leaves and rhizomes of *H. coronarium*. Error bars are standard deviation (n=5).

Total Antioxidant Activity

Determination of the total antioxidant activity of ethanolic leaf and rhizome extracts of *H. coronarium* was evaluated using the molybdenum blue approach. Phosphomolybdenum method is a quantitative assay that investigates the reduction rate among antioxidant, oxidant and molybdenum ligand. The method involves thermally generating auto-oxidation during prolonged incubation period at higher temperature [21]. The formation of the blue complex in the assay has been used as a sensitive test for reducing agents. The results of the total antioxidant activity expressed as milligram ascorbic acid equivalents (AAE) per gram sample of the leaves and rhizomes of *H. coronarium* are summarized in Table 2 and Figure 3.

Table 2. Mean total antioxidant activity of the ethanolic extract of the leaves and rhizomes of *H. coronarium*

Plant Part	mg AAE/ g sample (%RSD)
Leaves	2.94 (8.82)
Rhizomes	1.02 (15.19)

Values are reported as grand mean (RSD) (n=5). Significant difference at 0.05 level (2-tailed)

As shown in Table 2 and Figure 3, the total antioxidant activity of the ethanolic extracts of the leaves and rhizomes of *H. coronarium* were 2.94 and 1.02 mg AAE/ g sample. This implies that ethanolic extracts of the leaves and rhizomes of *H. coronarium* exhibited antioxidant activity via reduction of Mo (VI) to Mo (V). The mechanism involves electron transfer in the presence of a reducing agent (antioxidant). Natural bioactive compounds such as phenols and flavonoids can cause this reduction [2]. The reducing properties of the extracts as evaluated in this assay exhibits potential to react with certain precursors of peroxides in order to prevent peroxide formation [22].

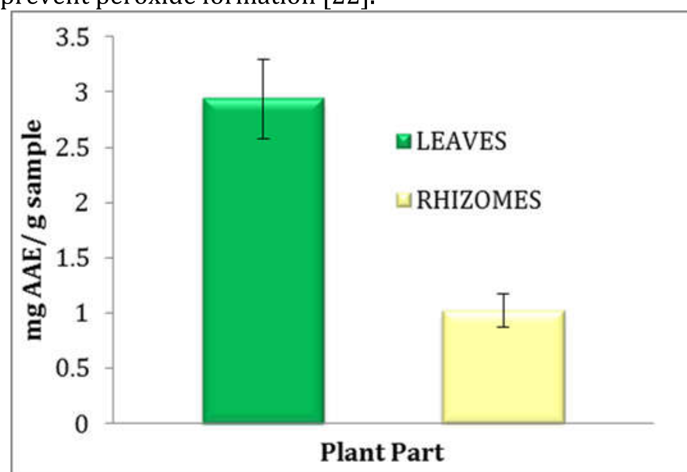

Figure 3. Graphical presentation of total antioxidant activity of the ethanolic extract of the leaves and rhizomes of *H. coronarium*. Error bars are standard deviation (n=5).

The leaves exhibited higher antioxidant activity than the rhizomes as can be observed on Figure 3. The t-test results showed statistically significant difference between the total antioxidant activity of the ethanolic extracts of the leaves and rhizomes of *H. coronarium*. Hence, the total antioxidant activity of *H. coronarium* is plant-part dependent.

Total Phenolic Content

Total phenolic content assay is a colorimetric method that uses Folin-Ciocalteau's reagent to quantify the total phenolic in the sample. The mechanism involves reduction of the molybdenum component in the phosphotungstic-phosphomolybdic complexing reagent. The phenolate anions present in the sample were believed to destroy the yellow Folin-Ciocalteau phenol reagent and reduced the reagent thereby producing the characteristic blue color of the resulting solution [19].

The total phenolic content, expressed as milligram gallic acid equivalents (GAE) per gram sample of the leaves and rhizomes of *H. coronarium*, are presented in Table 3 and Figure 4.

Table 3. Mean total phenolic content of the ethanolic extract of the leaves and rhizomes of *H. coronarium*

Plant Part	mg GAE/ g sample (%RSD)
Leaves	1.95 (3.27)
Rhizomes	1.48 (11.98)

Values are reported as grand mean (RSD) (n=5). Significant difference at 0.05 level (2-tailed)

As shown in Table 3, the total phenolic content of the ethanolic extracts of the leaves and rhizomes of *H. coronarium* are 1.95 and 1.48 (mg GAE/ g sample), respectively. Previous reports showed that methanolic extracts of *H. coronarium* contains a wide variety of phenolics ranging from flavonoids such as chrysin and teptochrysin to phenylpropanoids such as eugenol [8, 9, 12]. Phenolic compounds can be classified either as secondary antioxidants due to their ability to bind with pro-oxidant metal ions or primary antioxidants due to their antioxidant reactions based on hydrogen atom transfer and single electron transfer mechanisms [19].

The t-Test results revealed significant difference between the total phenolic content of the ethanolic extracts of the leaves and rhizomes of *H. coronarium* which can also be observed in Figure 4. This finding implies that the total phenolic content of *H. coronarium* is plant-part dependent wherein the total phenolic content is statistically higher in leaves than in rhizomes. This implies that there are generally more phenolic compounds found on the leaves of *H. coronarium* than the rhizomes of this plant.

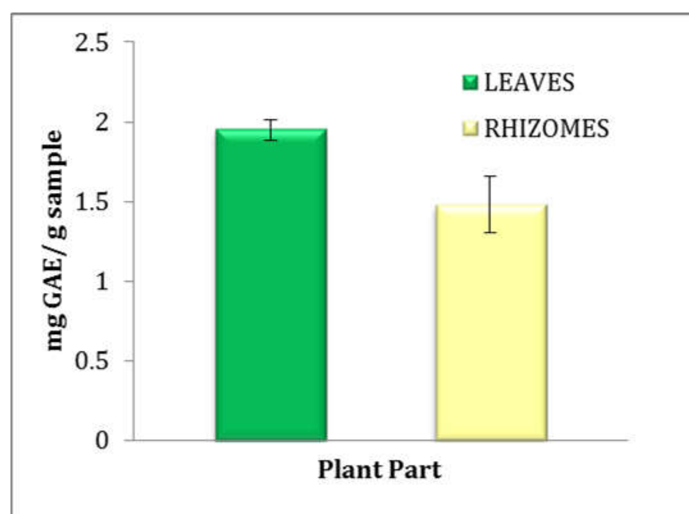

Figure 4. Graphical presentation of the total phenolic content of the leaves and rhizomes of *H. coronarium*. Error bars are standard deviation (n=5).

The rhizomes of *H. coronarium* have been widely known to have many medical applications in traditional medicine which can be attributed to the presence of phytochemicals [8, 23]. It was generally believed that antioxidants and other secondary metabolites would be transported and stored to the rhizomes and higher antioxidant activity should be observed in the rhizomes than the other plant parts [7]. However,

some results showed that leaves have significantly higher phenolic content and antioxidant activity than rhizomes in ginger plants [24]. This is similarly observed in this present study.

Screening of five wild and six cultivated ginger species exhibited higher total phenolic content in leaves than in rhizomes. *Etlingera elatior* (torch ginger) and *Etlingera maingayi* are both ginger species that showed outstanding leaf total phenolic content that were seven and eight times higher than those of the rhizomes [7]. *Hedychium* had been ranked third of the highest methanolic leaf total phenolic content among 26 ginger species belonging to nine genera and three tribes screened for total phenolic content. Majority of ginger species studied had significantly higher phenolic content and antioxidant activity in leaves than in rhizomes [24].

Previous reports showed higher concentrations of flavones and flavonols in vegetable leaves which are exposed to sunlight [25]. Flavones and flavonols can be formed by altered substitution and saturation patterns of flavonoids, the most common and widely distributed group of phenolic compounds found in plants [19]. Only trace amounts of these compounds were found in unexposed parts of plants below the surface of the soil such as roots and rhizomes [24]. This may also explain why the leaves of ginger plants such as *H. coronarium* have significantly higher phenolic content and antioxidant activity than rhizomes. Moreover, flavonoids such as quercetin and kaempferol were detected from the leaves of several ginger plants as evaluated by a metabolomics study [26].

Relationship of Reducing Power, Total Antioxidant Activity and Total Phenolic Content

Summary of the results of Pearson's correlation analysis are presented in Table 4. The total antioxidant activity of methanolic rhizome extracts of *H. coronarium* can be attributed to the chemical composition of this plant [27]. Statistical results of the Pearson's correlation presented in Table 4 indicates a significant positive correlation (0.873) which implies that the plant part with higher phenolic content also exhibited higher total antioxidant activity. Hence, the phenolic compounds present in the sample have a pronounced contribution to the total antioxidant activity of the sample extract. Phenolic compounds constitute the primary class of natural antioxidants present mostly in plants [28]. Furthermore, it was suggested that excellent total antioxidant activity might be due to the presence of bioactive compounds such as flavonoids, carotenoids and ascorbic acid [29].

Leaves of several species of gingers from different genera were found to contain shikimic acid [26] which is a precursor and a building block for the synthesis of phenolic compounds. Trans-4-caffeoylquinic acid and trans-3-coumaroylquinic acid are phenolic compounds that mitigates oxidative stress caused by gamma radiation were also detected in Zingibeaceae species [26]. A strong correlation between total antioxidant activity and classes of phenolic compounds such as hydroxycinnamic acids was previously reported [22].

Table 4. Correlation coefficients based on Pearson's correlation analysis

ASSAY	Total Phenolic Content	Total Antioxidant Activity
Total Phenolic Content	1	0.938**
Total Antioxidant Activity	0.938**	1

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

An inverse relationship was observed between the EC_{50} values for the reducing power with total phenolic content of the ethanolic leaf and rhizome extracts of *H. coronarium*. This indicates that the plant part with higher total phenolic content has lower EC_{50} value for the reducing power. Since lower EC_{50} value means stronger reducing power, the result simply means that phenolic compounds are largely responsible for the antioxidant activity in terms of reducing power within the sample [19].

The reducing power of the plant extracts is associated with the electron donating ability of the antioxidants present in the extract. The mechanism involved is single electron transfer where an antioxidant transfers a single electron that results in the reduction of the potential target compounds. It was suggested that the best phenolic antioxidants are compounds which contain electron donor groups directly attached to an aromatic ring [19]. A study examined the antioxidant potentials of a variety of phenolic antioxidants and revealed that antioxidant activity generally increases with increasing number of phenolic rings which indicates that polyphenols are more effective antioxidants than simple phenols [30]. Furthermore, the activity of flavonoids also increased linearly with a corresponding increase in free hydroxyl groups.

The principle for both reducing power assay and total antioxidant activity assay involved reduction of cations. It was expected that the plant part with stronger reducing power (lower EC_{50} value) would also exhibit stronger total antioxidant activity compared to the other plant. This assumption was confirmed by

the results of this study wherein an inverse relationship was observed between EC_{50} for the reducing power with the total antioxidant activity of the ethanolic extracts of the leaves and rhizomes of *H. coronarium*.

CONCLUSION

Significantly higher total antioxidant activity and total phenolic content were observed in the ethanol extracts of the leaves of *H. coronarium* than in rhizomes. Higher reducing power was also observed in the ethanol extracts of the leaves of *H. coronarium* than in rhizomes. There was an inverse relationship between the EC_{50} values for the reducing power and total phenolic content. Hence, reducing power is directly proportional to total phenolic content and total antioxidant activity. A positive correlation was observed between total antioxidant activity and total phenolic content. These findings imply that the ethanol extracts of the leaves of *H. coronarium* which has higher total phenolic content also exhibited higher reducing power and total antioxidant activity than the rhizomes. Hence, the phenolic compounds present in the ethanolic extracts of *H. coronarium* have a pronounced contribution to its antioxidant activity. This study shows that there is a significant variation in the amount phenolic compounds extracted from different plant parts of *H. coronarium*. The ethanolic extracts of the leaves of *H. coronarium* exhibited higher antioxidant activity and phenolic content than the rhizomes. Utilization of the leaves of *H. coronarium* rather than the rhizomes is less destructive to the plant. Hence, the higher level of phenolic compounds and the antioxidant activity in *H. coronarium* leaves may provide support to its potential as underutilized natural source of antioxidants.

ACKNOWLEDGEMENT

The authors are indebted to Aileen May G. Ang and Yuyen V. Tan for their valuable suggestions on the improvement of this paper. Special thanks go to Rainear A. Mendez and Rajane Faith Bautista for their helpful assistance in the conduct of experiments. The authors are also grateful to the Chemistry Department, Natural Science Research Center, and Natural Products Research and Development Center of Central Mindanao University, University Town, Musuan, Bukidnon, Philippines for the supporting the conduct of the experiments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CITATION OF THIS ARTICLE

Redondo, M. S. and Barbosa, G. B. Antioxidant activity and phenolic content of the ethanol extracts of *Hedychium coronarium* (Zingiberaceae) in Mindanao, Philippines. Bull. Env. Pharmacol. Life Sci., Vol 7 [10] September 2018: 97-105