Original Research Article

Antioxidant Activity and Phenolic Content of *Hornstedtia conoidea* (Zingiberaceae)

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ABSTRACT

Introduction: Several Zingiberaceae plants were reported to possess wide range of antioxidants. The Philippine endemic *Hornstedtia conoidea* is still poorly investigated. **Aim:** This study aimed to provide fundamental information on the total antioxidant activity and total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea*. **Methods:** The leaves and rhizomes of *H. conoidea* were collected from Kibawe, Bukidnon, Philippines, oven-dried at 40 °C and extracted using absolute methanol. The total antioxidant activity and total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea* were evaluated through Phosphomolybdenum method and Folin- Ciocalteu method, respectively. **Results:** The total antioxidant activity, expressed as milligram ascorbic acid equivalent per gram sample, was significantly higher in leaves (4.67) as compared to rhizomes (2.03). Furthermore, the determination of total phenolic content, expressed as milligram gallic acid equivalent per gram sample, revealed that leaves have greater amount of phenolics (1.67) than rhizomes (1.28). A positive correlation between total antioxidant activity and total phenolic content was observed. The present findings support that the leaves and rhizomes of *H. conoidea* are both potential sources of natural antioxidants.

Key words: Zingiberaceae *plants, rhizomes, leaves, Hornstedtia conoidea,* Total phenolic content, Total antioxidant activity

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INTRODUCTION

The quest for natural-based health enhancer and food preservatives has long been the subject of many researches in various fields of studies. In recent years, there have been accumulating amount of evidences showing enormous health benefits that can potentially be obtained from plants such as trees, fruits and vegetables[1] Several studies suggest that plants contain numerous components capable of preventing the development of oxidative stress-related chronic diseases due to Reactive Oxygen Species (ROS). ROS such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide are highly reactive and toxic molecules generated normally in cells during metabolism. They cause severe oxidative damage to proteins, lipids, enzymes and deoxyribonucleic acid by covalent binding and lipid peroxidation that cause subsequent tissue injury.[2]

Antioxidants play a major role in wiping up these reactive oxygen and nitrogen species by scavenging free radicals in biological system.[3] Secondary metabolites of plants such as phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids and alkaloids have been proven to neutralize oxidative stress due to their antioxidant activity.[4] According to Wojdyło, Oszmiański, Czemerys,[5] both edible and inedible plants contain polyphenolic compounds which have been reported to have multiple biological effects, including antioxidant activity.

Zingiberaceae plants, commonly known as gingers, are perennial herbs that produce aromatic rhizomes. Zingiberaceae is the largest family in the order *Zingiberales* consisting of about 50 genera and 1300 species worldwide.[6,7] It is commonly known as gingers and is widely distributed throughout the tropics particularly in Southeast Asia. Zingiberaceae family is a

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plant species endowed with antioxidative properties.[3] Members of the family yield spices, dyes, perfumes, medicines and numerous ornamental species are cultivated for their showy flowers. Recently these plants have acquired great importance in the present day world due to its anti-aging, anticancer, antioxidant, anti-alzheimer's diseases and variety of other medicinal properties.[8]

Zingiberaceae species grow naturally in damp, shaded part of the lowland or on hill slopes, as scattered plants or thicket. Zingiberaceae produces aromatic rhizomes that are above the ground or subterranean. Most members of the family are easily recognized by the characteristic aromatic leaves and fleshy rhizome when both are crushed and also by the elliptic to elliptic-oblong leaves arranged in two ranks on the leaf shoot. In Southeast Asian region, several species of Zingiberaceae are used as spices, condiment, traditional medicines, flavoring agents and as the source of certain dyes. The rhizomes are eaten either raw or cooked as vegetables. Some usually grown species are *Zingiber officinale, Curcuma longa* and *Alpinia galangal*.[3]

Hornstedtia conoidea Ridl. is a species of ginger belonging to the genus *Hornstedti*a Retz. *H. conoidea* is locally known as tagbak and is considered the most abundant Philippine *Hornstedtia* species in the province of Bukidnon, Mindanao, Philippines.[9] The image of *H. conoidea* plant with fruits, leaves and rhizomes are shown in Figures 1a, 1b and 1c, respectively.

Some local people of the province of Bukidnon, Mindanao, Philippines considered *H. conoidea* to be edible.[10] in which the leaves and rhizomes are used as condiments.[9] Ethno-medicinal uses were also reported on this plant. The fruit containing the ripe seeds can cure stomach disorders.[10] and diarrhea[9] while the rhizomes are used against fever and chills.[9] *H. conoidea* plant either bore flowers and/or fruits and its reproductive parts were frequently visited by ants, thus, a possible pollinator.[9]

This is the first study to determine the total antioxidant activity and total phenolic content of the leaves and rhizomes of *H. conoidea* using methanol as solvent.

Figure 1: Photographs of *H. conoidea* (a) plant with fruits, (b) leaves and (c) rhizome.

MATERIALS AND METHODS

Chemicals

The chemicals and reagents that were used in this study were analytical reagent grade absolute methanol, dibasic sodium phosphate, Folin-Ciocalteu reagent, sulfuric acid, ammonium molybdate, sodium carbonate, gallic acid standard and ascorbic acid standard.

Sample Collection

The leaves and rhizomes of *H. conoidea* were collected from Kibawe, Bukidnon (7°34'00.9"N 124°55'52.4"E). Mature and healthy rhizomes and leaves of *H. conoidea* (without infestation of insects) were placed in clean net bags and were transported to Chemistry laboratory, Chemistry Department, College of Arts and Sciences, Central Mindanao University (CMU), University Town, Musuan, Maramag, Bukidnon, Philippines. Plant identification was done at CMU Herbarium by Hannah Lumista and Florfe Acma.

Sample Preparation

Sample preparation was performed employing the method described by Wijekoon, Bhat, Karim[11] and Ibrahim, Mat, Lim, Ahmad.[12] The samples were cut into uniformly sized small pieces and were subjected to oven drying at 40°C for 72 hrs or until the loss on drying was less than 10%. After oven drying, the samples were ground to a fine powder using an osterizer (Oster). The powdered samples were then placed in clean and dry resealable plastic bags and stored at room temperature. The plastic bags containing the sample were covered with aluminum foil until further analysis to prevent direct exposure to light.

Extraction

Solvent extraction was carried out using the method described by Wijekoon, Bhat, Karim[11] with slight modifications in the amount of sample, amount of solvent and extraction time.

The solvent extraction of the leaves of *H. conoidea* was performed by using 126 g of accurately weighed pulverized sample per replicate. The solvent extraction was carried out using magnetic stirrer and hotplate at 1190 rpm for 3 hrs at room temperature. 3 successive extractions were performed. On the first extraction,

550 mL of absolute methanol was added in order to completely soak the powdered sample. After extraction for 3 hrs, the mixture was 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 2 more times following the same procedure. On the second and third extractions, 350 mL and 300 mL of absolute methanol, respectively were used. The total volume of solvent used was 1200 mL per replicate.

For the solvent extraction of the rhizomes of *H. conoidea*, the same method was employed. Eighty grams of accurately weighed pulverized sample was used per replicate. On the first extraction, 400 mL of absolute methanol was added into the powdered sample to completely soak the samples. On the second and third extractions, 250 mL of absolute methanol was used. The total volume of solvent used was 900 mL per replicate.

The collected filtrates from the three successive extractions of each plant part were pooled and placed in a separate Erlenmeyer flask (1000 mL) covered with aluminum foil to prevent exposure to light. The samples were then kept at 4°C. Removal of solvent from the filtrate was done using rotary evaporator (Yamato RE 210) at 40°C. The concentrated extracts were stored in glass vials covered with aluminum foil and stored at - 20°C.

Determination of Total Antioxidant Activity

Preparation of Standards and Samples

A stock solution of ascorbic acid with concentration 300 mg/L was prepared by dissolving 0.0150 g of the standard ascorbic acid in absolute methanol. The resulting solution was then diluted into 50 mL. Different concentrations of 0, 15, 30, 60, 90, 105, 150, 210, 255, 300 mg/L were prepared as working standards for calibration curve.

The stock sample solution with a concentration of 1000 mg/L was prepared by dissolving 0.1000 g of the extract in absolute methanol. The solution was then diluted to make a total volume of 100 mL. A 500 mg/L of test solution was prepared by obtaining a 100 µL aliquot of the sample solution with subsequent addition of 100 µL absolute methanol.

Assay

The total antioxidant activity of the methanolic extracts of the leaves and rhizomes of *H. conoidea* was determined by adapting the method previously described by Prieto, Pineda, Aguilar[13] with slight modification particularly on the use of eppendorf tubes and centrifuge. In a 1.5 mL eppendorf tube, 200 µL of the test solution was added with 600 µL of the reagent solution prepared by mixing equal amounts of 4 mM ammonium molybdate (100 ml), 0.6 mM sulfuric acid (100 mL) and 28 mM dibasic sodium phosphate. The mixture was then incubated at 95°C for 90 mins and centrifuged for 3 mins at 11,000 rpm. The absorbance of the solutions was determined at 695 nm wavelength using micro-plate spectrophotometer reader (Spectramax 250).

The same procedure was applied for the ascorbic acid working standards and the negative control in which absolute methanol was used instead of the test solution. The total antioxidant activity expressed as milligram ascorbic acid equivalent (AAE) per gram sample was calculated using linear regression equation of the line derived from the calibration curve using equation 1.

Total Antioxidant Activity,

$$\frac{\text{mg}}{\text{g sample}}\ \text{AAE} = \frac{A}{B} \times \frac{C}{D}. \qquad \text{Equation 1}$$

Where:

A = ascorbic acid concentration of the test solution determined from the calibration curve, mg AAE/ Liter

B = concentration of the test solution, g/L

C = weight of the concentrated extract, g

D = weight of the dried sample, g

Determination of Total Phenolic Content

Preparation of Standards and Samples

A stock solution of gallic acid with concentration of 300 mg/L was prepared by dissolving 0.0150 g of the standard gallic acid in absolute methanol. The resulting solution was then diluted to 50 mL. Different concentrations of 0, 30, 45, 60, 75 and 90 mg/L were prepared as working standards for the calibration curve. The stock sample solution with a concentration of 1000 mg/L was prepared by dissolving 0.1000 g of the extract in absolute methanol. The solution was then diluted to make a total volume of 100 mL. A 500 mg/L of test solution was prepared by obtaining a 100 µL aliquot of the sample solution with subsequent addition of 100 µL absolute methanol.

Assay

The total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea* was determined by adapting the methods previously described by Ainsworth, Gillespie[14] with slight modification by using eppendorf tubes and centrifuge. In a 1.5 mL eppendorf tube, 200 µL of the test solution was added with 200 µL of 10 % Folin-Ciocalteu reagent and 800 µL 10%

sodium carbonate. The mixture was then incubated at room temperature for 2 hrs and was centrifuged for 3 mins at 11,000 rpm. The absorbance of the solutions was determined at 750 nm wavelength using microplate spectrophotometer reader (Spectramax 250).

The same procedure was applied for the gallic acid working standards and blank (absolute methanol). The total phenolic content expressed as milligram gallic acid equivalent (GAE) per gram sample was calculated using linear regression equation of the line derived from the calibration curve using equation 2.

Total Phenolic Content,

$$\frac{\text{mg}}{\text{g sample}}\text{GAE} = \frac{A}{B} \times \frac{C}{D}. \qquad \text{Equation 2}$$

Where:

A = gallic acid concentration of the test solution determined from the calibration curve, mg GAE/Liter

B = concentration of the test solution, g/L

C = weight of the concentrated extract, g

D = weight of the dried sample, g

Statistical Analysis

The analysis of the sample was done in four replicates. The determination for each parameter was carried out in five trials per replicate. The data generated were subjected to *t*-Test at 0.05 level of significance and the correlation between the total antioxidant activity to the total phenolic content of *H. conoidea* was determined using Pearson's correlation at 0.01 level of significance. Grubb's test was used to carry out the test for outliers.

RESULTS

The mean extraction yield from the methanolic extraction of the leaves and rhizomes of *H. conoidea* was determined to be 5.79 % and 2.39 % from the leaves and rhizomes, respectively (Table 1).

Table 1: Mean extraction yield of leaves and rhizomes of *H. conoidea*.

Plant Part	Extraction yield, % (RSD)
Leaves	5.79 % (22.20)
Rhizomes	2.39 % (9.98)

Total Antioxidant Activity

In this study, the total antioxidant activity of the methanolic extracts of the leaves and rhizomes of *H. conoidea* was assessed through phosphomolybdenum method. The results of the determination, expressed as milligram ascorbic acid equivalents (AAE) per gram sample, are summarized in Table 2.

Table 2: Mean total antioxidant activity and total phenolic content of the methanolic extract of the leaves and rhizomes of *H. conoidea*.

Plant Part	mg AAE/g sample (% RSD)	mg GAE/g sample (% RSD)
Leaves	4.67 (8.83)	1.67 (7.83)
Rhizomes	2.03 (9.06)	1.28 (9.40)

Means are significantly different at 0.05 level of significance based on t-Test.

As presented in Table 2, the total antioxidant activity in the methanolic extracts of the leaves and rhizomes of *H. conoidea* are 4.67 and 2.03 mg AAE/g sample, respectively. The result of *t*-Test revealed that there is a significant difference (p <0.001) between the total antioxidant activity of the methanolic extracts of the leaves and rhizomes of *H. conoidea*. This implies that the total antioxidant activity of the leaves is significantly higher than that of the rhizomes.

Total Phenolic Content

The total phenolic content, expressed as milligram gallic acid equivalents (GAE) per gram sample, are presented in Table 2. As shown in Table 2, the total phenolic content in the methanolic leaf and rhizome extracts of *H. conoidea* are 1.67 and 1.28 mg GAE/g sample, respectively. It is apparent that leaves exhibit higher phenolic content as compared to the rhizomes of *H. conoidea*. The result of *t*-Test revealed that there is significant difference (p=0.001) between the total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea*. This implies that the total phenolic content of the leaves is significantly higher than that of the rhizomes.

DISCUSSION

Recently, gingers have received much attention from researchers due to their diverse medicinal and pharmacological effects that can be attributed to the various compounds present in these plants particularly antioxidants. Incorporating antioxidants in human diet would help reduce oxidative damage since they best counteract with free radicals. In addition, natural antioxidants are safe and were proven to increase shelf-life of food products containing fats and oils.[15] Both leaves and rhizomes of gingers are widely used as spice, condiment and traditional medicine.[16]

In phosphomolybdenum method, molybdenum (VI) is reduced to molybdenum (V) in the presence of a reducing agent (antioxidant), forming a green phos-

phomolybdate (V) complex, which can be evaluated spectrophotometrically at 695 nm. The assay involves an electron transfer mechanism. Many natural products, including phenols and flavonoids, can cause this reduction.[17]

The result of this study is consistent with the result obtained from the study of Jeyapragash, Subhashini, Raja, Abirami, Thangaradjou[18] wherein the aqueous ethanol extracts of *Halodule uninervis* and *Syringodium isoetifolium* possess higher total antioxidant activity in the leaves as compared to the rhizomes. Moreover, the methanolic extracts of the leaves of *Etlingera coccinea* also showed higher total antioxidant capacity as compared to the stems and the rhizomes.[19] Moreover, both water and ethanol extracts of *E. elatior* showed higher DPPH radical scavenging in leaves than in rhizomes.[20]

Different trend, however, was observed in the ethanol extracts of *Amomum muricarpum*. It was reported to exhibit higher antioxidant activity in terms of radical scavenging activity in its rhizomes than in leaves.[21] Higher reducing power was also observed from the ethanolic extracts of the rhizomes of *Acorus calamus* as compared to leaves.[22]

As plant secondary metabolites, the phenolics or polyphenols are very important due to their antioxidant activities. They are responsible in chelating redox-active metal ions, inactivating lipid free radical chains and avoiding the hydroperoxide conversions into reactive oxyradicals.[23] Under the basic reaction conditions, a phenol loses an H^+ ion to produce a phenolate ion, which reduces Folin-Ciocalteu reagent.[24,25] The change is monitored spectrophotometrically. As phenolics (including many flavonoids) contain polar phenolic hydroxyl groups, their high extraction into methanol and water is quite reasonable.[17]

The study of[26] reported that leaves of *H. conoidea* have significantly higher levels of kaempferol and quercetin than in the leaves of *Zingiber officinale*. Both kaempferol and quercetin belongs to the class of flavonoids particularly flavonols. In addition, chlorogenic acid level of the leaves of *H. conoidea* exceeded that of the leaves of *E. elatior* which is also a potential source of commercially available chlorogenic acid.[26] Phytochemical screening of the ethanolic and water extracts of both the leaves and rhizomes of *H. conoidea* revealed that there are alkaloids, flavonoids, saponins, tannins and steroids present.[21] Since Folin- Ciocalteu method measure the activity of only hydrophilic antioxidants,[19] the difference in the amount of total phenolic content between plant parts may be attributed to the difference in the amount of hydrophilic antioxidant present.

Moreover, it was reported by Herrmann[27] that there are much greater concentrations of flavones and flavonols in leaves of vegetables since they are exposed to sunlight. Only trace amounts were found in unexposed parts below the soil surface, which include roots and rhizomes. This could explain why leaves have significantly higher phenolic content and antioxidant activity than rhizomes in ginger plants.[28]

Similar study conducted by Chan *et al.* on 14 ginger species revealed higher total phenolic content and higher antioxidant activity in the leaves than rhizomes of those species belonging in the genera *Alpinia, Curcuma* and *Etlingera*. Chan, Lim, Omar[29] reported that in *Etlingera* species (*E. maingayi, E. elatior, E. littoralis and E. fulgens*), the total phenolic content was seven to eight times higher in the leaves than in the rhizomes.

Rhizomes of *Etlingera philippinensis* and *H. conoidea* were also found to contain almost similar levels of amino acids, ethanolamine, trehalose, galactosylglycerol, xylitol, galactinol, mucic acid, alpha- Dimethylfructofuranoside, 2-O-glycerol-beta-D-galactopyranoside, hydroquinone-beta-glucopyranoside, salicylaldehyde-beta-D-glucoside, pipecolic acid, ribonic acid, trans-4-hydroxycinnamic acid, 2,4,5-trihydroxypentanoic acid, monomethylphosphate, phosphate and unknown compounds. There is higher level of quinic acid, trans-3- caffeoylquinic acid, trans-3-coumaroylquinic acid and 2-deoxy- pentos-3-ylose dimethoxyamine in the rhizomes of *H. conoidea* as compared to rhizomes of *E. philippinensis*.[26]

The correlation between total antioxidant activity and total phenolic content on both plant parts was determined using Pearson's Correlation. The statistical result for the correlation of total antioxidant activity and total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea* revealed that there is a significant positive correlation (r=0.943, p<0.001) existing between the two. This result indicates that plant part with higher total antioxidant activity possess higher total phenolic content. This is an implication that most of the phenolic compounds in the leaves of *H. conoidea* are primarily responsible for the high total antioxidant activity.

Jing, Mohamed, Rahmat, Bakar[30] also noted that antioxidant activities are most probably contributed by polyphenol contents in the plant extracts. The antioxidant activity of the phenolics is essentially determined by their structures, in particular the electron delocalization over an aromatic nucleus. When these compounds react with free radicals, the delocalization of the gained electron over the phenolic antioxidant and

the stabilization by the resonance effect of the aromatic nucleus occurs, which prevents the continuation of the free radical chain reaction.[31]

CONCLUSION

This is the first study to provide fundamental information on the total antioxidant activity and total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea*. The methanolic extracts of the leaves of *H. conoidea* has significantly higher total antioxidant activity as compared to the rhizomes. Moreover, the methanolic extracts of the leaves of *H. conoidea* has significantly higher total phenolic content as compared to the rhizomes.

Correlation of total antioxidant activity and total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea* revealed that there is significant positive correlation existing between the two. This result indicates that the higher the total antioxidant activity would mean higher total phenolic content. This is an implication that most of the phenolic compounds in *H. conoidea* are primarily responsible for the high total antioxidant activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ROS: Reactive oxygen species ROS; **DPPH:** 1,1-Diphenyl-2-picrylhydrazyl; **CMU:** Central Mindanao University; **AAE:** Ascorbic acid equivalent; **GAE:** Gallic acid equivalent; **RSD:** Relative Standard Deviation.

SUMMARY

The total antioxidant activity and total phenolic content of the methanolic extracts of the leaves and rhizomes of *H. conoidea* collected from Kibawe, Bukidnon, Philippines were evaluated through Phosphomolybdenum method and Folin- Ciocalteu method, respectively. The methanolic extracts of the leaves of *H. conoidea* has significantly higher total antioxidant activity and total phenolic content as compared to the rhizomes. Significant positive correlation exists between total antioxidant activity and total phenolic content.

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