Antioxidant activities and phytochemical screening of *Amomum muricarpum*, *Hornstedtia conoidea* and *Etlingera philippinensis*

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**ABSTRACT**
Prime concern on human health preservation has escalated interest on search for more natural antioxidant sources. Despite the reported potential values of some Zingiberaceae species, less emphasis is still given to other Zingiberaceae plants especially those found in the Philippines. To the best of the authors’ knowledge this is the first scientific report on Philippine endemic *Hornstedtia conoidea* and *Etlingera philippinensis*. The water and ethanol extracts of the leaves and rhizomes of *Amomum muricarpum*, *H. conoidea* and *E. philippinensis* were subjected to in vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging (antioxidant) activity assay and phytochemical analysis. Ascorbic acid was used as standard. High DPPH radical scavenging activities were observed in the water extracts of *E. philippinensis* and *H. conoidea* leaves and the ethanol extract of *A. muricarpum* rhizome with percentage DPPH inhibition of \(92.07 \pm 0.51\), \(92.97 \pm 0.23\), and \(94.73 \pm 0.06\) respectively at 500 \(\mu\)g/mL. Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins and steroids in the plant extracts. This current study suggests that the extracts of these investigated plants are potential sources of antioxidants. Further investigations are needed to exploit other possible potential medicinal uses of these plants.

**Keywords:** DPPH radical scavenging activity, phytochemical screening, *Amomum muricarpum*, *Hornstedtia conoidea* *Etlingera philippinensis*

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**INTRODUCTION**
Reactive oxygen species (ROS) are implicated in the pathogenesis of several diseases [1]. Hence, the accumulation of reactive and highly cytotoxic ROS must be under tight control [2]. Endogenous antioxidants defend our body from such phenomena. However, these antioxidants are gradually consumed over time and risk of oxidative stress increases with age [3]. Prevention strategies, such as exogenous antioxidants supplementation, may help restore the balance. Hence, natural antioxidants play key roles in this strategy [3].

Addition of natural antioxidants can increase shelf-life of food products containing fats and oils. In addition, natural antioxidants are safe and impart health benefits to the consumer [4]. The antioxidant compounds can be derived from natural sources such as plants [5].

There is increasing interest in the radical-scavenging activities of some natural antioxidants, especially those found in edible plants, which may play a role in preventing various chronic diseases [6]. Modern consumers ask for natural products, free of synthetic additives [7]. Therefore, the application of natural antioxidants will probably continue even in the future.

The Ginger family, Zingiberaceae, is a monocotyledonous [8] and the largest family in the order Zingiberales that comprises generally 300 genera and 1000 species [9]. It is among the plant families which are widely distributed throughout the tropics particularly in Southeast Asia [10]. Zingiberaceae species grow naturally in damp, shaded parts of the lowland or on hill slopes, as scattered plants or thickets [10]. Most members of the family are easily recognized by the characteristic aromatic leaves and fleshy rhizome when both of them are crushed, the elliptic to elliptic–oblong leaves arranged in two ranks
on the leaf-shoot [10], and typically have large rhizomes [11]. Many plants belonging to the family are used for food, spice [11] and a history of medicinal use in systems of traditional medicine [8] and [11].

Z. officinale has shown significant efficacy in nausea, vomiting, motion sickness and arthritis, and active compounds with antioxidant, anti-mutagenic, antimicrobial and anticancer properties have been isolated and require further scrutiny [12]. Ginger rhizomes are used traditionally for management of different gastrointestinal disturbances. Several studies proved that the rhizome possesses diverse biological activities such as cytotoxic, antioxidant, and anti-inflammatory effects [13]. Zingiber zerumbet (L.) Smith rhizomes have been demonstrated to possess anti-inflammatory, antinociceptive, antiallergic, antioxidant, anticancer, antimicrobial, antihyperglycemic, antiallergic, antioxidant, and antiplatelet activities at different doses/concentrations [14].

Amomum is the second largest genus in the Zingiberaceae family and has 24 species. In 1909, H.N. Ridley in Leaflets of Philippine Botany reported three species of Amomum from South Negros, namely Amomum fusiforme Ridl., Amomum lepicarpa Ridl. and Amomum lepicarpa pubescens Ridl. Elmer (1915) described the following species of Amomum from the Philippines: Amomum propinquum Ridl. from Baguio; Amomum mindanaense Elm. from Todaya (Mt. Apo), District of Davao, Mindanao, June 1909; Amomum muricarpum Elm. from Todaya (Mt. Apo), District of Davao, Mindanao, June 1909; Amomum palawanense Elm. from Puerto Princesa (Mt. Pulgar), Palawan; Amomum pandanicarpum Elm. from Todaya (Mt. Apo), District of Davao, Mindanao, May and September, respectively, 1909; Amomum pubimarginatum Elm. from Todaya (Mt. Apo), District of Davao, Mindanao, May, 1909. In 1919, Elmer reported the presence of 4 species of Amomum found in the Sorsogon peninsula [15].

Amomum muricarpum Elmer (Fig. 1) belongs to class Equisetopsida C. Agardh subclass Magnoliidae Novák ex Takht., superorder Lilianae Takht. order Zingiberales Griseb., family Zingiberaceae Martinov and genus Amomum Roxb. It was discovered in Mt. Apo, Todaya, Davao, Philippines in 1909 by Adolph Daniel Edward Elmer. It is commonly known as tugis in Bukidnon and tagbak in Bisaya. A. muricarpum Elmer grows 1 – 1.5 m long and most of its leaves are found at the upper 2/3 portion while there are reduced leaves at lower 1/3 portion. Leaves are lanceolate, generally 23-28 x 6-7 cm, base rounded, apex acuminate, margins entire. Its petiole is sub-sessile and its ligules are wavy to truncate, 1.5 to 2.0 mm and smooth. It has obconic inflorescence, 7-6 x 3.5 – 4 cm, and its bracts are membranous, triangular and slightly pubescent, pinkish. The plant’s bracteoles are tubular, apex irregularly toothed, pinkish measuring 6-7 mm long. Its calyx is elongated, fused, tubular, pinkish in color but tips are light green and hairy. The dorsal lobe of A. muricarpum is oblong while lateral lobe narrow oblong, yellow with red marks at center. The fruits of A. muricarpum are rambutan-like, with spines, fruits reddish purplish when mature [15].

Figure 1. A. muricarpum plant (A) and its rhizome (B).

A. muricarpum could be found in the inner forest of Impalutao, Impasugong, Bukidnon, Philippines Forest Reserve (Center for Ecological Development and Recreation). It is distributed in Cuernos Mountains, Davao Oriental, Mt. Hamiguitan and Negros (new distribution record). This species is rare and vulnerable. Fruits are reported by the local people (Mandaya) to be edible. According to the local guide, Larry Cahilog, the fruits of this plant are eaten by wild cats (Viverra tagalonga) and rodents [15].

In China, A. muricarpum is commonly known as you guo dou kou (Pinyin, China). Distribution of A. muricarpum is in China to Indo-China and Philippines (Mindanao). Giang et al. [16] isolated a new natural diarylheptanoid, designated muricarpin, together with four diarylheptanoids from the rhizomes of A. muricarpum Elmer (Zingiberaceae) growing in Vietnam. Three known compounds, 1,7-bis(3',4'-dihydroxyphenyl)heptan-3-yl acetate, 1-(4'-hydroxyphenyl)- 7 - (3',4'-dihydroxyphenyl) heptan-3-yl acetate and 1-(3',4'-dihydroxyphenyl)- 7 - (4'-hydroxyphenyl)-

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heptan-3-one were isolated for the first time from the genus *Amomum*, meanwhile (5R)-5-hydroxy-1,7-bis(4-hydroxyphenyl)-heptan-3-one was found for the first time in plants.

*Hornstedtia conoidea* Ridl. (Fig. 2) is commonly known as panaon, pinoon, and panon in Bisaya, tagbak in Mandaya, and puso-puso in Tagalog. The plant grows up to 3 m tall with green to brownish rhizome. Its leaves are broad lanceolate, coriaceous, base cuneate, or obtuse while its petioles are sessile. The ligule of *H. conoidea* Ridl. is oblong and pubescent. It has spindleshaped inflorescence, 9 x 3 cm. Involucr is present in the plant and its bracts are triangular, 6 x 2 cm, red except at base which is whitish. Its bracteole is tubular, membranous, pink at upper part, white at base, and 3 cm long. The calyx of the plant is elongated, membranous and light pink, 40 x 5 cm. It has an oblong and red corolla, 1.5 x 0.5 cm. *H. conoidea* Ridl. has a red and oblong labellum, without staminodes, 1.5 x 0.8 cm. The spindle-shaped inflorescence, presence of an involucre, and absence of staminode qualifies this species to belong to the genus *Hornstedtia* [15].

*H. conoidea* was found at 105 and 190 m above sea level and was recorded growing in the secondary growth dipterocarp forest of Bislig Experimental Forest, Bislig, Surigao del Sur, Mindanao, Philippines. This was also recorded to be present beside the river of the Mt. Hamiguitan Range Natural Park, Davao Oriental, Philippines. It is also distributed in Cuernos Mountains (type locality), Negros Oriental, Philippines, Surigao del Sur, Davao Oriental, and is endemic in the Philippines. This species is common in Surigao del Sur but their number may decline due to over collection of its fruits and no efforts to propagate the plant. The fruits of this plant are reported by the local people (Cebuano, Kamaoy) to be edible [15].

![Figure 2](image1.png)

**Figure 2.** *H. conoidea* plant (A) and its rhizome (B).

*Etlingera philippinensis* (Ridl.) M. Sm. (Fig. 3) was discovered in 1904 in the district of Davao, Mindanao, Philippines by E.B. Copeland. *E. philippinensis* was also reported to be found in Laguna, Quezon, Sorsogon, Mindoro, Palawan, Panay, Negros, Biliran, Leyte, Philippines, specifically in low and medium elevation forests. It belongs to the genus *Etlingera* and has subterranean, creeping and aromatic rhizomes. *Etlingera* plants can grow up to 5-6 m tall, forming dense clumps with stout and pungent rhizomes (3-4 cm diameter). Its leaves (100-150 cm long) are lanceolate and green. Crushed leaves emit a pleasant sour fragrance. The species is native to Malaysia and Indonesia, and is widely cultivated in the tropics for its inflorescences as spice [17].

![Figure 3](image2.png)

**Figure 3.** *E. philippinensis* Plant (A) and its rhizome (B).

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Work published so far covers many aspects but it is limited to the best-known fruits and vegetables [18]. Existing research work indicates that utilization of underexploited sources and better evaluation of ethnic and traditional foods can offer many benefits in the promotion of human health [18]. Despite the reported potential value of commonly known Zingiberaceae plants such as *Z. officinale* and *C. longa*, other plant species in this family especially those found in the Philippines, are not yet explored well. To the best of the authors' knowledge, there is no available scientific study on the antioxidant activity and phytochemical screening of *A. muricarpum* and Philippine endemic *E. philippinensis* and *H. conoidea*.

This study aimed to determine the radical scavenging activity using DPPH assay and phytochemicals present in the water and ethanol extracts of the leaves and rhizomes of the *A. muricarpum*, *H. conoidea*, and *E. philippinensis*.

MATERIALS AND METHODS

Chemicals and Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical was purchased from Wako Chemical Co., Tokyo, Japan. All other chemicals used were of analytical reagent grade.

Plant materials

*H. conoidea* and *E. philippinensis* were collected in June 2014 from Kibawe, Bukidnon, Philippines (7°34'7.77"N 124°55'21.284"E and 7°33'33.8976"N, 24°55'58.7964"E, respectively) in June, 2014. *A. muricarpum* plants were collected from Center for Ecological Development and Recreation (CEDAR), Impalutao, Impasugong, Bukidnon, Philippines (8°14'57.714"N, 125°01'50.7792"E) in October, 2014. Gratuitous permis was properly secured from the Department of Environment and Natural Resources (DENR) - Region 10. Plant samples were identified by Dr. Florfe M. Acma and Prof. Hannah P. Lumista of the Center of Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University, University Town, Musuan, Bukidnon. Voucher specimens of the plant samples were deposited at Central Mindanao University Herbarium (CMUH).

The plant leaf and rhizome samples were air-dried for three to four weeks, powdered using a blender, and stored inside airtight plastic container prior its extraction.

Preparation of plant extracts

**Water extract preparation.** Freshly collected leaves and rhizomes of the plant samples were cut into small pieces and boiled in sufficient distilled water for five minutes. The mixture was then filtered. The filtrate was freeze dried to remove water and the residue was stored at least at -15 °C until analysis.

**Ethanol extract preparation.** Powdered leaf and rhizome samples were separately soaked in 95% ethanol for 48 hours and filtered. The solvent in the filtrate was removed in vacuo using a rotary evaporator at a temperature below 40°C. The ethanol extract was stored inside refrigerator prior its usage.

**DPPH Radical Scavenging Activity**

DPPH radical scavenging activities of the water and ethanol extracts were determined using the method of Lee and Shibamoto (2001) as cited by [19]. Briefly, various amounts of the samples (500 µg/mL, 100 µg/mL, 50 µg/mL, 10 µg/mL) were mixed with 3 mL of methanolic solution of DPPH (0.1 mM). The mixture was shaken vigorously in a vortex mixer for 10 s and allowed to stand in the dark at room temperature for one hour. Then absorbance was measured at 517 nm against methanol as a blank in the Lasany double beam UV-Vis spectrophotometer model LI-2800 (Haryana, India). The DPPH solution alone in methanol was used as control. Each sample was assayed in triplicate and mean values were calculated. L-ascorbic acid was used as standard. The radical scavenging activity of samples corresponds to the intensity of quenching DPPH. The percent of DPPH discoloration of the samples was calculated and the results was expressed as percentage inhibition using the formula (equation 1) shown below.

\[
\% \text{ Inhibition} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100 \tag{1}
\]

where *A*_\text{control} and *A*_\text{sample} are the absorbance values of the control and test sample, respectively.

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The effective concentration of sample required to scavenge DPPH radical by 50% (EC\textsubscript{50}) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentration.

**Phytochemical Screening**

Phytochemical screening was performed following the method described by Aguinaldo et al., [20].

**RESULTS**

**DPPH Assay**

The data (Table1) represent the percentage inhibition of DPPH radical by the scavenging activity of the water and ethanolic extracts of the leaves and rhizomes of the studied Zingiberaceae plants. This is visibly indicated by the discoloration of the DPPH solution from purple to yellow.

Notable radical scavenging activities were observed in the water extracts of the rhizomes (9.79 ± 0.27) and leaves (6.64 ± 0.06) of _E. philippinensis_ which were significantly higher than that of ascorbic acid at 10 µg/mL. Ethanol extract of the _A. muricarpum_ rhizomes exhibited the highest radical scavenging activity of 94.73 ± 0.06 at 500 µg/mL. This radical scavenging activity is nearly the same when compared to that of the standard ascorbic acid (97.27 ± 0.40). This was followed by the water extract of _H. conoidea_ leaves (92.97 ± 0.23), water extract of _E. philippinensis_ leaves (92.07 ± 0.51), ethanol extract of the _A. muricarpum_ leaves (49.37 ± 0.23), ethanol extracts of the _E. philippinensis_ leaves (40.61 ± 0.87), water extract of the _A. muricarpum_ leaves (39.90 ± 0.82), water extract of the _A. muricarpum_ rhizomes (35.36 ± 0.18), and ethanol extract of _H. conoidea_ leaves (27.74 ± 0.07).

At 500 µg/mL, radical scavenging activities were significantly higher in the leaves of _H. conoidea_ and _E. philippinensis_ than in its rhizomes in both water and ethanolic extracts, and in the water extract of _A. muricarpum_. Ethanol extract of _A. muricarpum_ had higher radical scavenging activity in its rhizomes than its leaves. However, varying trends in the radical scavenging activities were observed at lower extract concentrations.

The concentration of the extracts to scavenge 50% of the DPPH radical (EC\textsubscript{50}) ranged from 251.4054 µg/mL in the water extract of the _E. philippinensis_ leaves to 1821.964 µg/mL in its rhizomes. These values are not comparable to that of the standard ascorbic acid which has EC\textsubscript{50} of 87.48 µg/mL.

**Phytochemical Screening**

Phytochemical screening of water and ethanolic extracts of the leaves and rhizomes of _A. muricarpum, H. conoidea, and E. philippinensis_ showed the presence of alkaloids, flavonoids, saponins, tannins, steroid (Table 2).

**DISCUSSION**

**DPPH Radical scavenging activity**

Free radicals cause lipid peroxidation and production of highly toxic lipid derivatives, which in turn can modify cell functions and even may lead to cell death [1]. Cellular damage or oxidative injury arising from free radicals or ROS now appears the fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders [21].

DPPH radical scavenging assay has been widely used to evaluate the radical scavenging ability of the plant extracts as it is simple and highly sensitive [22]. The DPPH assay utilizes DPPH* radical, one of the few stable organic nitrogen radicals, which bears a deep purple color [23]. DPPH radical is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [24]. This assay is based on the measurement of the reducing ability of antioxidants toward DPPH* [23]. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants [24]. DPPH was reduced to a pale yellow color due to the abstraction of hydrogen atom from antioxidant compound [24]. The more antioxidants occurred in the extract, the more the DPPH reduction will occur [24].

An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules [25], protects the body from reactive species [26], and may, therefore, have health-promoting effects in the prevention of degenerative diseases [25]. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells [21].

Thus, an increase in the consumption of dietary antioxidants, which can scavenge free radicals, may be a strategy to prevent free radical-induced damage to biomolecules of lipids, proteins, and DNA, including low density lipoprotein oxidation and cancer cell initiation, an important beginning stage of carcinogenesis [27]. Antioxidant research is a key topic in both the medical and food industry today [26].
Zingiberaceae plants have received much attention since they produce many complex compounds that are useful in food as herbs and spices, flavoring and seasoning, and in the cosmetics and medicinal industries as antioxidant and antimicrobial agents [28]. Several past antioxidant studies on gingers species were confined to rhizomes [29, 10, 30, 14, 31, 32]. Although leaves of ginger species have been used for food flavoring and in traditional medicine, little research has been done on their antioxidant properties until recent years [17].

Table 1. DPPH radical scavenging activity of the water and ethanol extracts of the leaves and rhizomes of *A. muricarpum*, *H. conoidea*, and *E. philippinensis*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plant Part</th>
<th>Extraction Solvent</th>
<th>Average % Inhibition*</th>
<th>EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 µg/mL</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Rhizomes</td>
<td>Water</td>
<td>5.97 ± 1.07</td>
<td>44.50 ± 1.21</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Ethanol</td>
<td>2.74 ± 0.07</td>
<td>4.44 ± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>0.00 ± 0.00</td>
<td>12.02 ± 0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>2.89 ± 0.50</td>
<td>4.44 ± 0.07</td>
</tr>
<tr>
<td><em>H. conoidea</em></td>
<td>Rhizomes</td>
<td>Water</td>
<td>9.79 ± 0.27</td>
<td>10.69 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Ethanol</td>
<td>0.50 ± 0.07</td>
<td>1.13 ± 0.06</td>
</tr>
<tr>
<td><em>E. philippinensis</em></td>
<td></td>
<td>Leaves</td>
<td>6.64 ± 0.06</td>
<td>15.25 ± 0.35</td>
</tr>
<tr>
<td><em>A. muricarpum</em></td>
<td>Rhizomes</td>
<td>Water</td>
<td>0.62 ± 0.00</td>
<td>4.02 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Ethanol</td>
<td>1.59 ± 0.27</td>
<td>8.92 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>1.90 ± 0.16</td>
<td>3.30 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>1.49 ± 0.20</td>
<td>3.67 ± 0.33</td>
</tr>
</tbody>
</table>

* - mean of 3 replicates and expressed as mean ± RSD

Table 2. Phytochemical profile of the water and ethanolic extracts of the leaves and rhizomes of *A. muricarpum*, *H. conoidea*, and *E. philippinensis*

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th><em>H. conoidea</em></th>
<th><em>A. muricarpum</em></th>
<th><em>E. philippinensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>A</td>
<td>E</td>
<td>A</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>(2-deoxysugars)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cyanogenic</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend: "A" Water extract, "E" Ethanolic extract, "+" Present, "−" Absent, "++" poor, "+++" moderate, "++++" heavy

In Zingiberaceae, it is generally believed that antioxidants and other secondary metabolites are transported to the rhizomes where they are accumulated [33]. This implies that rhizomes would have higher antioxidant activity than would other plant parts [33]. Rhizomes of cultivated species have been reported to possess radical-scavenging compounds comparable to commercial antioxidants on a weight per weight basis [33].

In this study, ethanol extract of *A. muricarpum* has higher radical scavenging activity in its rhizomes than its leaves. However, *A. muricarpum* water extract and both water and ethanol extracts of *H. conoidea* and *E. philippinensis* have higher antioxidant activity than in its rhizomes.

Findings on the DPPH radical scavenging activities of *H. conoidea* and *E. philippinensis* and on the water extract of *A. muricarpum* were similarly observed in some previous studies. A study on 26 ginger species, belonging to nine genera and three tribes, showed that antioxidant properties of leaves were strongest in *Etlingera* followed by *Alpinia* and *Hedychium*. Eleven out of 14 species (78%) had significantly higher values in leaves than in rhizomes. Similar trends were also observed in other species of *Zingiber*, *Boesenbergia* and *Elettariopsis* [17]. Another study on the *Etlingera elatior* revealed significantly higher antioxidant properties in leaves than inflorescences and rhizomes [17].

Moreover, ethyl acetate extracts from *Alpinia zerumbet* leaves showed higher DPPH radical scavenging activities than those from rhizomes [34]. The antioxidant activities of methanol extracts from two *Z.*
officinale varieties (Halia Bentong and Halia Bara) as determined by the DPPH assay and the total amounts of phenolics and flavonoids were higher in leaves than those of the rhizomes and stems. On the other hand, the ferric reducing/antioxidant potential activity of the rhizomes was higher than that of the leaves [35].

In comparison between the extraction solvents, higher antioxidant activities were observed in the water extracts of H. conoidea leaves and E. philippinenses leaves and rhizomes than in its ethanol extracts. Better activities were observed in the ethanol extracts than water extracts of H. conoidea rhizomes and both A. muriarum leaves and rhizomes. Varying results were also observed in the study of Yeh et al. [36]. The antioxidant effect of the ethanol extracts of two Z. officinale Roscoe varieties, namely: Guangdongginger and Chu-ginger in Taiwan, were found to be more effective than water extracts in Trolox equivalent antioxidant capacity and Ferric reducing ability of plasma. Contrarily, ginger water extracts were more effective in free radical scavenging activities and chelating abilities [36].

Ten water extracts from herbs (i.e. Aegle marmelos L., Andrographis paniculata Nees, Chrysanthemum indicum L., Cymbopogon citratus Stapf., Hibiscus sabdariffa L., Jubliang and Z. officinal Rosc.) prepared by boiling in hot water for 10 min displayed to be good sources of water soluble antioxidants, phenolic compounds and antimutagens [37]. Comparative study on the antioxidant activity of E. elatior flower from Indonesia using DPPH assay revealed that E. elatior in the methanol extract has higher antioxidant potential as compared to ethyl acetate extract [38].

Ethanol is more efficient in cell wall and seed degradations which have non-polar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of water extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive [39]. These may explain higher antioxidant activity in some ethanol extracts in this study. Antioxidant activities were found to be not comparable with the standard ascorbic acid. These results were similar to that obtained in the study of Peteros and Uy [40] on the crude methanol extract of four Philippine medicinal plants namely: Brucea amarissima (Lour.) Merr, Bark, Intsia bijuga (Coebr.) O. Kuntze, Laportea meyeniana Warb, and Pipturus arborescens (Link) C.B. Rob leaves. This is understandable, since L-ascorbic acid is already in a pure form, while the crude plant extracts still need to be processed in order to isolate the compounds responsible for their antioxidant activity [40]. The DPPH radical scavenging abilities of the Z. officinale varieties (Halia Bentong and Halia Bara) rhizomes extracts were less than those of butylated hydroxytoluene (96.21%) and α-tocopherol (89.57%) at 45 μg/mL [41].

Phytochemical analysis

Natural products are gaining attention owing to their rich phytochemistry and risk-free use [42]. The phytochemical constituents of plants have received much attention due to their potential utilization in the nutraceutical and drug industries [43].

Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds [44]. These natural antioxidants present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress [44]. Fruits and vegetables are also known to contain various forms of phytochemicals and antioxidants, particularly polyphenol compounds (e.g., flavonoids and anthocyanins). Their frequent consumption has been associated with a lowered risk of heart disease, cancer, hypertension, and stroke [14].

Antioxidant activity of the plants is due to the presence of flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins [5]. Phenolic compounds play key role as antioxidants due to the presence of phenolic groups (hydroxyl substituents next to their aromatic structures), which enable them to scavenge free radicals [43].

Flavonoids, alkaloids, saponins, tannins and steroids found in the water and ethanol extracts of H. conoidea, E. philippinensis and A. muriarum were similarly observed in other Zingiberaceae plants. Z. officinale was reported to contain alkaloid [45-46], tannin [45-46], carotenoids [45], saponin [45, 47], flavonoids [45-46], and cardiac glycosides [46]. Flavonoid compounds (quercetin, apigenin, luteolin, and myricetin) and phenolic acids (gallic acid, vanillic acid, ferulic acid, tannic acid and caffeic acid) were identified with different concentration in leaves and rhizomes of ginger varieties. The most abundant phenolic acid in ginger was gallic acid, and flavonoids were quercitin and apigenin [48].

Methanol and ethyl acetate extracts of the flower of E. elatior revealed the presence of tannins, flavonoids, saponin and steroid [38]. The methanol extract of the leaves of Curcuma alismatifolia Gangnep were found to contain flavonoids, alkaloid and gum [49].
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The antioxidant activities revealed in this study may be attributed to the presence of flavonoids in all the water and ethanol extracts of H. conoidea, E. philippinensis and A. muricarpum leaves and rhizomes. Several studies revealed the association of flavonoids to the antioxidant activity of plant extracts. Z. officinale is extensively reported to possess antioxidant activity against a variety of free radicals [5]. The high level of total phenolic and flavonoid in Halia Bara (Z. officinale variety) indicated high antioxidant activities [35]. Essential oil from E. elatior has high total phenolic content and total flavonoid content and possessed antioxidant activity when evaluated using the DPPH assay [50]. Oral administration of water extract of Z. officinale along with paraben significantly (p ≤ 0.05) ameliorates paraben-induced lipid peroxidation in the liver of mice [51].

The total phenolic and flavonoid contents appear to be responsible, at least in part, for the Ficus virens var. sublanceolata and Ficus auriculata extracts’ excellent antioxidant capacity and all the extracts exhibited dose-dependent antioxidant activity employing 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid and DPPH radical scavenging capacities, ferric reducing power and lipid peroxidation inhibition properties [52].

Positive and significant relationship (r²=0.942) with high significance (P<0.001) was obtained between total flavonoid content and vitamin C equivalent antioxidant capacity in the six cultivars of plums studied [25]. The high alkaloid and flavonoids contents of the plants, suggests their antioxidant potentials and justifies their therapeutic actions, which could be used in drug formulation. The antioxidant potentials of plants have been linked with their flavonoids contents [53].

The phytochemical analysis of the crude extracts 12 traditionally used Indian medicinal plants indicated the presence of major phytocompounds, including phenolics, alkaloids, glycosides, flavonoids, and tannins, which may have been responsible for the observed antioxidant activity [54]. It was suggested that there seemed to be a good correlation among gallic acid, and other phenolic and flavonoid compounds and antioxidant activity and other biological activity in Citrus aurantium [26].

Flavonoids are a group of polyphenolic compounds, diverse in chemical structure and characteristics, found ubiquitously in plants [55]. They constitute an enormous collection of biologically active compounds that are ubiquitous in plants, many of which have been used in traditional Eastern medicine for thousands of years [41]. Flavonoids are potent antioxidants, free radical scavengers [55, 41], metal chelators and inhibit lipid peroxidation [55], thus displaying anti-aging properties and reducing the risk of cancer [41]. The structural requirements for the antioxidant and free radical scavenging functions of flavonoids include a hydroxyl group in carbon position three, a double bond between carbon positions two and three, a carbonyl group in carbon position four, and polyhydroxylation of the A and B aromatic rings [55]. The presence of phenolic compounds such as eugenol, shogaols, zingerone, gingerdiols, gingerols, diacetoxy-[6]-gingerdial, etc., in ginger oil and oleoresins may be responsible for their antioxidant properties [56]. They were found to be better antioxidants than butylated hydroxyanisole [56].

Several alkaloids of various structural types have been found to be potent inhibitors of O2 [57]. Two quinoline alkaloids, 3,8-dihydroxyquinoline and 2,8-dihydroxy-3,4-dimethoxyquinoline, and 2,4-diterbutylphenol isolated from the dried body of Scolopendra subspines showed DPPH radical scavenging activity [58]. The alkaloids cepharanthine and fangchinoline from Stephania rotunda have effective antioxidant and radical scavenging activity [59]. Coptidis Rhizoma and the alkaloids contained therein clearly have beneficial uses in the development of therapeutic and preventive agents for Alzheimer’s disease and oxidative stress-related disease [60].

Tannins, a unique group of phenolic metabolites with molecular weights between 500 and 30000 Da, are widely distributed in almost all plant foods and beverages [61]. Tannins were found to have redox potentials similar to those of structurally related simple phenolics but 15-30 times more effective at quenching peroxyl radicals than simple phenolics or Trolox. Polygalloyl glucose, a tannin, reacted an order of magnitude more quickly with hydroxyl radical than mannitol. These results suggest that tannins, which are found in many plant-based foods and beverages, are potentially very important biological antioxidants [62].

Using the Folin-Ciocalteu and DPPH assays, respectively, highest total phenolic content and ascorbic acid equivalent capacity were observed in the polymeric tannin fraction of Alpinia galanga rhizomes, in the crude extract and non-polymeric phenolic fractions fraction of Curcuma longa rhizomes, and in the polymeric tannin fractions fraction of E. elatior leaves [17].

The presence of alkaloids and tannins in the A. muricarpum, H. conoidea, and E. philippinensis extracts possibly contribute to antioxidant activities demonstrated in these plants.
CONCLUSION
DPPH radical scavenging activity and phytochemical analysis of the *A. muricarpum*, *H. conoidea*, and *E. philippinensis* were done for the first time. *A. muricarpum* rhizomes showed considerable high radical scavenging activity. It is noteworthy that the leaves of *H. conoidea* and *E. philippinensis* exhibited better radical scavenging activity than its rhizomes. The presence of flavonoids and tannins and the observed radical scavenging activity in this current study may suggest the antioxidant property of these studied plants. Investigation at the molecular level on this Zingiberaceae species is needed to exploit its potential medicinal uses.

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Conflict of interest
The authors declare no conflict of interest.

REFERENCES


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