Research Article

DPPH Radical Scavenging Potential of Ginger Leaves and Rhizomes

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ABSTRACT

Introduction: Gingers, belonging to the family Zingiberaceae, are popularly known for their beneficial uses in medicine and culinary applications. **Aim:** This study was conducted to evaluate the DPPH radical scavenging activity of the leaves and rhizomes of *Zingiber officinale* Rosc., *Curcuma longa* L., and *Etlingera elatior* (Jack) R.M. Smith. **Methods:** The plant samples were collected from Bukidnon, Mindanao, Philippines. Both water and ethanolic extracts were prepared separately from its leaves and rhizomes. The extracts were subjected to the determination of DPPH radical scavenging activity relative to ascorbic acid. **Results and Discussion:** Leaves, in general, had higher radical scavenging activity in water than in ethanol extracts. On the other hand, rhizomes had generally higher radical scavenging activity in ethanol than in water extracts except for *E. elatior*. Among the leaf extracts, *E. elatior* possessed the highest radical scavenging activity. In both water and ethanol, *E. elatior* displayed higher radical scavenging activity in its leaves that its rhizomes. **Conclusion:** Findings of this study suggest the potential of *E. elatior* leaves as source of antioxidants.

Key words: Gingers, *Curcuma longa, Etlingera elatior*, *Zingiber officinale,* DPPH.

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INTRODUCTION

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 reactive oxygen species now appears to be the fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders.[2]

An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules,[3] protects the body from reactive species[4] and may, therefore, have health-promoting effects in the prevention of degenerative diseases.[3] Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells.[2]

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 deoxyribonucleic acid, including low density lipoprotein oxidation and cancer cell initiation, an important beginning stage of carcinogenesis.[5] Antioxidant research is a key topic in both the medical and food industry today.[4] The DPPH assay is a very popular[6] and simplest method which is considered the first approach to evaluate the antioxidant potential of an extract, compound or biological sources.[7] DPPH assay utilizes DPPH$^{\bullet}$ radical, one of the few stable organic nitrogen radicals, which bears a deep purple color. It is commercially available and does not have to be generated before assay. This assay is based

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on the measurement of the reducing ability of antioxidants toward DPPH•. The ability can be evaluated by electron spin resonance or by measuring the decrease of its absorbance.[8]

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.[9] In Zingiberaceae, it is generally believed that antioxidants and other secondary metabolites are transported to the rhizomes where they are accumulated.[10] This implies that rhizomes would have higher antioxidant activity than would other plant parts.[10] Rhizomes of cultivated species have been reported to possess radical-scavenging compounds comparable to commercial antioxidants on a weight per weight basis.[10] With these, several past antioxidant studies on ginger species were confined to rhizomes.[11-16] The most common Zingiberaceae plant is the *Zingiber officinale*, commonly known as ginger. *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.[17] Another rhizomatous herbaceous perennial plant of the Zingiberaceae family is *Curcuma longa* L. Once a native to South Asia, it is now widely cultivated in the tropical and subtropical regions of the world[18] Commonly known as the golden spice turmeric, *C. longa* has been popular because of its component curcumin. Some promising effects have been observed on patients with cancer, arthritis, ulcerative proctitis, ulcerative colitis, psoriasis, atherosclerosis, diabetes, lupus nephritis, renal conditions, acquired immunodeficiency syndrome, gastric inflammation, vitiligo, Crohn's disease, irritable bowel disease, tropical pancreatitis, acquired immunodeficiency syndrome and cholecystitis.[19] *Etlingera elatior* (Jack) R. M. Smith is a natural species in Sumatra, Indonesia and has been distributed throughout Southeast Asia. In Peninsular Malaysia, its young flowers shoots can be eaten raw and used for flavoring in local dishes.[20] It is traditionally used for flavoring[20-21] and medicine.[21]

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.[22] It is imperative that scientific attention should also be given to ginger leaves. In this study, the DPPH radical scavenging activity of the leaves and rhizomes of *Curcuma longa*, *Etlingera elatior* and *Zingiber officinale* were evaluated.

MATERIALS AND METHODS

Plant Sampling

E. elatior plant samples were collected in June 2014 while *C. longa* and *Z. officinale* were collected in October, 2014 in Musuan, Maramag, Bukidnon, Philippines.

Preparation of plant extracts

Preparation of plants extracts was done as previously reported in Barbosa *et al.*[23] Briefly, cut plant samples of freshly collected leaves and rhizomes were boiled in sufficient distilled water for five minutes and filtered. The residue left after freeze drying was stored at least -15°C until analysis. This served as the water extract. Ethanol extracts were prepared by soaking separately leaves and rhizomes in 95 % ethanol for 48 hrs. The ethanol extract obtained after solvent removal *in vacuo* using a rotary evaporator at 40°C 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.[24] Briefly, various amounts of the samples (500-, 100-, 50- and 10 µg/mL) were mixed with three mL of methanolic solution of DPPH (0.1 mM). The mixture was shaken vigorously in a vortex mixer for 10 seconds and allowed to stand in the dark at room temperature for one hour. Absorbance was then measured at 517 nm against methanol as a blank in a Lasany double beam UV-Vis spectrophotometer (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 results expressed as percentage inhibition using the formula shown below:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

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

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

RESULTS

DPPH Radical Scavenging Activity

Percentage inhibition represents the DPPH radical scavenging activity of the sample extract. Figure 1 depicts a representative concentration-percent (%) inhibition curve for the DPPH radical scavenging activity. Median effective concentration of each extract, that is, the concentration of the extract required to scavenge 50 % of the DPPH radical (EC_{50}), was calculated from the linear equations of the regression lines. In general, a linear trend was observed between the plant extract concentration and DPPH radical scavenging activity; that is, an increase plant extract concentration resulted to an increase percentage inhibition.

The data in Table 1 shows the percentage inhibition of DPPH radical by the scavenging activity of the ethanol and water extracts of the leaves and rhizomes of the studied Zingiberaceae plants and the EC_{50} values. The lower the EC_{50} value, the higher is the radical scavenging activity of the plant extract.

In order to illustrate direct relationship between EC_{50} and antioxidant activity, anti-radical power, $1/EC_{50}$, was calculated by getting the reciprocal of EC_{50} value. High DPPH radical scavenging activity was manifested by the higher $1/EC_{50}$ and lower EC_{50} values. The anti-radical power ($1/EC_{50}$), graphically presented in Figures 2 and 3, was visibly indicated by the discoloration of the DPPH solution from purple to yellow.

As depicted in Figure 2, the leaf ethanol extract of *E. elatior* had the highest anti-radical power among the leaf

Table 1: DPPH radical scavenging activities of the ethanol (E) and water (W) extracts of the selected Zingiberaceae plants.

Sample Code	Average % Inhibition (Mean ± SD of three trials)				EC_{50}
	10 mg/L	50 mg/L	100 mg/L	500 mg/L	
Ascorbic Acid (AA)	8.20 ± 0.23	48.43 ± 0.29	93.46 ± 0.50	97.04 ± 0.06	72.56
EERE	0.00 ± 0.00	1.14 ± 0.06	2.57 ± 0.21	10.54 ± 0.06	2376.47
EELE	1.89 ± 0.06	6.54 ± 0.28	12.90 ± 0.54	75.28 ± 0.71	335.30
ZORE	2.25 ± 0.20	13.72 ± 0.60	26.83 ± 0.77	82.06 ± 0.15	286.16
ZOLE	0.68 ± 0.39	1.77 ± 0.28	4.80 ± 0.06	17.10 ± 0.39	1487.87
CLRE	2.33 ± 0.12	10.79 ± 0.18	22.20 ± 0.47	79.59 ± 0.37	303.38
CLLE	0.54 ± 0.13	3.41 ± 0.29	7.14 ± 0.13	36.09 ± 0.20	692.02
EERW	29.22 ± 0.35	30.61 ± 0.12	31.97 ± 0.05	44.52 ± 0.30	675.00
EELW	29.09 ±0.79	40.88 ± 0.28	52.86 ± 1.12	95.44 ± 0.08	128.74
ZORW	0.83 ± 0.17	1.44 ± 0.13	4.30 ± 0.56	23.73 ± 0.39	1034.55
ZOLW	2.02 ± 0.13	3.54 ± 0.17	5.56 ± 0.23	21.70 ± 0.23	1201.91
CLRW	1.65 ± 0.00	1.89 ± 0.20	2.99 ± 0.38	5.51 ± 0.27	6266.74
CLLW	0.42 ± 0.07	3.32 ± 0.07	5.02 ± 0.57	35.26 ± 0.85	684.64

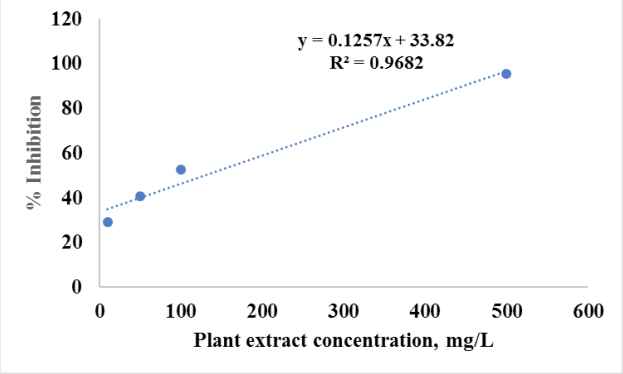

Figure 1: A typical dose -response curve of the water extract *E. elatior* leaves.

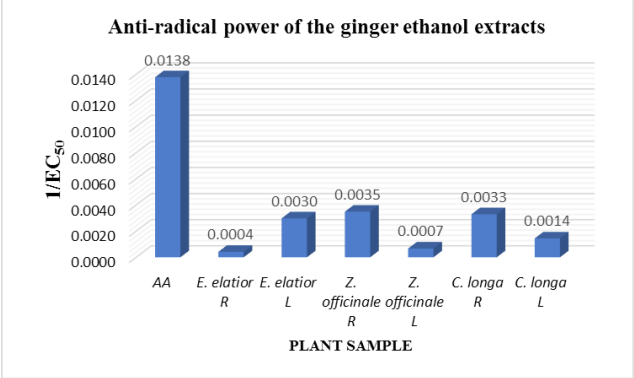

Figure 2: Anti-radical power (1/EC50) of the ethanol extracts of the Zingiberaceae plants (R - Rhizomes; L –Leaves; AA-Ascorbic Acid).

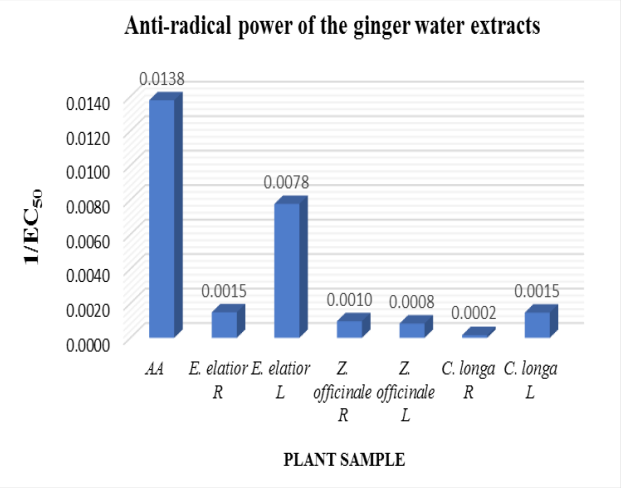

Figure 3: Anti-radical power (1/EC50) of the water extracts of the Zingiberaceae plants (R - Rhizomes; L - Leaves).

samples while *Z. officinale* has the least. Among the rhizome ethanol extracts, ZO has the highest anti-radical power followed by *C. longa*, then *E. elatior* having the lowest.

Figure 3 revealed that among the water extracts, *E. elatior* leaf extract consistently possessed the highest anti-radical power. Among the rhizome water extracts, *E. elatior* still ranked first in terms of anti-radical power.

Overall, *E. elatior* leaf extracts had the highest radical scavenging activity among the plant leaf samples in both water and ethanol solvents. *E. elatior* had higher radical scavenging activity in leaves than its rhizomes in both ethanol and water extracts while the opposite was observed in *Z. officinale*. In *C. longa*, rhizome has higher anti-radical power in rhizomes in ethanol but the reverse was true in water extract.

DISCUSSION

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.[25] The DPPH assay utilizes DPPH• radical, one of the few stable organic nitrogen radicals, which bears a deep purple color.[8] DPPH radical is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[26] This assay is based on the measurement of the reducing ability of antioxidants toward DPPH•.[8] The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants.[26] DPPH was reduced to a pale yellow color due to the abstraction of hydrogen atom from antioxidant compound.[26] The more antioxidants present in the extract, the more the DPPH reduction will occur.[26]

In this study, the water and ethanol extracts of the studied ginger species displayed radical scavenging activity. In comparison between the extraction solvents, that is, between water and ethanol, varying results were observed among samples in this study. Varying results were also observed in the study of Yeh *et al.*[27] The antioxidant effect of the ethanol extracts of two *Z. officinale* Roscoe varieties, namely: Guangdong ginger 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. In contrast, ginger water extracts were more effective in free radical scavenging activities and chelating abilities.[27]

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. officinale* Rosc.) prepared by boiling in hot water for 10 minutes exhibited to be good sources of water soluble antioxidants, phenolic compounds and antimutagens.[28]

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[29] 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.[29] 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.[30]

In Zingiberaceae, it is generally believed that antioxidants and other secondary metabolites are transported to the rhizomes where they are accumulated.[10] This implies that rhizomes would have higher antioxidant activity than would other plant parts.[10] Rhizomes of cultivated species have been reported to possess radical-scavenging compounds comparable to commercial antioxidants on a weight per weight basis.[10]

In this study, higher radical scavenging activity in leaves than in rhizomes was consistently observed in *E. elatior*. Higher antioxidant activities in leaves than rhizomes 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*.[22] Another study on the *Etlingera elatior* revealed significantly higher antioxidant properties in leaves than inflorescences and rhizomes.[22] Varying results, however, was observed in *C. longa* and *Z. officinale* wherein only in water extracts of *C. longa* that the leaves had higher radical scavenging activity. This observation was similarly observed in previous studies. For instance, ethyl acetate extracts from *Alpinia zerumbet* leaves showed higher DPPH radical scavenging activities than those from rhizomes.[31] 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. However, the ferric reducing/antioxidant potential activity of the rhizomes was higher than that of the leaves.[32]

Phytochemical analysis of ginger plants revealed the presence of phytochemicals. For instance, a number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids and polyphenols such as flavonoids and flavones glycosides were found in *Z. officinale* Roscoe.[32] The ethanol extract of the rhizomes *A. purpurata*, another member of ginger family, was reported to have antioxidant activity and contained flavonoids, alkaloids, saponins, carbohydrates, proteins, phenols, resins, glycosides and tannins.[33] The acetone extract of *A. galanga* L. rhizomes revealed higher antioxidant activity and higher phenolics and flavonoids content than the ethanol extracts. Galangin and kaempferide, isolated flavonoids from *A. galanga* L. rhizomes, scavenged hydroxyl radicals in a concentration dependent manner. Both flavonoids were found to protect DNA from radiation induced lesions resulting from radiation exposures under *in vitro* and *ex vivo* conditions.[33] Phytochemical screening of the ethanol extract of *Curcuma zedoaria* rhizomes showed the presence of tannins, flavonoids, saponins, alkaloids, terpenoids, carbohydrates and steroids. Moreover, the extract exhibited anti-pyretic effect using the Brewer‟s yeast administered fever inducing method.[35] Metabolomic study revealed the presence of antioxidant compound such as quercetin, shikimic acid, citric acid and kaempferol in the leaves of *E. elatior*, *C. longa* and *Z. officinale*.[36] The leaves of *E. elatior* and *C. longa* were also found to contain chlorogenic acid.[36]

CONCLUSION

It is noteworthy that the water extract of *E. elatior* had the highest radical scavenging activities in leaves than its rhizomes. The opposite was observed in the water extract of *C. longa*. For the ethanol extracts, higher radical scavenging activities in leaves were similarly demonstrated for *E. elatior* only. Ethanol extracts of *C. longa* and *Z. officinale* had higher radical scavenging in rhizomes than its leaves. Leaves, in general, had higher radical scavenging activity in water than in ethanol extracts. On the other hand, rhizomes had generally higher radical scavenging activity in ethanol than in water extracts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study reports on the antioxidant activity of water and ethanol extracts of the leaves and rhizomes of the common gingers, namely: *Zingiber officinale* Rosc., *Curcuma longa* L., and *Etlingera elatior* (Jack) R.M. Smith. Leaves, in general, had higher radical scavenging activity in water than in ethanol extracts. On the other hand, rhizomes had generally higher radical scavenging activity in ethanol than in water extracts except for *E. elatior*. Among the leaf extracts, *E. elatior* possessed the highest radical scavenging activity. In both water and ethanol, *E. elatior* displayed higher radical scavenging activity in its leaves that its rhizomes. Findings of this study suggest the potential of *E. elatior* leaves as source of antioxidants.

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