Research Article

Cyclic Voltammetry and Spectrophotometric Determination of Antioxidant Activities of Selected Ginger Species

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ABSTRACT

Introduction: *Zingiberaceae* rhizomes are commonly investigated due to its applications in food and traditional medicine use. However, less scientific attention was given to its leaves. **Aim:** This study primarily aimed to compare the antioxidant activity of the leaves and rhizomes of common *Zingiberaceae* plants namely: *Zingiber officinale*, *Curcuma longa* and *Etlingera elatior.* **Methods:** Antioxidant activity of the water and ethanol extracts of the leaves and rhizomes of the studied plants was determined using cyclic voltammetry (CV) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. **Results:** Very low positive correlation (r = 0.22) of antioxidant activities was observed between DPPH and CV methods. In both DPPH and CV methods, *E. elatior* has generally higher antioxidant activity in leaves than in rhizomes and water extract than ethanol extracts. Higher antioxidant activity was observed in leaves than in rhizomes for *Z. officinale* and *C. longa* for both water and ethanol extracts. In general, water extracts of the three ginger plants have higher antioxidant activity than its ethanol extracts. **Conclusion:** Both CV and DPPH assay revealed that leaves of common gingers studied are potential sources of antioxidants.
Key words: DPPH radical scavenging activity, Cyclic voltammetry, Gingers.

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INTRODUCTION

The role of assessing the antioxidant activity of plant sources is pivotal in the search for more plant sources of antioxidants. Hydrogen atom transfer, single electron transfer, reducing power and metal chelation are among the mechanisms involved in the monitoring of antioxidant activity.[1] A number of methods proposed to measure antioxidants in botanicals has increased considerably.[2] Antioxidant activity can be measured spectrophotometrically and electrochemically. However, there are problems encountered in using spectrophotometric methods for the determination of antioxidant activity of turbid extracts of vegetables, fruits or herbs. Electrochemical approach such as voltammetry and polarography has certain advantages, such as the possibility of direct measurement, quickness and high sensitivity.[3]
The measurement of antioxidant activity using electrochemical methods surfaced in the past decade. Among these methods, Cyclic Voltammetry (CV) attracted much attention as an alternative method to conventional chemical assays.[1] In CV, electron donation capability (redox potential) of antioxidants is measured. Antioxidants respond to a voltammetric scan according to their redox potential. The oxidation potential of a specific antioxidant compound or functional group (usually phenyl group) and its concentration is reflected in the cyclic voltammogram generated.[1]
Cyclic Voltammetry (CV) is rapid, simple[4] and does not require sophisticated chemical reagents or solvents1 and special or advanced sample preparation.[1,4] The CV tracing provides the total reducing power of the sample without the necessity to measure the specific antioxi-

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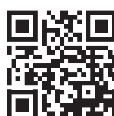

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dant capacity of each component alone.[5] Labor-intensive characterization of the activity of each component against a specific Reactive Oxygen Species (ROS) is not required in CV. This serves as the basis for other methodologies measuring the total antioxidant capacity.[4] CV has been employed in evaluating the total (integrated) antioxidant capacity of low molecular weight antioxidants in human plasma, animal tissues and edible plants.[4] CV methodology is suitable for screening studies and its sensitivity is sufficient for determining the physiological concentrations of antioxidants.[4]

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a spectrophotometric assay which utilizes DPPH● radical, one of the few stable organic nitrogen radicals, which bears a deep purple color. DPPH assay is based 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.[6]

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.[7] Several past antioxidant studies on gingers species were confined to rhizomes.[8-13] Little research has been done on the antioxidant properties of ginger leaves until recent years despite for their uses in food flavoring and traditional medicine.[14]

Zingiberaceae plants, commonly known as gingers, typically have large rhizomes that are used for food, spice, or traditional medicines.[15] Moreover, the rhizomes are eaten raw, or cooked as vegetables and as flavoring. A study on the Philippine endemic *Zingiberaceae* plants such *Etlingera philippinensis* revealed that phenolic compounds have profound contribution to the antioxidant activities.[16] This was similarly observed on the ethanolic extract of the *Zingiberaceae* plant *Hedychium coronarium*.[17] Metabolomics study of Amomum muricarpum, *E. philippinensis* and *Hornstedtia conoidea* showed the presence of chlorogenic acid and shikimic acid in its leaves.[18] It was also previously reported that both leaves and rhizomes of the commercial ginger is the cultivated species *Zingiber officinale* (ZO).[19] *Curcuma longa* L. (CL) is another rhizomatous herbaceous perennial plant of the ginger family. Once a native to South Asia, but is now widely cultivated in the tropical and subtropical regions of the world.[20] Commonly known as the golden spice turmeric, *C. longa* has been popular because of its component curcumin. Curcumin can modulate multiple cell signaling pathways. 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.[21] *Etlingera elatior* (Jack) R. M. Smith (EE) 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.[22] It is traditionally used for flavoring[22,23] and medicine.[23]

This study primarily aimed to determine and compare the antioxidant activity using cyclic voltammetry and DPPH assay of the water and ethanol extracts of the leaves and rhizomes of selected *Zingiberaceae* plants grown in Mindanao, Philippines.

MATERIALS AND METHOD

Chemicals and Reagents

All chemicals used were of analytical reagent grade. DPPH radical was purchased from Wako Chemical Co., Tokyo, Japan.

Plant materials

Sample collection was done within the province of Bukidnon, Mindanao, Philippines. In particular, ZO was collected from Portulin, Pangantucan, Bukidnon (7° 51' 13.91"N 124° 51' 31.45"E); CL from Upper Gutapol, Kibawe, Bukidnon (7°33'30.0384"N 124°56'6.558"E); EE from Lower Lumbayao 1, Gutapol, Kibawe (7°34'0.6852"N 124°56'0.1536"E). 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 were deposited at the herbarium of the same University.

Preparation of plant extracts

Water and ethanol extracts were prepared as previously reported by Barbosa, Peteros, Inutan.[24] Briefly, freshly collected leaves and rhizomes of plant samples were boiled in distilled water for five minutes and the filtrate was freeze-dried to remove the solvent. For the ethanol extract, the pulverized air-dried leaf and rhizome samples were separately soaked in 95% ethanol for 48 hrs. After which, in vacuo solvent removal using rotary evaporator was done at a temperature below 40°C. The extracts were stored at least -15°C prior analysis.

DPPH Radical Scavenging Activity

The method of Lee, Shibamoto[25] was employed in the determination of DPPH radical scavenging activities of the water and ethanol extracts. Varied concentrations of 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. Using Lasany double beam UV-Vis spectrophotometer model LI-2800 (Haryana, India), the absorbance was measured at 517 nm. Methanol served as blank while methanolic DPPH solution was used as control. Each sample was assayed in triplicate with L-ascorbic acid used as standard. Percentage inhibition was calculated using the formula (equation 1) shown below.

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad (1)$$

Where Acontrol and Asample 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.

Cyclic Voltammetry

The voltammetric method described by Zielinska, Szawara-Nowak, Zielinski[26] was followed with slight modification. In particular, the volume of the extract solution and buffer used was 20 mL instead of 100 μL to allow immersion of electrodes in the sample solution.

Cyclic voltammetric experiments were performed in water and ethanol extracts. The extracts were separately mixed with 0.2 M sodium acetate-acetic acid buffer (pH 4.5) at the ratio of 1:1 (v/v). The sodium acetate-acetic acid buffer acted as supporting electrolyte for the voltamperometric measurements. The measurements were carried out using a conventional three electrode system: (a) a glassy carbon electrode as working electrode, (b) a Ag/AgCl electrode as reference electrode and (c) a platinum electrode as counter electrode. The voltammetric experiments were performed at room temperature using a voltammetric apparatus cell, to which analyzed extract mixed with the supporting electrolyte was introduced.

Exactly 20 mL of the extract and 20 mL of buffer was used. To avoid any deposition, the working electrode was repeatedly cleaned. After washing, the electrode was ready for further tests. Cyclic voltammograms were acquired and recorded using a Bruker AUTOLAB PGSTAT302N Potentiostat/Galvanostat (Netherlands) by scanning the potential from -100 to +1300 mV at a scanning rate of 100 mV/s. The total charge below the anodic wave curve of the voltammogram was measured. The method was based on the correlation between the total charge below the anodic wave of cyclic voltammograms and the antioxidant capacities of the sample and the reference substance. Eighty percent (80 %) methanol solutions of Trolox within the concentration range 0.1-1.25 mM were used and the results were expressed as mmol Trolox/μg extract).

RESULTS

In this study, antioxidant activity of selected sample extracts was determined by both electrochemical and spectrophotometric methods. Twelve extracts, comprising the water and ethanol extracts of the leaves and rhizomes of *Zingiberaceae* species, were subjected to the cyclic voltammetry and DPPH assay.

The data (Table1) represent the scavenging activity and the mmol trolox/μg extract of the water and ethanol extracts of the leaves and rhizomes of the studied *Zingiberaceae* plants. The DPPH radical inhibition is visibly indicated by the discoloration of the DPPH solution from purple to yellow and the radical scavenging activity is expressed as anti-radical power. Anti-radical power is the inverse of the concentration of the extract to scavenge 50 % of the DPPH radical (EC_{50}). EC_{50} was calculated from the linear equations of the regression lines. Anti-radical power of the extracts is graphically presented in Figure 1.

As shown in Figure 1 and Table 1, EELW has the highest anti-radical power followed by ZORE then CLRE. EERE has the lowest anti-radical power. In general, EE consistently revealed the highest anti-radical power among the plant samples except for the ethanolic extract of its rhizome. Among the ethanolic extracts of rhizome, ZO showed the highest anti-radical power followed by CL. EE leaves consistently have higher anti-radical power than rhizomes in both water and ethanol extracts. CL and ZO have higher anti-radical power in rhizomes for ethanol but the opposite is true for water extracts. For the leaf samples, water extracts of the three ginger plants have higher anti-radical power than ethanol extracts. For rhizome samples, higher anti-radical power was observed in ethanol than in water except for EE samples.

Representative cyclic voltammograms for the standard trolox and sample extracts are presented in Figures 2 and 3, respectively. The antioxidant activity of the selected plant extracts was electrochemically evaluated based on the integrated peak area of the cyclic voltammogram

Table 1: Comparison of antioxidant activity of the selected *Zingiberaceae* plants determined by DPPH assay and cyclic voltammetric method (CL – *C. longa*, EE – *E. elatior*, ZO – *Z. officinale*, R – Rhizome, L – Leaves, W – Water, E – Ethanol).

Sample	Mean mmol trolox / µg extract ± SD	DPPH Radical Scavenging Activity, EC_{50}	Anti-radical Activity, 1/ EC_{50}
ZORW	0.0140 ± 0.0001	2042.96	0.00049
ZOLW	0.0211 ± 0.0002	1188.54	0.00084
ZORE	0.0244 ± 0.0023	281.49	0.00355
ZOLE	0.0067 ± 0.0000	1509.58	0.00066
CLRW	0.0127 ± 0.0000	5559.38	0.00018
CLLW	0.0175 ± 0.0003	858.06	0.00117
CLLE	0.0076 ± 0.0001	879.10	0.00114
CLRE	0.0100 ± 0.0008	333.80	0.00300
EERW	0.0165 ± 0.0001	911.88	0.00110
EELW	0.0157 ± 0.0003	153.60	0.00651
EERE	0.0065 ± 0.0001	6731.78	0.00015
EELE	0.0066 ± 0.0001	278.47	0.00360

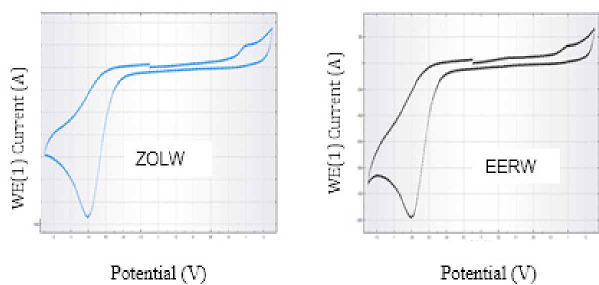

Figure 3: Figure 3: Representative cyclic voltammograms of sample extracts. (Legend: ZOLW - Water extract of *Z. officinale* leaves; EERW - Water extract of *E. elatior* rhizomes).

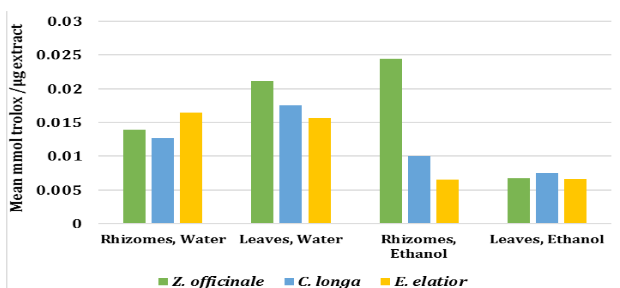

Figure 4: Antioxidant activity of selected ginger species using cyclic voltammetry.

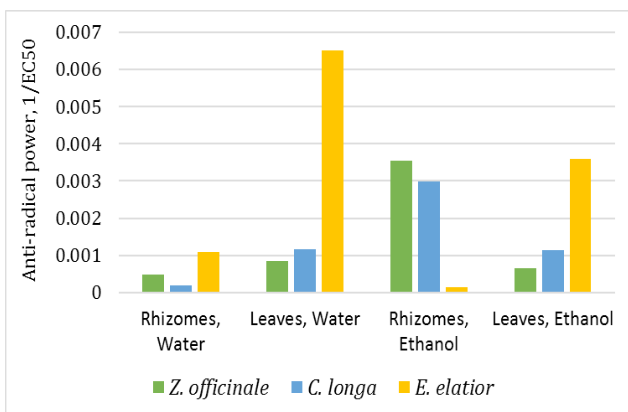

Figure 1: Antioxidant activity of the selected *Zingiberaceae* plants determined by DPPH Assay.

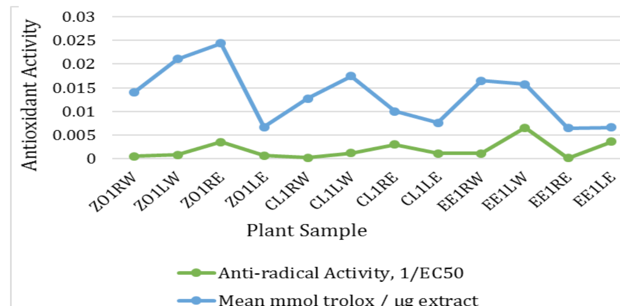

Figure 5: Graphical presentation of the comparison of antioxidant activity determined by DPPH assay and cyclic voltammetric method. Legend: (CL – *C. longa*, EE – *E. elatior*, ZO – *Z. officinale*, R – Rhizome, L – Leaves, W – Water, E – Ethanol).

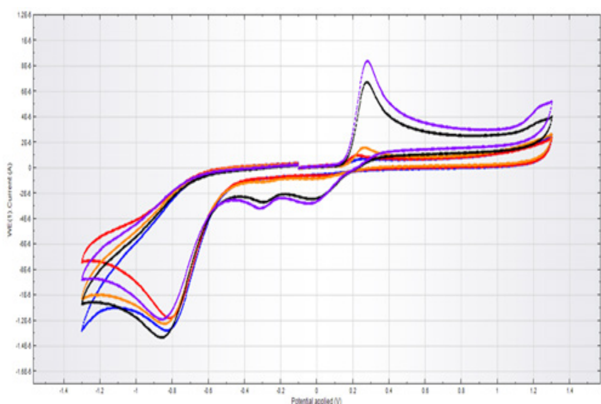

Figure 2: Cyclic voltammograms of standard solutions of Trolox.

obtained from the cyclic voltammetry of the water and ethanol extracts. Antioxidant activity was expressed as mmol trolox per µg extract based on the trolox calibration standards.

Antioxidant activity determined electrochemically and the comparison of the results obtained using the two methods, DPPH and CV, are presented in Figures 4 and 5, respectively.

As shown in Figure 5, it is noteworthy that among the selected sample extracts, varying trends in the antioxidant activity were demonstrated between the electrochemical (cyclic voltammetric) and the spectrophotometric (DPPH assay) methods. For instance, cyclic

voltammetric results showed that the ethanol extract of *Z. officinale* rhizome exhibited the highest antioxidant activity (ZORE, 0.0244 ± 0.0022). This was followed by the water extract of *Z. officinale* leaves (ZOLW, 0.0211 ± 0.0002), water extract of the *C. longa* leaves (CLLW, 0.0175 ± 0.0003).

A different ranking order was revealed in the spectrophotometrically derived antioxidant activities. In the DPPH assay, anti- radical power ($1/EC_{50}$) was highest in the water extract of *E. elatior* (EE1LW) with $1/EC_{50}$ of 0.0065, followed by the ethanol extract of *E. elatior* leaves (EE1LE, 0.0036). Lowest anti-radical activity across the twelve sample extracts was observed in the ethanol extract of *E. elatior* rhizomes (EE1RE) with $1/EC_{50}$ of 0.0001. It is noteworthy that among the twelve (12) plant extracts, EERE consistently possessed the lowest antioxidant activity as determined in both assay. That is, EERE has the lowest mmol trolox per μg extract (0.006499 ± 0.01) and lowest anti-radical power (0.000149).

Correlation analysis between the anti-radical power ($1/EC_{50}$) and mmol trolox per μg extract resulted to a very low positive correlation of 0.22. Low correlation may be accounted to the use of different positive controls in the two methods. The graphical presentation in Figure 6 verified this correlation.

DISCUSSION

The results obtained in using different methods in the assessment of antioxidant activity in various reaction systems are inconsistent and are often not comparable.[27] For instance, the results of the analysis of antioxidant capacities of flavonoids exhibited inconsistent results among the DPPH, Folin-Ciocalteu Reagent (FCR), Ferric Reducing Ability of Plasma (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC) assays. Upon comparison of the four methods, DPPH assay showed the lowest correlation with the charge under anodic wave obtained in CV method. Among TEAC, FCR, DPPH and FRAP, DPPH is the only method which uses methanol. This organic solvent has lower dielectric constant than water, which may cause transformation of the radical-scavenging mechanisms.[27]

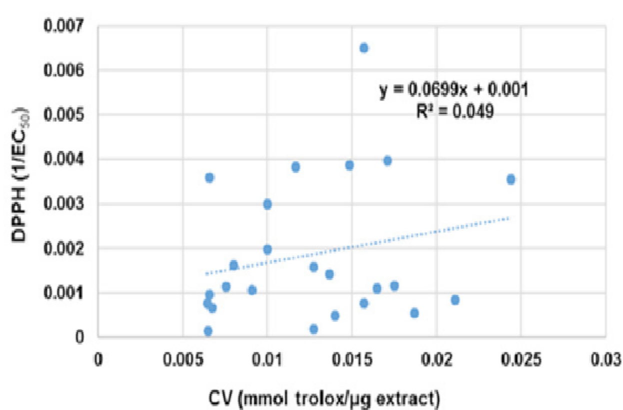

Figure 6: Correlation between DPPH assay and cyclic voltammetric method.

The voltammogram obtained from the cyclic voltammetry run provides the integrated antioxidant capacity based on the analysis of anodic current waveform. The anodic current waveform is a function of the reductive potential of a given compound in the sample and/or a mixture of components. In here, the contribution of each individual component in a sample is not specifically determined. Biological oxidation potential and the anodic current intensity (Ia) are the two parameters that define the total antioxidant capacity. *Ia* reflects the concentration of the components in a mixture or samples.[26] The area under the anodic current wave, designated as S which is related to the total charge, has been proposed as a better parameter reflecting the antioxidant capacity of the sample.[4]

CV is a redox-based method.[28] The electrochemical behavior of the natural compounds with antioxidant activity depends on their structural features. As such, useful information on their antioxidant functionality can be deduced.[29] Integral to electrochemical techniques are their inherent sensitivity and selectivity. This could remarkably improve the total phenolic indexes obtained by spectrophotometric protocols.[30]

CV was reported to be rapid,[28,31] simple,[31,32] sensitive[28,32] and reliable.[32] The sensitivity of cyclic voltammetric method depends on the antioxidant compound analyzed.[33] CV was found to be a cheap method in the determination of the total antioxidant capacity of cold-pressed edible oils.[31] Moreover, CV does not require so sophisticated chemical reagents or solvents and special sample preparation is not needed.[28] It is applicable to samples of any hydrophilicity or lipophilicity, as well as turbidity, which are restricted in many spectrophotometric methods.[28] Noteworthy to mention is the capability of CV to provide stable tracing for fresh samples or those stored frozen for long durations.[32]

CV was reported as valuable method in evaluating the contribution of low molecular weight antioxidants to the total antioxidant capacity of a fruit juice, extract or wine.[34] Antioxidant capacity was measured using a new electrochemical method without pretreatment. This was based on the measurement of the anodic area of a cyclic voltammogram - used in testing the antioxidant capacity of dried extract and infusions of green tea, black tea, rosemary, coffee, acerola and açaì, herb teas consisting

of a mixture of natural antioxidants called "Herb Tea" and "Quality Tea" without pre-treatment.[35]

CV has been used in the measurement of antioxidants in milk,[36] evaluation of the antioxidant capacity of low molecular weight antioxidants in plasma and the severity of oxidative stress exerted on the plasma,[32] determination of the antioxidant capacity of edible plants,[37] characterization of the antioxidant properties of thioctic acid[38] and in the evaluation of the antioxidant activity of 14 flavonoid compounds by cyclic voltammetry.[27]

CONCLUSION

The antioxidant activities obtained from the electrochemical (cyclic voltammetric method) and the spectrophotometric method, (DPPH assay) showed different ranking order. Very low positive correlation (r = 0.22) was obtained between DPPH and CV methods, which may be attributed to the different positive controls used in the two methods. Interestingly, both CV and DPPH assay revealed that leaves of common gingers studied are potential sources of antioxidants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CV: Cyclic voltammetry; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ROS:** reactive oxygen species**;** **ZO:** *Zingiber officinale*; **CL:** *Curcuma longa*; **EE:** *Etlingera elatior*; **CEBREM:** Center of Biodiversity Research and Extension in Mindanao; **EC_{50}:** Effective Concentration of sample required to scavenge DPPH radical by 50%; **FCR:** Folin-Ciocalteu Reagent; **FRAP:** Ferric Reducing Ability of Plasma; **TEAC:** Trolox Equivalent Antioxidant Capacity; **UV-VIS:** Ultraviolet Visible; **ZORE:** Ethanol Extract of *Zingiber officinale* Rhizomes; **CLRE:** Ethanol Extract of *Curcuma longa* Rhizomes; **EERE:** Ethanol Extract of *Etlingera elatior* Rhizomes; **ZOLE:** Ethanol extract of *Zingiber officinale* Leaves; **CLLE:** Ethanol Extract of *Curcuma longa* leaves; **EELE:** Ethanol Extract of *Etlingera elatior* Leaves; **ZORW:** Water extract of *Zingiber officinale* Rhizomes; **CLRW:** Water Extract of *Curcuma longa* Rhizomes; **EERW:** Water extract of *Etlingera elatior* rhizomes: **ZOLW:** Water Extract of *Zingiber officinale*; Leaves; **CLLW:** Water extract of *Curcuma longa* Leaves; **EELW:** Water Extract of *Etlingera elatior* Leaves.

SUMMARY

This study compared the antioxidant activity of the common *Zingiberaceae* plants, namely: *Zingiber officinale, Curcuma longa* and *Etlingera elatior*. Cyclic Voltammetry (CV) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to determine the antioxidant activity of the water and ethanol extracts of the leaves and rhizomes of the studied plants. Different ranking order was observed between CV and DPPH. In both DPPH and CV methods, *E. elatior* has generally higher e antioxidant activity in leaves than in rhizomes and water extract than ethanol extracts. Higher antioxidant activity was observed in leaves than in rhizomes for *Z. officinale* and *C. longa* for both water and ethanol extracts. In general, water extracts of the three ginger plants have higher antioxidant activity than its ethanol extracts. Interestingly, both CV and DPPH assay revealed that leaves of common gingers studied are potential sources of antioxidants.

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