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# Voltammetric and spectroscopic determination of polyphenols and antioxidants in ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

Ginger (*Zingiber officinale*) is widely consumed as an important spice or a common condiment in food and beverages. This study focuses on the determination of pungent and bioactive components in ginger and their antioxidant activity using voltammetric and spectroscopic methods. Gas chromatography-mass spectroscopy analysis revealed that the major components of the pungent compounds were zingerone, shogaols, gingerols, paradols, wikstromol, and carinol. Using spectroscopic methods, the antioxidant capacity of ginger aqueous extract was found to be 16.0 μmol gallic acid equivalent (GAE) per gram of ginger extract, and the total phenolic and flavonoid content was estimated to be 7.8 mg GAE/g ginger extract and 15.4 mg Quercetin equivalent (QE) per gram of ginger extract, respectively. Electroanalytical quantification estimated the antioxidant capacity of the ginger infusion to be 23.5 μmol GAE/g ginger extract, which is slightly higher than that estimated using chemical assay. The results may provide useful information for the development of ginger processing and utilization as a flavoring agent, and for our understanding of ginger as a source of natural antioxidants.

## 1. Introduction

The rhizome of ginger (*Zingiber officinale* Roscoe) is an important spice or a common condiment widely used for a variety of foods and beverages [1]. Traditionally, ginger is used alone or mixed with other biological substances in remedies for treating colds, osteoarthritis [2], cardiovascular disease [3], asthma [4], dyslipidemia [5], nausea, and vomiting [6, 7]. The major active components in fresh ginger have been identified to be zingerone and gingerols, while shogaols, paradols, and gingerdione are produced from the corresponding zingerone and gingerol during thermal processing [8, 9]. All the active components and related compounds consist of 4-hydroxy 2-methoxyphenyl moiety [10] and exhibit anti-inflammatory [11, 12], anti-platelet [13], anti-tumor [14], and high antioxidant properties [15, 16]. The bioactivity of ginger rhizome varies depending on the composition of its active compounds [17, 18].

There has been great attention focused on simple and accurate quantification methods of active compounds in ginger rhizome, and most analyses use gas chromatography (GC) and high performance liquid chromatography (HPLC) [19]. However, these techniques have their limitations, for example, in GC, the heating may induce some chemical reactions of the active components. HPLC, on the other hand, has a high cost and requires extensive sample preparations. Recently, electroanalytical detection has been demonstrated to be the simplest, most facile, accurate and fast detection method for the analysis of the active compounds in ginger sample [20]. Here, cyclic voltammetry has been employed as a technique for detection of gingerols [21]. This electrochemical method has also been applied to evaluate the antioxidant capacity of lipophilic compounds, such as carotenoids, chlorophylls, tocopherols, and capsaicin present in vegetables and beverages [22, 23].

The composition of active components in ginger depends on its geographical origin, post harvesting treatment, processing, drying conditions, and temperature [17, 18, 24]. In this paper, we report the antioxidant capacity of the infusion of ginger rhizome locally marketed in Brunei Darussalam. To determine the antioxidant capacity, we used chemical assays coupled with spectroscopic methods and compared our findings with the electroanalytical approach, namely differential pulse voltammetry (DPV). The major active components in the ginger were identified using gas chromatography-mass spectroscopy (GC-MS) and compared with those reported in literature. Electrochemical study of ginger infusion has been previously reported by Chaisiwamongkhol et al. [20], although the study focuses on the enhancement of sensitivity using

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a modified working electrode surface. In our study, we investigated the redox properties, including oxidation and reduction potentials, diffusion coefficient, redox properties, and pKa. These parameters can be related to the antioxidant strength of the compounds in the ginger infusion by using cyclic voltammetry (CV) with polished glassy carbon electrodes.

## 2. Materials and methods

### 2.1. Sample preparation

Commercially available ginger was obtained from the local market in Brunei Darussalam. The ginger was initially washed and blotted to dry. The sample was cut into thin slices and dried using a vacuum oven at 50 °C until constant weight was achieved. The dried sample was blended into a fine powder and stored in an air-tight container at room temperature prior to use for GC-MS, antioxidant, and electrochemical analyses.

Ginger infusion was prepared according to Ziyatdinova *et al.* [25] and Chaisiwamongkhol *et al.* [20] with slight modifications. Briefly, dried ginger powder (2.0 g) was infused in 200 mL double distilled water with stirring at 95 ± 1 °C for 5 min, and pH of the infusion was measured. The infusion was then filtered and left to cool to room temperature prior to centrifugation at 4000 rpm for 20 min to remove particulates. The supernatant was collected, followed by vacuum filtration. A clear infusion was obtained and it was used for antioxidant and electrochemical analyses.

For GC-MS analysis, similar infusion of 4.0 g dried ginger sample was prepared. The clear infusion collected after the vacuum filtration was then heated in an oven at a temperature between 40 to 50 °C until the infusion was completely dried. The solidified sample was then dissolved in methanol, followed by sonication for 10 min and gravity filtration. The filtrate was further centrifuged at 4000 rpm for 20 min. The supernatant was transferred into vials for GC-MS analysis. Pure methanol was used as a blank.

### 2.2. Chemicals and reagents

All chemicals and reagents were of analytical grade and were used without further purification. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid monohydrate, and quercetin hydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA), while aluminium chloride, Folin-Ciocalteu reagent, glacial acetic acid, methanol, ethanol, sodium acetate, sodium carbonate, sodium chloride, and sodium nitrite were purchased from Merck (Darmstadt-Germany). Double distilled water was used throughout the sample preparations and measurements. Stock standard solutions of gallic acid and quercetin were each prepared in double distilled water and ethanol.

### 2.3. Electrochemical apparatus

Electrochemical analyses of the ginger extracts were performed using Autolab system which includes a μAutolab type III potentiostat/galvanostat (Metrohm Autolab B.V., Netherlands) connected to an AC automatic voltage regulator (Elim Wellness Enterprise, Metrohm Sdn, Bhd., Malaysia) and Nova software version 1.10 to plot the voltammograms. A three electrode system includes a 3-mm diameter polished glassy carbon electrode as working electrode, a platinum wire counter electrode, and a silver/silver chloride (in 3 M NaCl) as reference electrode. The ginger infusion (1000 μL) was diluted with 9 mL of 0.1 M acetate buffer and placed in an electrochemical cell. In the experiments, pH of the supporting electrolyte was adjusted to be in the range of 2.0–7.0 by adding glacial acetic acid or sodium hydroxide. For a standard reference, an experiment with similar sample conditions and electrochemical analyses for gallic acid dissolved in the same supporting electrolyte was performed.

Typically, the CVs were recorded at a potential range between +0.1 V and +0.8 V with a scan rate of 100 mV $s^{-1}$. All experiments were carried out at room temperature (25 °C). In order to evaluate irreversible redox reactions and the electrolysis mechanisms of compounds in the ginger extract, the scan rate was varied at 5, 10, 20, 50, 80, 100, and 200 mV $s^{-1}$. To ensure that the observed peak potentials are consistently presenting the redox reactions of the same compounds in the ginger extract, we changed the concentration of the extract from 100 to 1000 ppm. The electrodes were gently polished with 0.3 micron alumina and rinsed with double distilled water prior to each voltammetric scan.

### 2.4. Differential pulse voltammetry (DPV)

In this experiment, 1 mL of the ginger extract solution was spiked into the supporting electrolyte. To quantify the total antioxidant capacity, a calibration curve passing through 8 data points was plotted using the standard gallic acid with concentrations ranging from 0.127 to 17 ppm. The total antioxidant content was expressed as milligrams of gallic acid equivalent per gram of dried ginger (mg GAE/g dried ginger). The result was validated by the detection limit of this DPV technique, which was determined based on the signal-to-noise ratio for the gallic acid measurement [26].

### 2.5. Gas chromatography mass spectrometry (GC-MS)

A Shimadzu GC/MS-QP2010 system equipped with a split/split less injector was utilized to characterize the compounds in the ginger infusion. The separation of the compounds was performed on a DB-5 ms column (length 30 m, diameter 0.25 mm, and thickness 0.25-μm film). Helium was used at a flow rate of 1.00 ml/min and a split ratio of 100.0. The oven temperature was programmed on heating from 50 to 140 °C at a rate of 20 °C/min. The temperature was then increased stepwise from 140 to 300 °C at a rate of 10 °C/min and held at this final temperature for 10 min. Helium was used as the carrier gas at a constant flow of 1.69 mL/min. Mass-selective detector was set at capillary direct interface temperature of 200 °C, ionization energy of 70 eV, and full scan mode with a mass range of 2–500 m/z. The compounds in the ginger extract were identified by comparing their retention index and mass spectra with those of reported compounds in the NIST 2008 version database and published literature data.

### 2.6. Antioxidant assays

#### 2.6.1. DPPH analysis

The antioxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to spectroscopic method reported by Yeh *et al.* [27]. 500 μL of the ginger extract (100 ppm–1,000 ppm, respectively) or standard gallic acid solution was added into freshly prepared 1 mL methanolic solution of DPPH (50 ppm). The mixture was shaken and allowed to stand in the dark at room temperature for 30 min. The absorption of the mixture then was recorded at 517 nm against methanol. A range of concentrations of standard gallic acid (0.1–5 ppm) were prepared to establish a calibration curve passing through 10 data points with the linear regression coefficient of 0.997. A DPPH solution without the sample extract was used as a control. The percentage DPPH radical scavenging activity (*RSA*) of the ginger extract was calculated using:

$$\%\ RSA = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100\%$$

where $A_{control}$ is the absorbance of the control solution and $A_{sample}$ is the absorbance after addition of the ginger extract [28]. The $IC_{50}$ value which is defined as the concentration of the extract required for 50% *RSA* was determined from the plot of RSA as a function of concentration. The antioxidant activity of the ginger extract was calculated based on $IC_{50}$ of standard gallic acid. The antioxidant activity was then expressed in μmol of gallic acid equivalent per gram of dried ginger (μmol GAE/g dried

ginger).

#### 2.6.2. Total phenolic content (TPC) analysis

The TPC in the ginger infusion was determined according to the Folin-Ciocalteu method [8]. In this analysis, 500 μL of diluted infusion (1,000 ppm) or standard gallic acid solution was introduced into a sample vial containing 2.5 mL of ten-fold diluted Folin-Ciocalteu reagent. After 5 minutes, 2 mL sodium carbonate (7.5% w/v) was added. The mixture was allowed to stand in the dark for 30 minutes at room temperature. Absorption of the mixture then was recorded at a single wavelength of 760 nm. The TPC of the infusion was calculated from linear regression of the calibration curve of gallic acid which was plotted in the range of 0.1–20 ppm. The TPC of the ginger infusion was then expressed in milligrams of gallic acid equivalent per gram of ginger extract (mg GAE/g ginger extract).

#### 2.6.3. Total flavonoid content (TFC) analysis

The TFC of the ginger infusion was measured using a slightly modified colorimetric method [8]. Two mL of the diluted infusion (1,000 ppm) or ethanolic quercetin standard solution was mixed with 0.3 mL sodium nitrite (5% w/v), followed by the addition of 0.3 mL aluminium chloride (10% w/v). The mixture was further incubated for 6 min. Subsequently, 2 mL NaOH (4% w/v) was added and the solution was vortexed, and it then was allowed to stand at room temperature. For comparison, we prepared standard ethanolic solutions of quercetin in the same way. Absorption of the mixture and the standard solutions was recorded at a single wavelength of 510 nm. The TFC of the infusion was determined from linear regression of a calibration curve, which was obtained from quercetin of different concentrations in the range of 1–200 ppm. The TFC of the ginger infusion was then expressed in milligrams quercetin equivalent per gram of dried ginger (mg QE/g dried ginger).

## 3. Results and discussion

### 3.1. Characterization of constituents in ginger extract

The chemical constituents of the ginger extract were identified by GC-MS analyses. GC TIC spectrum is presented in Figure S1, and the tentative chemical composition, retention time, retention index, and respective percentages are summarized in Table S1 in the Supplementary Information section. From the GC peaks, a total of 18 compounds were identified, from which pungent compounds, such as zingerone, shogaols, gingerols, and paradols were the major components. The major components were similar to those reported in literature [8, 27, 28, 29, 30, 31, 32, 33, 34], and most of them are the active constituents of ginger attributed to the antioxidant, anti-cancer, and anti-inflammatory properties. Interestingly, small amounts of wikstromol and carinol were detected, contributing to 2.2–2.7% of the overall composition. It is noteworthy that the two compounds contain two 4-hydroxy 2-methoxyphenyl moieties. Although the two compounds have been identified as marker compounds in different plant families from *Taccaceae* to *Zygophyllaceae* [35], there is no strong evidence for the presence of the two compounds in the ginger rhizome. Therefore, we may speculatively consider that the two compounds could be the products of associative combination of two zingerone or gingerols during thermal processing in GC-MS measurement, in addition to the formation of shogaols and paradols produced from the corresponding zongerone and gingerols. We also detected other components in the ginger infusion, including methyl β-D-glucopyranoside, pyranone, propionate, and furanone. The remainder were unidentified compounds made up of low-intensity peaks of less than 0.5% of the total area.

### 3.2. Antioxidant capacity, total phenolic content (TPC) and total flavonoid content (TPC)

TPC, TFC, and $IC_{50}$ are representative of the antioxidant capacity and therefore important for quantification of potential sources of antioxidant compounds in ginger. The most effective compounds that contribute to the antioxidant capacity are phenolic and flavonoid compounds. From the absorbance value of 0.120 for the ginger infusion (1,000 ppm) and the calibration curve of the gallic acid, the TPC value of the infusion was found to be 7.8 mg GAE/g dried ginger. This value is comparable to that of fresh ginger infusion reported in the literature; i.e. 6.3 mg GAE/g dry weight [36], and 8.46 mg GAE/g dry weight [32], but it is about 3-fold lower than that in dried ginger (27.40 mg GAE/g dry weight) or stir-fried ginger (22.24 mg GAE/g dry weight) [32]. This implies that the concentration of phenolic compounds in the extract of fresh ginger is much lower than the dried ginger due to either less amount of principal phenolic compounds, such as gingerols, shogaols, paradols, and phenolic acids in the fresh ginger or less moisture in dried and stir-fried ginger.

In general, principal flavonoids include flavanones, flavanols, flavones, and flavonols, such as catechin, epicatechin, and quercetin [37]. The TFC of the ginger infusion was first carried out using a diluted mixture (1,000 ppm) with an absorbance value of 0.025. Using the calibration curve of the quercetin, the TFC value of the infusion was calculated to be 15.4 mg QE/g dried ginger. The TFC in the ginger extract is roughly similar to the value reported in literature (0.0789 mmol of trolox/g dry weight equivalent to 9.0 mg QE/g dry weight) and it is also almost comparable with that found in chili and green cardamon [36]. Since the extracted phenolic volatile oils and acids, flavonoids, and aromatic volatile compounds contribute to the antioxidant capacity via proton or electron transfers [38], the low TPC and TFC in the ginger extract may indicate its low antioxidant capacity.

The antioxidant capacity of the ginger infusion was represented by its DPPH radical scavenging activity. Upon the DPPH analysis, the absorbance values obtained ranged from 0.563 to 0.952. The $IC_{50}$ of the ginger infusion was estimated to be 625.7 ± 4.8 μg/mL, equivalent to the antioxidant capacity of 16.0 ± 0.1 μmol GAE/g ginger extract. In comparison, the $IC_{50}$ of ginger in this study is about four-fold higher than that reported by Koch et al. (160 μg/mL) using the DPPH analysis for a fresh rhizome [39]. The discrepancy could be attributed to the different freshness levels and treatment of the ginger. In this study, the antioxidant activity of the ginger infusion was also determined by DPV method based on the electrochemical oxidation at the glassy carbon electrode. Under the optimized conditions (pH 3.8), a linear response for gallic acid concentrations between 0.127 to 17 ppm with $R^2$ coefficient of the linear regression being 0.9902 was obtained. Fig. 1A shows the DPVs with the oxidation peak approximately at +0.32V and the inset displays the calibration curve of the gallic acid. From the calibration curve, the detection (LOD) and quantitation limits (LOQ) of our DPV method were calculated to be 0.14 and 0.48 ppm, which are comparable to those reported in literature [40]. With the same experimental condition, DPV of ginger infusion (1000 ppm) was measured. As shown in Fig. 1B, DPV shows two oxidation peaks at +0.13 and +0.38 V. From the peak current at +0.13 V, we estimated the antioxidant activity of the ginger infusion to be 4.00 ± 0.05 mg GAE/g ginger extract or 23.5 ± 0.5 μmol GAE/g ginger extract, slightly higher than that estimated using DPPH method. Therefore, for determining antioxidant activity, we consider the DPPH method to have some limitations and low sensitivity. Hence, the DPV technique provides better quantification of the antioxidant capacity than the DPPH method, which has been previously utilized to quantify the antioxidant capacity of several crops [41]. Upon comparing the antioxidant capacity of the different sources, we found that the antioxidant capacity of the ginger infusion to be much lower when compared to those of, for instance, highbush blueberries (*V. corymbosum* bluecrop) or wild blueberries (*V. myrtillus* L.) being 2.08 mmol GAE/g dry weight and 4.63 mmol GAE/g dry weight [41]. This finding evidenced the low antioxidant capacity of the ginger extract.

### 3.3. Electrochemical properties of the antioxidants in ginger

In finding the optimum electrochemical characteristics of the pKa,

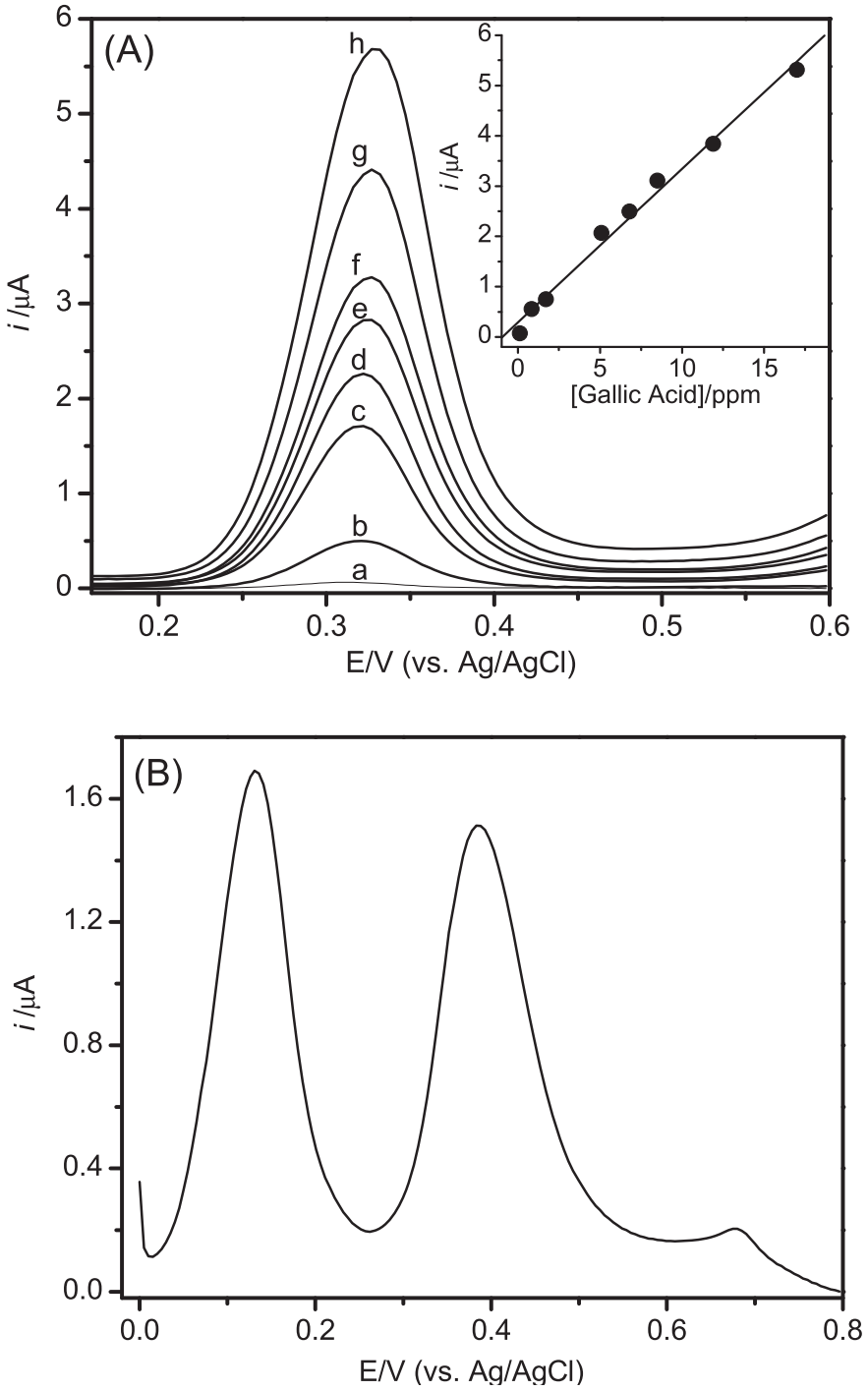


**Fig. 1.** (A) Overlaid differential pulse voltammograms for different concentrations of gallic acid in 0.1 M acetate buffer (pH 3.8) measured at 3-mm diameter glassy carbon electrode with modulation amplitude of 70 mV and scan rate of 20 mV/s. Inset: peak current as a function of concentration of gallic acid. (B) The differential pulse voltammograms of ginger extract (1000 ppm) in 0.1 M acetate buffer pH 6.5 measured at the same conditions.

diffusion coefficient and redox properties of the antioxidants present in the ginger extract, we investigated the cyclic voltammetry (CV) of the ginger infusion as shown in Fig. 2. As comparison, the CV of the supporting electrolyte is also depicted in Fig. 2. The oxidative scan of the ginger infusions was initially started at -1.0 V up to +1.2 V vs. Ag/AgCl, at which point, the oxidative voltammetric response of the electrode was recorded. Subsequently, the reverse voltammogram was scanned down to -1.0 V. It is clearly observed that the oxidative scan of the ginger infusions shows a peak at +0.56 V with peak current of 1.82 µA. Upon reversing the scan direction, a reductive peak is observed at +0.03 V with a peak current of 0.67 µA. In other words, the CV scans are almost similar to each other but the peak current is continuously decreased. This indicates that some of the compounds contained in the ginger infusions undergo irreversible redox reaction, which may be related to non-electroactive products upon electrooxidation of the active compounds possessing the 4-hydroxy-3-methoxyphenyl moiety [20, 42]. As a result, peak currents of the subsequent voltammetric cycle of the ginger infusions tend to decrease, while the oxidation and reduction potentials remain unchanged.

The redox mechanism of ginger infusion should be associated with

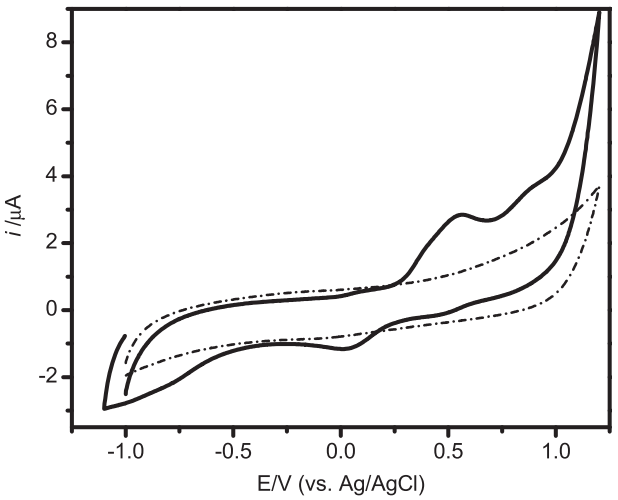


**Fig. 2.** Cyclic voltammograms of double distilled water (dotted line) and ginger extract in double distilled water (solid line) measured at 100 mV/s at 3-mm diameter polished glassy carbon electrode.

the redox processes of the electroactive species, such as gingerols, shogaols, paradols, and zingerone; where the presence of these compounds in the ginger infusions have been evidenced by GC-MS analyses, as described in Section 3.1. The redox mechanism of these electroactive species is related to the oxidation and reduction of their 2-methoxyphenol moiety [43]. We recall that gingerol, for instance, shows the oxidation and reduction potential peak at +0.68 V and +0.37 V, respectively. The different substituents attached to 2-methoxyphenol moiety are responsible for the variation of the oxidation and reduction potential peaks of the electroactive species in the ginger infusion. The electrochemical reaction of 2-methoxyphenol moiety involves irreversible hydrolysis, resulting in o-benzoquinone moiety as intermediate and dihydroxybenzyl derivative as a final product [20, 21].

### 3.4. Effect of voltage scan rate

Fig. 3A shows the CV and the magnitudes of the oxidative and reductive peak currents of ginger infusion recorded in 0.1 M acetate buffer, at scan rates between 5 and 200 $mVs^{-1}$. The concentration of the ginger infusion was 1000 ppm, and pH of the supporting electrolyte was 4.0. At these conditions, the CV showed a maximum peak current. All of the CV presented in each case were from the first scan. Interestingly, as shown in Fig. 3A, separation of the peak-to-peak potential is slightly larger at higher scan rates. This indicates that the voltammetric response to some extent is distorted by the scan rate through the changes in capacitance of the supporting electrolyte.

As plotted in Fig. 3B, the oxidative and reductive peak currents ($i_c$ and $i_a$) of the ginger infusion versus the square root of scan rate ($v^{1/2}$) exhibit a straight line with an intercept of nearly zero current. The linear relation of $i_c$ and $i_a$ versus $v^{1/2}$ immediately indicates that the reversible redox process on the electrode was controlled by diffusion, where the diffusion of the electroactive compounds took a longer time as the scan rate was decreased. The linearity of $i_c$ and $i_a$ with $v^{1/2}$ is also used to evaluate the presence of a surface bound redox process. This can be rationalized by considering a reversible electron transfer reaction in terms of the diffusion layer thickness and the time taken to record the scan. In this sense, the diffusion layer tends to grow larger from the electrode in a slower scan rate because the current function ($i/v^{1/2}$) should be constant for all scan rates at which Nernstian equilibrium is maintained on the electrode surface. Consequently, the flux to the electrode surface becomes smaller at a slower scan rate leading to lower oxidative and reductive peak currents due to the linear proportion of the current with the flux towards the electrode. Our finding further indicates that the reversible electron

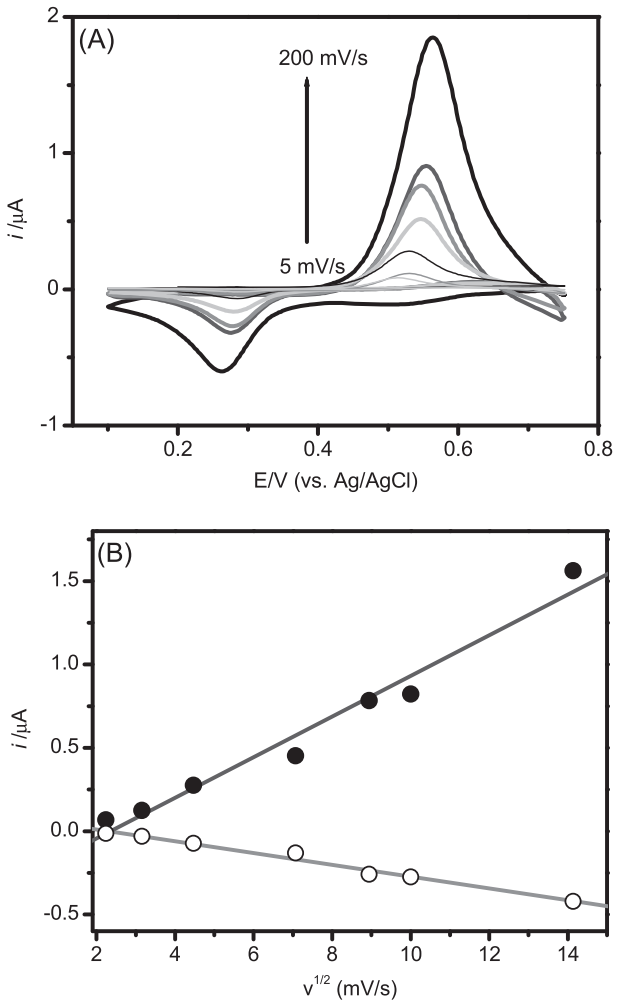


**Fig. 3.** (A) Cyclic voltammograms of ginger extract (1000 ppm) of infusion in 0.1 M acetate buffer (pH 4) measured from 5 to 200 mV/s at a 3-mm polished glassy carbon electrode. (B) A plot of anodic (filled circles, $R^2 = 0.9689$) and cathodic (open circles, $R^2 = 0.9833$) peak current against square root of scan rates.

transfer of the ginger extract on the electrode surface involves rapid diffusion. From the slope of the forward potential scan as a function of $v^{1/2}$, we determined the diffusion coefficient ($D$) of the electroactive compounds by using the Randles-Sevcik equation (at 25 °C),

$$i_p = 2.69 \times 10^5 \, n^{3/2} \, ACD^{1/2} \, v^{1/2}$$

where $n$ is number of electrons, $A$ is electrode area, and $C$ is concentration of the infusion. Taking into account that there are two electrons involved in the redox reactions of the active compounds, as described in section 3.5, we calculated their average diffusion coefficient to be $11.4 \times 10^{-6}$ $cm^2 \, s^{-1}$. This value is slightly larger than that of quercetin $3.18 \times 10^{-6}$ $cm^2 \, s^{-1}$ [44], suggesting that the average molecular size of the active compounds is slightly smaller than that of quercetin. In this sense, gingerols, shogaols, paradols, and phenolic acids which are the major phenolic compounds in the fresh ginger is indeed smaller than that of quercetin.

### 3.5. Effect of pH

Fig. 4A shows the effect of pH on the voltammetric response monitored for ginger infusion (1000 ppm) in acetate buffer in the pH range of 2.0–7.0. It is clearly seen that the anodic and cathodic peak potentials of the CV are shifted toward less positive values, and their peak currents are

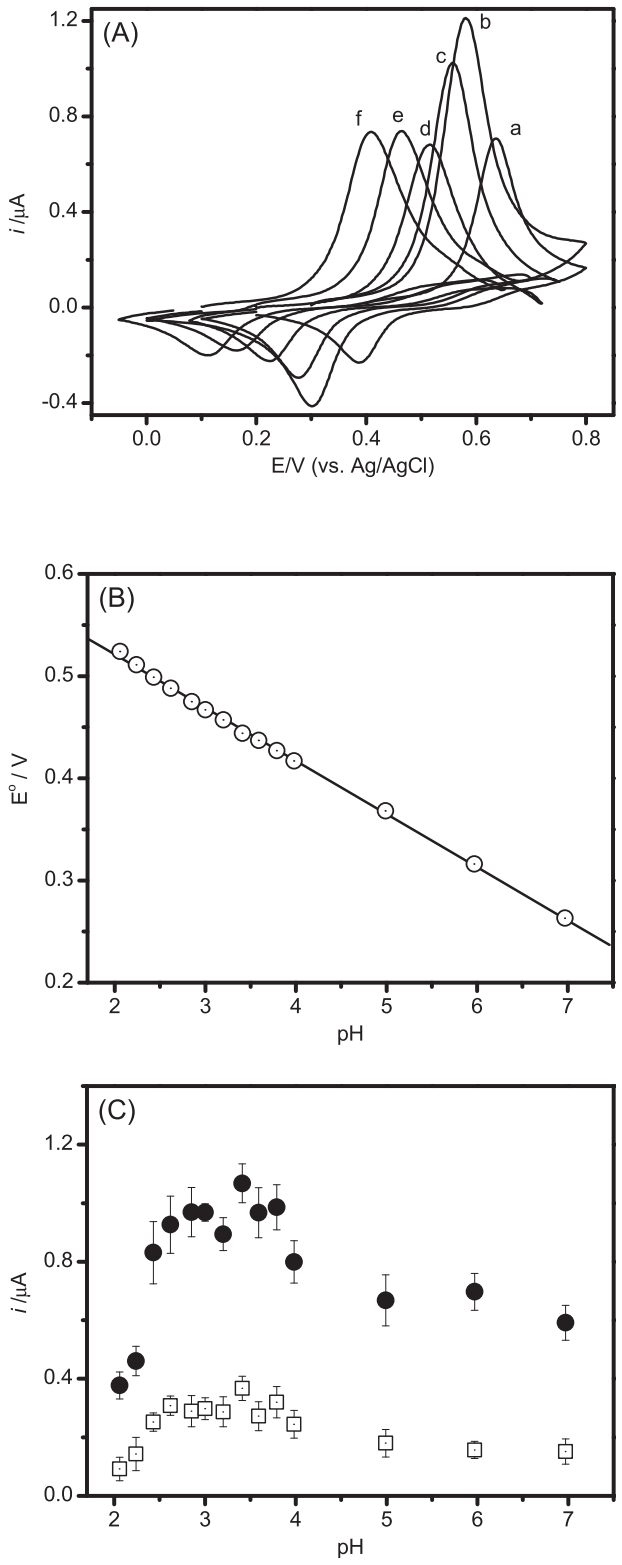


**Fig. 4.** (A) Cyclic voltammograms of 1000 ppm ginger extract in 0.1 M acetate buffer at pH (a) 2.2, (b) 3.4, (c) 4, (d) 5, (e) 6, and (f) 7 measured at 100 mV/s at 3-mm diameter polished glassy carbon electrode. (B) A graph of E° (V) as a function of pH, and (C) A plot of anodic (filled circles) and cathodic (open circles) peak current as a function of pH.

strongly pH-dependent. This indicates that the redox process is influenced by protonation of the electroactive compounds in high pH solution [44], where the thermodynamic driving force for the catalysis is modified with pH, leading to changes in the peak currents and the shapes of the CV. For further analysis, in Fig. 4B we show the plot of the formal peak potential (E°) versus pH, demonstrating that the peak potential decreased linearly with increasing pH ($E_p = 0.63–0.052$ pH; $R^2 = 0.999$). The slope of the linear relationship is 52 mV per unit pH, which is close to the Nernstian value (59.2 mV per unit pH) involving two-electron and two-proton process [45]. This finding is in agreement with reported values for quercetin [44], hydroxylamine [46], and other phenolic antioxidants [47]. This suggests that the redox process of the electroactive compounds in the ginger infusion involves an equal number of electron and proton transfer reactions. This similar electroanalytical finding has been observed for 6-gingerol with a bare BPPG electrode [20].

We may note that the pH dependence of the anodic and cathodic peak currents of the ginger extract are similar to one another, but the relationship is not so straightforward. As shown in Fig. 4C, the peak current increased rapidly as the pH rose from 2.0 to 2.8, reached the maximum at the pH from 2.8 to 3.8, and then decreased gradually for higher pH values. This type of pH-dependent peak current represents the protonation behavior, and to some extent can be considered as a characteristic sigmoid curve, from which the $pK_a$ of electroactive compounds may be determined [48]. By locating the inflection point [49], we estimated $pK_a$ of the ginger infusion to be around 2.3. Thus, we may consider that the electroactive compounds in the ginger infusion are protonated at pH 2. In the protonated condition, redox reaction generally involves two electrons and two protons producing monocation radical or monocation species [20], which is reduced when the scan direction was reversed [48]. A part of the ionic species is converted to the o-benzoquinone species via the irreversible hydrolysis reaction. As pH was increased from 2.0 to 2.8, an abrupt increase in the peak current suggests that some of the electroactive compounds may be readily deprotonated. In this sense, for instance, we may consider deprotonation of organic acids and volatile compounds which are most probably contained in the ginger infusion. However, we could exclude deprotonation of the pungent compounds as $pK_a$ of 4-hydroxy-3-methoxyphenyl derivatives in aqueous media is ca. 9.3.

## 4. Conclusions

In this paper, we have investigated the chemical constituents and antioxidant activity of the aqueous extract of commercially-available fresh ginger obtained in Brunei Darussalam. By using GC-MS analyses we identified and characterized the major constituents in ginger infusion to be the pungent compounds including zingerone, shogaols, gingerols, paradols, wikstromol, and carinol which are responsible for its antioxidant capacity. We speculate that the two compounds which contain two 4-hydroxy 2-methoxyphenyl moieties could be the products of associative combination of two zingerone or gingerols during thermal processing in GC-MS measurement, as well as the formation of shogaols and paradols from the corresponding zongerone and gingerols during thermal processing. The antioxidant capacity of the ginger infusion was estimated to be 16.0 μmol GAE/g ginger extract, and TPC and TFC of the infusion were found to be 7.8 mg GAE/g ginger extract, respectively. The redox properties allow the electroanalytical quantification of the antioxidant capacity of the ginger infusion, and it is estimated to be 23.5 μmol GAE/g ginger extract. This is slightly higher than that estimated value found using the DPPH method. This suggests that the DPV technique is more sensitive, providing better quantification of the antioxidant capacity. The results may provide useful information for the development of ginger processing and utilization as a flavoring agent and a source of natural antioxidant.

## Declarations

### *Author contribution statement*

Nursyahidah Alawiyah Idris: Performed the experiments.

Hartini M. Yasin: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Anwar Usman: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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### *Competing interest statement*

The authors declare no conflict of interest.

### *Additional information*

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