Hindawi
Journal of Chemistry
Volume 2019, Article ID 8047191, 10 pages
https://doi.org/10.1155/2019/8047191

Research Article

Subcritical Water Extraction of Antioxidants from *Curculigo latifolia* Root

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Received 28 December 2018; Revised 10 July 2019; Accepted 20 August 2019; Published 18 September 2019

Academic Editor: Leonardo Palmisano

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This study aims to extract antioxidant compounds from *Curculigo latifolia* root by subcritical water extraction (SWE). The influence of extraction temperature and time on the antioxidant activity of *C. latifolia* root extract was investigated in terms of extraction yield, total phenolic content (TPC), total flavonoid content (TFC), 2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The highest extraction yield (36.5%), TPC (92.55 mg GAE/g), TFC (13.26 mg RE/g), and antioxidant activities by ABTS (66.8 mg trolox equivalent/g sample) and DPPH (128.7 mg trolox equivalent/g sample) were detected at 180°C and 30 minutes extraction time. Based on the results, the optimum condition was selected at 180°C and 30 minutes of extraction. The sample was screened by using liquid chromatography-mass spectrometry (LC-MS) analysis where the phenolic compounds detected were structured based on their [M-H]-peak. The detected phenolic compounds were monobenzone, hydroquinone, phloridzin, pomiferin, mundulone, scandenin, and dimethyl caffeic acid. According to these findings, SWE is a promising, environmentally friendly, and efficient technology in the exploitation of natural products for the development of food and nutraceutical commodities.

1. Introduction

Phenolic compounds derived from natural sources have been associated with health benefits due to their antioxidant properties [1]. Several studies have shown that these natural antioxidants can be used to treat and prevent different types of diseases including cancer, hypertension, inflammation, degenerative disorders associated with ageing, and diabetes mellitus [2, 3]. In fact, all of these diseases are mainly caused by excessive production of reactive oxygen species (ROS) and an imbalanced proportion between antioxidant and ROS in the human body [4]. Several mechanisms of antioxidant action revealed that these mechanisms can be divided into two classes, which are preventive antioxidants and chain-breaking antioxidants that allow them to reduce and scavenge the radicals in order to alleviate the disease [5]. Furthermore, processed food that has synthetic antioxidants must be developed as a healthy product that naturally contains many kinds of health benefits [6]. In addition to these, consumption of a nutritional plant-based diet will play an important role in decreasing or managing the incidence of chronic disease including diabetes mellitus, cancer, Alzheimer's disease, and even ageing [7].

In the last few decades, the conventional extraction technique of using different organic solvents was used to isolate phenolic compounds from natural sources. Recently, there is an increasing awareness to limit the uses of organic solvents in extracting natural sources for therapeutic

activities due to the hazardous effect on human health caused by the residue from these hazardous solvents remaining in the final product [8]. Additionally, optimization of the extraction parameter must be investigated in order to preserve the structure of the extracted compounds. Hence, much interest has shifted towards the use of novel extraction techniques that are more environmentally safe than conventional extraction methods [9]. Subcritical water extraction (SWE) is one of the newest methods of extraction that can significantly increase the quality of the extracts and happens to be one of the extraction techniques that are favourable in extracting bioactive compounds from different kinds of natural sources [10]. This newest technology has been developed in an attempt to develop a cost-effective process, ensuring greater sustainability, safety, and quality of the products over conventional extraction techniques [11]. Basically, subcritical water extraction (SWE) uses water as the solvent in extracting phenolics using a temperature-dependent dielectric constant [12]. The polarity decreases with the increase of water temperature and throughout the whole process of extraction, input of high pressure is enough to maintain water in a liquid state [13]. In this work, the influence of temperature was investigated and optimized in the SWE of phenolic compounds from *C. latifolia*.

Curculigo latifolia Dryand. ex W. T. Aiton, is a rhizome geophyte that is categorised under the family Hypoxidaceae [14]. There are approximately 20 known species of *Curculigo*. Some of these species are said to have medicinal properties, such as acting as an anticancer agent and preventing gastrointestinal, cardiovascular, and heart-related diseases [15]. In our previous study, *C. latifolia*, which can be found in West Malaysia, was shown to exhibit antidiabetic properties in diabetic-induced rats. Nutrigenomic properties found in the fruit and root sections of *C. latifolia* that regulate responsible genes in glucose metabolism have been determined. All of the results demonstrate a high potential use of *C. latifolia* in diabetic therapy [16]. Additionally, the root has been certified to contain various compounds, such as cinnamic acid and benzoic acid-derived and flavonoid-derived compounds such as hesperetin, apigenin, dryopteric acid, and isorhamnetin [17]. According to the available literature, there were no reports on the use of SWE for the recovery of the bioactive compounds from *C. latifolia*. Thus, the aim of this study was to evaluate the efficiency of SWE for obtaining *C. latifolia* extract by varying on the effect of temperature and time of extraction in determining the extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities including 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

2. Materials and Methods

2.1. Plant Materials. The *C. latifolia* plant was collected in Selangor, Malaysia. A voucher specimen of the plant (SK 1709/09) was confirmed and deposited in the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The roots were cleaned with tap water and subsequently dried overnight in an oven at 40°C. The dried roots were ground into a fine powder that passed through a 30-mesh sieve. The sieved powdered roots were sealed and kept at 4°C for future analysis.

2.2. Materials and Reagents. Methanol, acetonitrile, and formic acid were of HPLC grade and purchased from Merck (Darmstadt, Germany). Folin–Ciocalteu reagent, 2,2-diphenyl-1 picrylhydrazyl hydrate (DPPH), sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$), trolox, rutin, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and potassium persulphate were obtained from Sigma-Aldrich (Steinheim, Germany). Methanol and ethanol were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade purity. All aqueous solutions were prepared using ultrapure water.

2.3. Subcritical Water Extraction. Subcritical water extraction (SWE) was carried out using a batch system comprised of a SEPAREX piston pump (P200 LGP50, France), an electrical control panel, and an extraction vessel. The total capacity of the high-pressure stainless steel vessel volume was 50 ml, and thus it was filled with about 2 g of the *C. latifolia* root. The independent variables were temperature (100, 150, 180, and 200°C) and time (30, 60, 90, and 120 min), while all other parameters were held constant. These independent variables were comprising extraction pressure (100 bar), flow rate (0.5 ml/min), and the water to solvent ratio (1 : 10 w/v). After extraction, the extraction vessel was cooled and depressurized. The obtained extracts were concentrated to dryness using a freeze dryer to remove water completely, and the pure extract was stored in a dark place at 4°C until used for analysis.

2.4. Determination of Total Phenolic Content. The total phenolic content (TPC) of *C. latifolia* root extract was measured according to the method reported by [17], using the Folin–Ciocalteu reagent with some modification. The crude extract was mixed with 0.5 mL of Folin–Ciocalteu's phenol reagent and 7.5 mL of deionized water. The mixture was kept at room temperature for 10 min before 1.5 mL of 20% (w/v) sodium carbonate was added [18]. Absorbance was measured at 765 nm using a microplate reader by a 96-well plate. Amounts of TPC were calculated using the gallic acid calibration curve within the range of 10–100 ppm [18]. Results were expressed as GA equivalents per gram of dry extract (mg GAE/g DE). All samples were analysed in triplicate and results averaged.

2.5. Determination of Total Flavonoid Content. Total flavonoid content (TFC) of the extracts was determined by the colourimetric assay following the procedure outlined in [19] with some modification. In brief, 100 μL of the properly diluted sample (1 g in 10 mL) was reacted with 2% $AlCl_3$ for 10 min at room temperature in a 96-well plate. Rutin was used as a positive control, and the TFC of the samples was expressed as milligram rutin equivalents (mg RE)/g sample. All samples were analysed in triplicate and results averaged.

2.6. Determination of Antioxidant Activity

2.6.1. DPPH Free Radical Scavenging Activity. The DPPH free radical scavenging activity of the sample was determined following the procedure described with minor modifications [19]. In short, 50 μL of diluted samples was reacted with 200 μL of 0.1 mM DPPH radical methanolic solution in a 96-well plate, gently swirled, and incubated in dark for 1 h. Absorbance was measured at 765 nm using a microplate reader by a 96-well plate. The percentage inhibition of the samples was calculated, and samples were expressed as milligram trolox equivalents (mg TE)/g sample where trolox was used as a positive reference.

2.6.2. ABTS Radical Cation Scavenging Activity. The ABTS radical cation scavenging activity was measured according to the methods with minor modifications [19]. The ABTS radical cation was generated by mixing 1 mL of 7 mmol/L ABTS stock solution with 1 mL of 2.45 mmol/L potassium persulate for 12 h in the dark at room temperature. The mixture was subsequently diluted with deionized water to form a solution with a final absorbance of 0.70 ± 0.02 at 734 nm. The scavenging activity was determined by reacting 20 μL of the properly diluted sample with 200 μL of the adjusted ABTS radical cation solution in a 96-well plate. The absorbance of the sample mixture was recorded at 734 nm after 10 min using a microplate reader by a 96-well plate. The percentage inhibition of the samples was calculated and expressed as milligram trolox equivalents (mg TE)/g sample where trolox was used as a positive reference.

2.7. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis. LC-MS analysis was performed using an MS-Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, USA) coupled to an HPLC system (Agilent Technologies, Santa Clara, USA). A C18 column (125×2 mm i.d., 5 μm particle size) was used. The mobile phase was composed of water (A, 6% acetic acid) in acetonitrile (B, 1% acetic acid), and the gradient programme of which was 2–5.00 min, 95 to 75% A and 5 to 25% B; 5–10.00 min, 60% A and 40% B; 15.00 min, 50% A and 50% B; and finally 20.00 min, 50% A and 50% B. The injection volume of the sample was 10 μL, and the flow rate of mobile phase was 0.5 mL/min. The chromatographic column was kept at 30°C. For MS analysis of samples, the following settings were used according to the procedure described with minor modification [20]: CID gas pressure to 2.40 mTorr, EIS needle voltage to 1000 V, EIS shield voltage to 600 V, drying gas temperature to 200°C, API housing temperature to 50°C, nebulizer gas pressure to 35 psi, and drying gas pressure to 40 psi. The flavonoids and other phenolic compounds in were identified based on the mass spectra and by comparison of the spectra of available standards.

2.8. Statistical Analysis. The extraction yields of the sample were calculated from the calibration curves of the standard compounds. The data were expressed as mean ± SD values. The Prism 7 was used for statistical analyses. The cutoff for a significant variation was $p < 0.05$ in Duncan's test. Spearman's rank correlation coefficients were calculated in order to characterize the relationship between antioxidant capacities detected by different assays for phenolic and flavonoid contents.

3. Results and Discussion

3.1. Influence of Process SWE Variables on the Extraction Yield. The extraction yields, total phenolic content, and total flavonoid content of the extracts obtained at different extraction conditions of temperature and time are presented in Table 1. Determining the yield is an essential step in order to determine the total extracted sample over the raw sample. Based on the results, the yield varied from 14.2 g/100 g to 36.5 g/100 g. The highest yields were achieved at 180°C although the differences were not significant in some cases. The increase in temperature also leads to the increase of yield for all different times. The yield varied from 14.0 to 36.5 g/100 g of the sample extract. The obvious yield was noticed for the first 30 min of extraction time at every extraction temperature. Additionally, at 100°C, a significant difference was noted between 30 and 60 min, but there is no significant difference in the result after that until it reached 120 min. All the yields were noted to increase from 30–120 min at 150°C, and simultaneously the amount of extracted sample was also increased to 26.5 g/100 g at 180°C with 30 min extraction time which was noted as the highest yield obtained.

In addition, at 200°C with 120 min extraction times, the yield was only 12.0 g/100 g of the dried root extract, which was recorded as twofold lower than the yield at the optimum condition. However, a significant difference was noticed at 180°C from different extraction times. In theory, a higher temperature causes a reduction of polar forces and hydrogen bonding between water molecules and this also reduces water polarity as well. At a high temperature, there is a possibility for the solution to become saturated with the solute, which can affect the mass transfer rate and prevent the dissolution of solids into the solution and decreases the yield [10]. In fact, from the reduction of water density and viscosity, this resulted in an increased mass transfer of the solvent into the matrix of the sample. However, as the sample approaches 200°C, there is a reduction of yield even after increasing the temperature. This fact was previously confirmed by Song et al., who stated that there was degradation of a compound as the temperature increases from 180°C to 210°C [21].

3.2. Effect of Temperature and Time on TPC and TFC of Root Extracts. The results in Table 1 also shows the total phenolic and flavonoid content obtained under SWE extraction conditions at various ranges of temperatures and times. Under each of the conditions, the phenolic and flavonoid compound was measured for every 30 min. The content of extractable phenolic compounds in the root of *C. latifolia* extract was determined through a linear gallic acid standard curve ($y = 0.0156x + 0.0416$, $R^2 = 0.9962$). The phenolic

Table 1: Extraction yields, total phenolic content, and total flavonoid content of *C. latifolia* root extracts by subcritical water extraction (SWE) at different extraction conditions.

Temperature (°C)	Time (min)	Yield (g/100 g)	TPC (mg GAE/g)	TFC (mg RE/g)
100	30	18.4 ± 0.31^{a}	34.43 ± 0.25^{a}	5.72 ± 0.81^{b}
100	60	15.3 ± 0.15^{b}	39.61 ± 0.13^{a}	8.5 ± 2.24^{a}
100	90	14.2 ± 0.11^{b}	38.79 ± 0.14^{a}	9.79 ± 1.17^{a}
100	120	15.5 ± 0.32^{b}	40.75 ± 0.55^{a}	8.3 ± 0.92^{a}
150	30	25.35 ± 0.68^{a}	72.89 ± 1.14^{a}	7.83 ± 0.38^{b}
150	60	20.0 ± 0.52^{b}	68.37 ± 4.79^{ab}	6.99 ± 0.51^{b}
150	90	19.5 ± 1.41^{b}	61.47 ± 1.13^{b}	11.1 ± 0.52^{a}
150	120	21.8 ± 0.38^{b}	64.60 ± 3.22^{ab}	10.4 ± 0.28^{a}
180	30	36.5 ± 0.91^{a}	92.55 ± 2.83^{a}	13.26 ± 0.3^{a}
180	60	31.4 ± 0.55^{b}	78.79 ± 0.69^{b}	11.05 ± 1.3^{ab}
180	90	22.6 ± 0.49^{c}	66.60 ± 0.57^{c}	8.68 ± 0.44^{b}
180	120	23.5 ± 0.11^{c}	48.58 ± 0.57^{d}	9.08 ± 0.3^{b}
200	30	14.0 ± 0.26^{b}	84.65 ± 1.97^{a}	7.8 ± 0.44^{b}
200	60	16.0 ± 1.48^{a}	87.97 ± 0.13^{a}	10.71 ± 0.4^{a}
200	90	16.5 ± 0.52^{a}	86.39 ± 2.01^{a}	8.52 ± 0.5^{ab}
200	120	12.0 ± 0.07^{c}	68.75 ± 0.76^{b}	9.86 ± 0.26^{ab}

The values are expressed as mean ± standard deviation ($n = 3$). Different letters in the same column indicate that the means differ significantly by the Tukey test ($p \leq 0.05$). TPC: total phenolic content; TFC: total flavonoid content; GAE: gallic acid equivalents; RE: rutin equivalents.

content for root extracts varied from 34.43 to 92.55 mg GAE/g. The highest fraction of phenolic content was collected for the first 30 min (92.55 mg GAE/g) at 180°C. In addition, while increasing the temperature, the phenolic content also increased. This finding is in agreement with other studies where it was reported that at 180°C, the highest phenolic content (26.26 g/L) was obtained [22]. Nevertheless, when the temperature increased to 200°C, the amount of phenolic content recovered decreased, and at this temperature, the lowest value of phenolic content was recorded (68.75 mg GAE/g) for 2 hours of extraction time. After achieving the highest phenolic and flavonoid compound, increasing the temperature will possibly lead to further degradation, and the reaction of some phenolic substances and the contribution of decreasing dielectric constant will make total phenolic content less effective [23]. Furthermore, a previous study reported by Erşan et al. observed that the degradation of the phenolic compound occurred above 180°C extraction temperature by qualitative confirmation with HPLC-DAD-ESI/MS analysis for every extraction temperature condition [24]. Furthermore, the results show no significant difference at 200°C with the increasing of extraction time.

The TFC of *C. latifolia* root extract was evaluated by using the aluminium colourimetric method, and the rutin standard was used as a standard curve ($y = 0.0074x + 0.0043$, $R^2 = 0.9993$). The flavonoid content of root extract varied from 5.72 to 13.26 mg RE/g. The highest quantity of flavonoid was obtained at 180°C in 30 min extraction times with 13.26 mg RE/g. The lowest flavonoid content was achieved during 30 min of extraction time at 100°C. Starting from 100°C, the flavonoid content increased until it reached the highest flavonoid fraction which was at 180°C. Increases in the extraction temperature leads to sudden decreases on the water viscosity, water permittivity, and also surface tension but this will increase in its diffusivity properties. Additionally, SWE at higher temperatures increased the thermal agitation and hence, decreased the strength of the hydrogen bonds, which led to the more efficient extraction of nonpolar hydrophobic compounds compared with polar hydrophilic compounds [25].

In fact, as the temperature of the water increased less energy was needed to disrupt the solute matrix interactions in order to achieve the enhancement of solute contact with the solvent [26]. Afterwards, the flavonoid content decreased as the temperature reached 200°C. So, it was easy to conclude that the bond in the flavonoid compound was unstable and began to break down as the temperature exceeded 200°C. For the first 30 min, the results showed a significant difference within the temperature range between 100°C and 180°C. Furthermore, the total phenolic content obtained was sevenfold more compared with the total flavonoid content in the *C. latifolia* root extract. Additionally, the influence of extraction time on the efficiency of SWE was studied previously [27], and according to the conducted study, the first 30 min of extraction time was considered as the optimal extraction time of phenolic compounds. At this condition, the polarity of water is reduced to approach the property of organic components and then hydrolysis might occur. This may lead to degradation of the cell wall phenolic compounds in the lignin network and, under this condition, the dielectric constant of subcritical water ($\varepsilon = 31$) was close to the permittivity of ethanol ($\varepsilon = 25$) or methanol ($\varepsilon = 33$) [25].

In terms of achieving high phenolic and flavonoid content under SWE extraction, there is no previous study yet that can be compared to the study of the *C. latifolia* root which uses subcritical water extraction. But a previous study reported that under conventional extraction using aqueous fraction, the TPC and TFC obtained was 4.62 mg GAE/g and 1.62 mg RE/g extract, respectively, and for the *n*-butanol fraction, the TPC and TFC obtained was 24.96 mg GAE/g and 10.84 mg RE/g extract, respectively [28]. Thus, by comparing the results, it shows the TPC by SWE is fourfold higher compared with butanol fraction extracts.

3.3. Total Antioxidant Capacity (TAC). For instance, phenols are well-known antioxidants while flavonoids are mostly known for their powerful free radical scavenging activity with strong antioxidant activity. The antioxidant activity of SWE of the *C. latifolia* root was measured using DPPH and ABTS assays, and a comparison was performed to understand the correlation of antioxidant activity with TPC and TFC values. The results for both antioxidant activities are displayed in Figures 1 and 2. In this study, the effect of temperature and time of SWE on antioxidant activities was determined. The DPPH radical scavenging activities ranged between 56.30 and 128.70 mg trolox equivalents/g samples and the ABTS scavenging activity varied from 42.59 to 66.78 mg trolox equivalents/g sample. In terms of process, DPPH scavenging activity increases as the temperature rises to 180°C and at 30 min under the same temperature exhibits the highest scavenging activity (128.70 mg trolox/g sample). The lowest free radical scavenging activity was recorded at 100°C and was also noted at 30 min of extraction time. Similarly, for the ABTS scavenging activity, the results indicate that as the temperature increased, the antioxidant activity also increased until it reached the maximum value at 180°C temperature. After that, antioxidant activity decreased when it approached 200°C. Theoretically, maximum antioxidant activity was achieved at the optimum extraction temperature by donating high electron or hydrogen in scavenging the free radicals. Afterwards, the antioxidant activity gradually decreases as the temperature approaches 200°C. Temperatures higher than 180°C give low antioxidant activity and, according to the literature, the main antioxidant constituents of the flavonoid compound will start degrading [29]. In addition, there is no significant difference reported at each temperature within the range from 100°C to 180°C for both antioxidant assays.

The DPPH and ABTS assay offered a cost-effective, efficient, and rapid solution in evaluating antioxidant assay based on calculating percentage inhibition which is equivalent with antioxidant capacity content in the sample. These applied methods are widely used in the evaluation of antioxidant abilities for both natural and synthetic molecules [30]. The smaller the concentration of antioxidants and the faster the antioxidant reacts with free radicals, the greater the scavenging activity [31]. According to the results of the scavenging activity by DPPH and ABTS assay, these extracts were significantly affected at various extraction temperatures and times. The result shows that the DPPH assay gives a twofold higher scavenging activity than the ABTS assay. This result is also supported by other previous studies that act under subcritical water extraction in which the trolox equivalent antioxidant capacity (TEAC) value is also twofold higher than the ABTS assay [32]. High TFC also can be one of the effective agents to scavenge free radicals by DPPH and also the ABTS assay [11]. From the observation at 200°C, the result shows that for both assays there is no significant difference in scavenging activity as increasing extraction time. This proves that low amount of phenolic and flavonoid content cannot inhibit the free radical, resulting in low antioxidant value at this point.

3.4. Correlation of Antioxidant Activities in Terms of the Content of Total Phenolics, Flavonoids, and Phytochemicals. To investigate the factors that are responsible for the antioxidant activity of *C. latifolia* root extracts, correlation analysis was carried out with the total phenolic content, total flavonoid content, and antioxidant activity. The experimental results were verified using linear correlation analysis. The ABTS and DPPH values of the extracts followed a similar trend as for the total phenolic and flavonoid contents [18], and a strong correlation among all the phytochemicals and antioxidant activity was obtained (Table 2). Based on the results, the highest correlation coefficient of 0.819 and significant correlation ($p < 0.001$) was between DPPH and TFC. The slightly higher free radical scavenging activity of the aqueous extract indicates the presence of a higher content of flavonoids in the extracts. Besides, the TPC finding correlated well with the results of ABTS and DPPH assays. There was also a moderate positive correlation that has a significant correlation ($p < 0.05$) between ABTS and TPC in root extracts. These results suggest that the DPPH assay better reflects the antioxidant contents of the *C. latifolia* root extract for SWE than the ABTS assay for total phenolic and flavonoid content. This correlation between phenolic and antioxidant activity substantiates the well-known free radical scavenging potential of these natural products.

3.5. LC-MS Analysis. The LC-MS profile from the screening of *C. latifolia* root extract is shown in Figure 3 and peaks were labelled according to the order of their retention time as shown in Table 3. Several bioactive compounds were detected based on accurate masses and fragmentation pattern aided by the existing literature in the mass spectrometry libraries. In particular, these compounds were phenolic (monobenzone) and identified as phenolic from benzene derivative (hydroquinone), flavonoid glycoside (phloridzin), prenylflavonoids (pomiferin), isoflavonoid (mundulone and scandenin), and in particular cinnamic acid derivative (dimethyl caffeic acid).

The negative molecular ion at [M-H]- for each of the targeted compound was also shown in Figure 4. The analysis of phenolic compounds was identified and characterized by the negative ionization mode [33]. In the analysis, the peak with a negative molecular ion at [M-H]- at an m/z of 109 was identified as hydroquinone. The second identified peak has shown an m/z of 477, indicating that this compound is phloridzin. The next peak exhibited an m/z of 341, which corresponds to monobenzone. The identified peak that had an m/z of 433, 477, and 683 were corresponding to scandenin, pomiferin, and dimethyl caffeic acid, respectively. The mundulone compound corresponds to two m/z value points which are 433 and 867, respectively. Previous reports evidenced that the identified compounds are able to exert a strong antioxidant activity. In fact, from the previous study it shows that hydroquinone acts as an antioxidant and helps in the protection of human cells against oxidative damage, and it was also reported that the antioxidant activity of hydroquinone is mainly due to

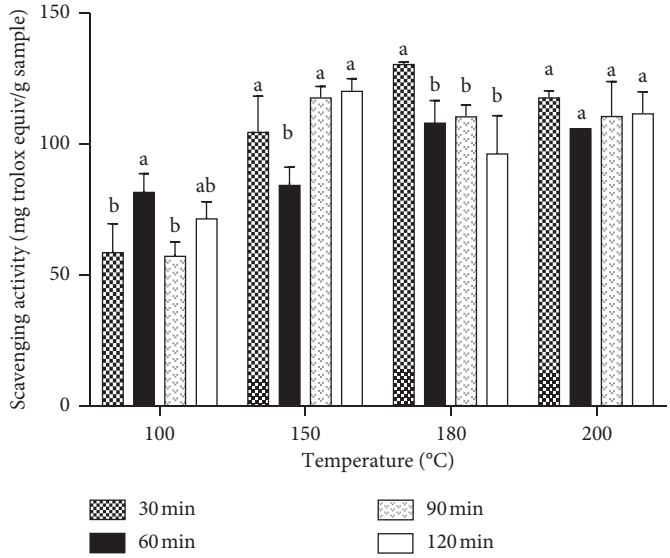


Figure 1: Effects of temperature and time on antioxidant activity (DPPH scavenging activity) of *C. latifolia* root extracts by SWE. Different letters indicate significant differences ($p < 0.05$) within the same extraction temperature.

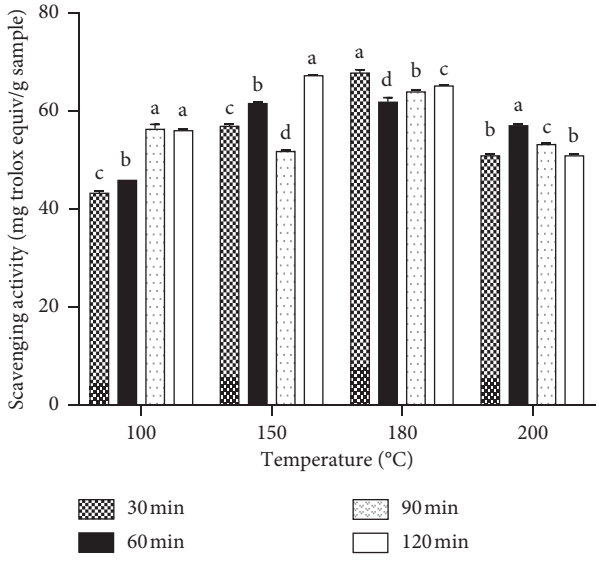


Figure 2: Effects of temperature and time on antioxidant activity (ABTS scavenging activity) of *C. latifolia* root extracts by SWE. Different letters indicate significant differences ($p < 0.05$) within the same extraction temperature.

Table 2: Correlations between TPC, TFC, ABTS, and DPPH of *C. latifolia's* extract.

R	TPC	TFC	DPPH	ABTS
TFC	0.451*	1		
DPPH	0.819***	0.591*	1	
ABTS	0.373*	0.512*	0.436*	1

R: correlation coefficient, TPC: total phenolic content, TFC: total flavonoid content, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to the control.

the activity of OH and the functional group in its structure [34]. Isoflavones are a large group of phenolic compounds synthesized through the central phenylpropanoid pathway. Higher antioxidant capacities were mainly attributed to the increase in the concentration of isoflavones present in the sample and also due to the regulation of enzymes involved in the phenylpropanoid pathway which has been proposed as a plant defense mechanism against selenium-induced oxidative stress [35]. In addition, phloridzin is known as a flavonoid glycoside which belongs to the chemical class of dihydrochalcones, phenylpropanoids with structures closely related to those of the immediate flavonoid precursors, the chalcones [36]. Previous studies show that the phloridzin compound detected in the sample also contributes to the antioxidant activity, and it was discovered that phloridzin also decreases the absorption of glucose, thus reducing postprandial blood glucose levels; hence, this will be beneficial to the treatment of diabetes mellitus [37]. Besides, we also looked at other compounds as a side compound such as hordatine A, 3-methylsuberic acid, ubiquinone, frangulin B, rubratoxin B, and emmotin A that were also detected in the sample extract. Coenzyme Q10 or mostly known as ubiquinone, also identified as one of the nonenzymatic endogenous antioxidants, plays an important role in the cellular metabolism and acts as an antioxidant through preventing the formation of lipid peroxy radicals [6]. The LC-MS analysis was effective in the screening of important polyphenolic compounds that contribute to the antioxidant capacity of the *C. latifolia* root extract, as assessed by TPC, TFC, and antioxidant assays. In the present study, the antioxidant compounds such as phenolic derivatives of benzoic acid, cinnamic acid derivatives, and flavonoids were reported for the first time in the *C. latifolia* root extract. These findings can be one of the benchmarks to show that the SWE method is capable of extracting other valuable phenolics that were present in the crude extracts. The results given by the SWE method

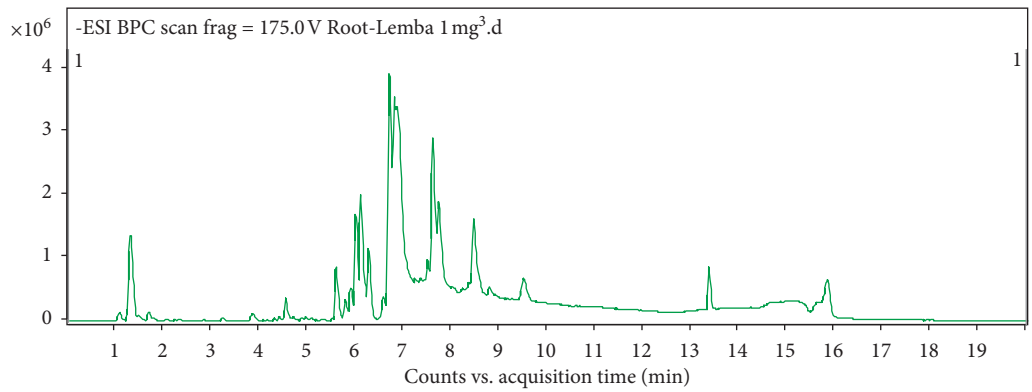
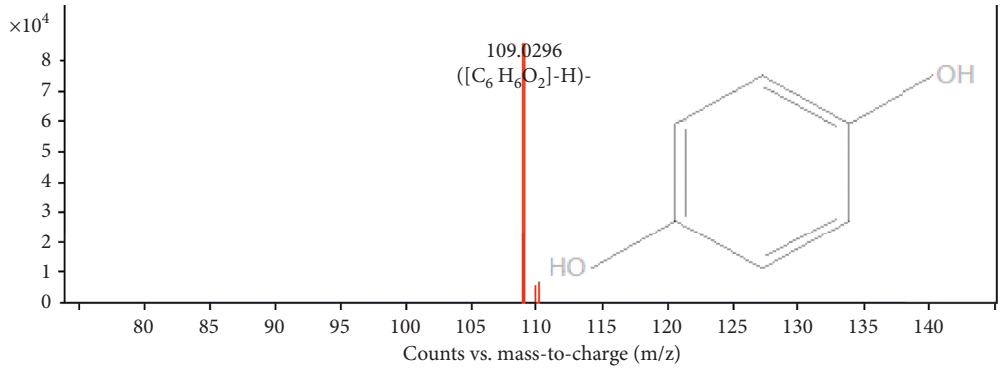


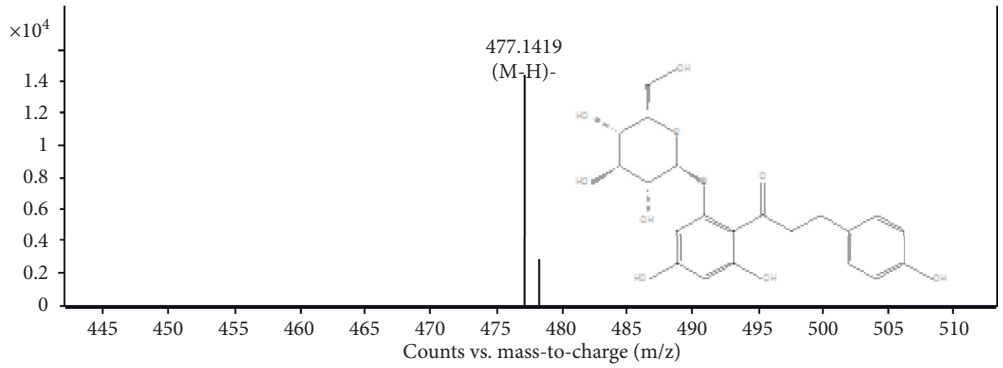
Figure 3: LC-MS chromatogram screening of the *C. latifolia* root extract.

Table 3: LC-MS analysis of *Curculigo latifolia* root extract.

Sample	Retention time (min)	Compound name	Formula
Root extract	5.185	Phloridzin(phenolic)	C_{21} H_{24} O_{10}
	5.916	Pomiferin(phenolic)	C_{25} H_{24} O_6
	6.589	Scandenin(phenolic)	C_{26} H_{26} O_6
	6.725	Mundulone(phenolic)	C_{26} H_{26} O_6
	7.322	Monobenzone(phenolic)	C_{13} H_{12} O_2
	7.529	Hydroquinone(phenolic)	C_6 H_6 O_2
	8.447	Dimethyl caffeic acid(phenolic)	C_{11} H_{12} O_4
	10.74	Hordatine A	C_{28} H_{38} N_8 O_4
	12.962	3-Methylsuberic acid	C_9 H_{16} O_4
	13.07	Ubiquinone	C_{14} H_{18} O_4
	13.698	Frangulin B	C_{20} H_{18} O_9
	15.95	Rubratoxin B	C_{26} H_{30} O_{11}
	16.960	Emmotin A	C_9 H_{10} O_5

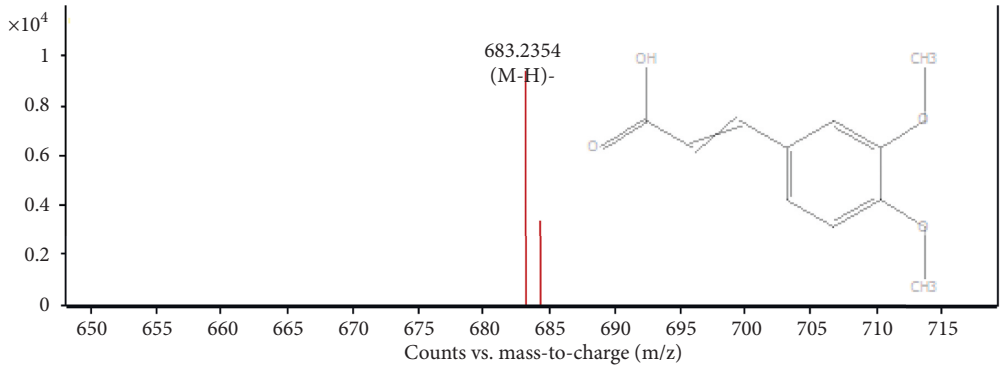


(a)

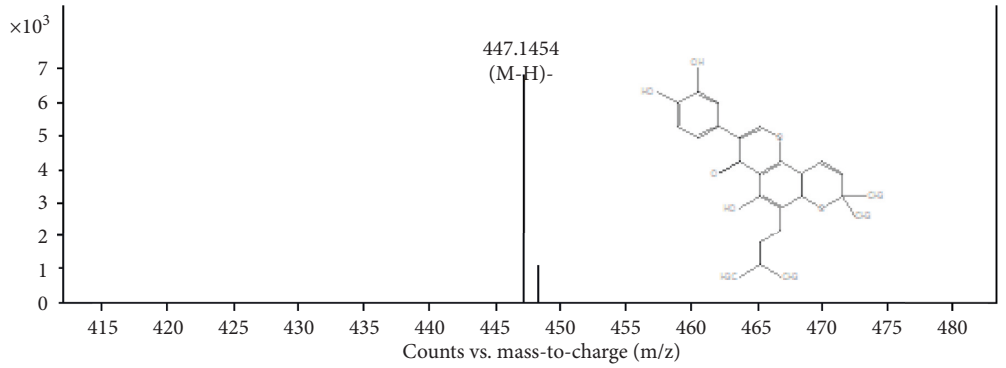


(b)

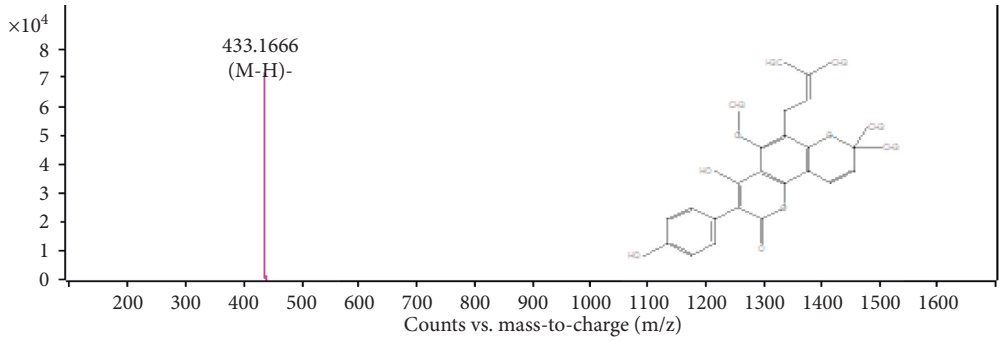
Figure 4: Continued.



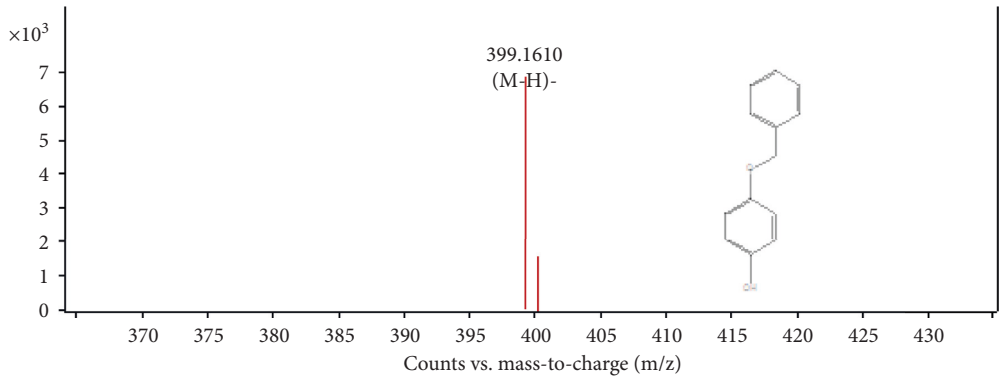
(c)



(d)

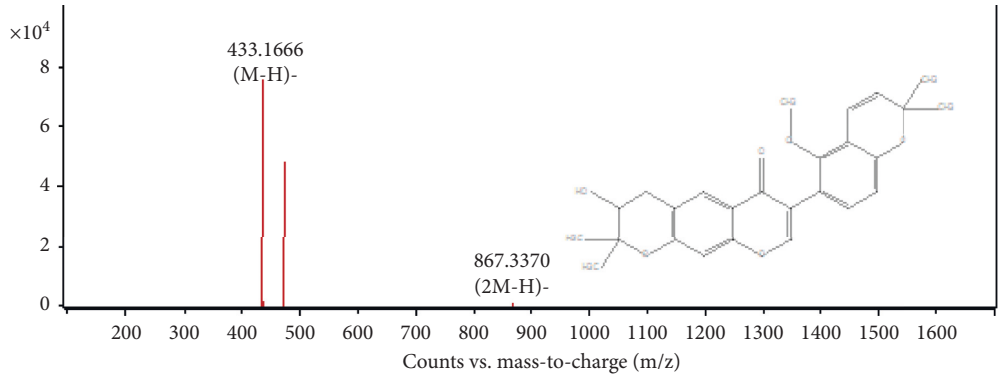


(e)



(f)

Figure 4: Continued.



(g)

Figure 4: Molecular ion from LC-MS chromatogram screening of the *C. latifolia* root extract. (a) hydroquinone, (b) phloridzin, (c) dimethyl caffeic acid, (d) pomiferin, (e) scandenin, (f) monobenzone, and (g) mundulone.

established in this study revealed that SWE is a green technique for powerful extraction of *C. latifolia* extracts without using toxic solvents, which can be widely applied in pharmaceutical studies, even at an industrial scale.

4. Conclusions

Temperature and time obviously affected the total phenolic, total flavonoid, and antioxidant activity of the *C. latifolia* root extract. The results demonstrated the highest phenolic, flavonoid, and antioxidant activity at 180°C and at 30 min of extraction time. At this point, the result gives the highest antioxidant activity with the highest percentage of inhibition activity. On the other hand, these results were well correlated with the TPC and TFC. Furthermore, it is worthy to note that the DPPH assay gives better correlation results for TPC and TFC compared with the ABTS assay. The optimum condition of SWE was demonstrated at 180°C at 30 min of extraction time and that above 180°C, degradation of phenolic and flavonoid compounds occurred. The *C. latifolia* root extract could be useful as a good source of antioxidative materials. Furthermore, SWE can be one of the most efficient extraction techniques for the production of antioxidative materials from a plant extract. The results are given in LC-MS analysis and the antioxidant activity for *C. latifolia* plants make us reflect on the ethnomedical usages as well as the therapeutic values that could be useful in promoting better nutrition and preventing oxidative stress-related diseases. A further investigation of this natural product is still required, particularly the bioactive components that could be an attractive source of nutraceutical ingredients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors acknowledge the financial support of the Malaysian Ministry of Higher Education for Fundamental Research Grant Scheme, FRGS (Project no: 04-01-16-1861FR), and the Institute of Bioscience, Universiti Putra Malaysia, for providing laboratory facilities.

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