

Research Article

Supercritical Fluid Extraction of Polyphenols from Vietnamese *Callisia fragrans* Leaves and Antioxidant Activity of the Extract

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Vietnamese *Callisia fragrans* (*C. fragrans*) has been considered as a valuable traditional plant with various medicinal properties. In this study, polyphenols were extracted from Vietnamese *C. fragrans* leaves by supercritical carbon dioxide (SC-CO₂) extraction method using ethanol as a cosolvent. The investigation of four factors influencing the total polyphenol content (TPC) and antioxidant activity of the extracts obtained from each single-factor experiment was conducted including ethanol concentration, CO₂ flow rate, extraction temperature, and pressure. Besides, the extraction efficiency of the SC-CO₂ method under the best extraction conditions, namely ethanol concentration of 14%, CO₂ flow rate of 20 g/min, extraction temperature of 45°C, and pressure of 200 bar was compared to that of the Soxhlet extraction (SE) method in terms of the TPC and antioxidant activity of the extracts. The results showed that using SC-CO₂ method, the TPC and the half-maximal inhibitory concentration value obtained were of 87.42 ± 1.33 mg/g and 243.83 ± 5.30 μM TE/g, respectively, with much less time and solvent amount required while that obtained using SE method were of 85.34 ± 4.27 mg/g and 236.33 ± 7.66 μM TE/g, respectively. This indicated that SC-CO₂ would be suitable for the industrial production of polyphenols with high antioxidant activity of the extracts obtained due to the restrictions of using the SE method and advantages of applying SC-CO₂ method. Therefore, SC-CO₂ method could be regarded as a potentially upcoming extraction technique which might be employed to replace the conventional SE method.

1. Introduction

Over 500 species belonging to Commelinaceae family, especially Vietnamese *Callisia fragrans* (*C. fragrans*), have been widely grown in eastern Australia to have their leaves and stems harvested [1]. Containing a great variety of bioactive components such as flavonoids, phytosteroids, vitamins, and microelements, this plant has been well known for many pharmaceutical uses including anti-inflammation, pain killers, burn treatment, and cardiovascular drugs [2, 3].

Polyphenols, known as secondary metabolites, present commonly in all higher plants with more than 8,000 phenolic structures that were identified and classified into 10 classes in association with its structures [4]. These compounds consist of flavonoids and nonflavonoids. It is reported that there are more than 4,000 flavonoid structures of which 143 are proanthocyanidins that have been found [5]. In terms of nonflavonoids, phenolic acids play a pivotal role and have been seen as derivatives of benzoic acids comprising gallic acid, protocatechuic acid, and derivatives of

cinnamic acid [6]. In addition, polyphenols have been considered as an antioxidant agent and an anticancer component owing to biological characterizations for the activation of a variety of enzymes and cell receptors [7]. The polyphenols have been widely applied for sunburn prevention because their aromatic-ring structures could affect the absorbance of the UV radiation ranging from 280 to 315 nm [8].

The solid-liquid extraction method has been divided into two types encompassing conventional and modern technologies. Conventional extraction techniques such as maceration, percolation, and decoction have long been applied in the fields of pharmacy and food industry [9]. Since the fundamental principles of the Soxhlet extraction (SE) method rely on exhaustive extraction leading to higher yields, this technique has been usually utilized in the laboratory due to its limitations of raw materials and well employed mainly for vegetable oil extraction compared to other traditional techniques such as maceration [10]. With the advantage of the amount of solvent reduction, this method could take long extraction time, contribute to pollution problems because of flammable and hazardous solvent consumption, and require strictly high-purity solvents [11]. Because of the development and enhancement of green extraction technologies, the application of ultrasound, microwave, enzyme, and pressurized liquid has been widely used in many parts of the world [12]. Among these methods, the employment of supercritical fluid, especially supercritical carbon dioxide (SC-CO₂) extraction method, has been globally investigated to extract natural products from plants based on its distinct capacities [13]. For the utilization of supercritical fluid technology, it has been gained the interest of food and pharmaceutical industries due to the prevention of thermal degradation of target compounds [14]. For instance, the industrial levels of SC-CO₂ extraction have been started since the 1970s for decaffeination of tea and coffee [15]. In contrast with polar phenolic structures, the nonpolar carbon dioxide (CO₂) compound may modify its polarity by the integration of other solvents called cosolvent or modifier such as ethanol to horn the selectivity and extraction yield of final products [16]. According to preliminary studies, temperature and pressure play a pivotal role in the extraction process using SC-CO₂ [10]. Besides, cosolvent concentration and CO₂ flow rate have been reported as positive factors, having great impacts on extraction yields [17].

In this study, from Vietnamese *C. fragrans*, based on total polyphenol content (TPC) determination and antioxidant activity investigation of the extracts obtained, the effect of single operational parameters including ethanol concentration, CO₂ flow rate, extraction temperature, and pressure on extraction process was evaluated by utilizing ethanol as a cosolvent for SC-CO₂ extraction method. In addition, SE method was carried out in comparison with the SC-CO₂ extraction method to determine the efficient modern extraction method for polyphenols.

2. Materials and Extraction Methods

2.1. Materials and Reagents. The leaves of Vietnamese *C. fragrans* were collected from Hoc Mon District, Ho Chi Minh City, Vietnam. After harvesting, the leaves were cleaned and dipped in 70.0% ethanol before being air-dried for 1 h. Then the leaves were stored in dark bags to prevent polyphenol degradation due to sunlight and transferred immediately to the laboratory in 1 to 1.5 h. By using a dry air oven, the leaves were dried at a temperature of 70°C for 3–4 h until a constant weight was obtained. Before extraction, the leaves were ground to a fine powder with a commercial-grade blender and stored at –20°C for further analysis.

Ethyl acetate (CH₃COOC₂H₅), ethanol (C₂H₅OH), potassium persulfate (K₂S₂O₈), and *n*-hexane (C₆H₁₂) were supplied by Chemsol, Vietnam. Folin–Ciocalteu reagent and sodium carbonate (Na₂CO₃) were supplied by Xilong, China. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), and gallic acid (3,4,5-trihydroxybenzoic acid) were obtained from Aldrich Chemical Co., Gillingham, Dorset, UK.

2.2. Extraction Methods

2.2.1. The Removal of Nonpolar Organic Molecules. 500.0 g of leaf powder of Vietnamese *C. fragrans* leaves was extracted with *n*-hexane by maceration for 14 days (1.5 L of *n*-hexane solvent, each day) at room temperature to remove nonpolar impurities. After that, the leaf powder was shade-dried at room temperature for 1 day and marked as LH.

2.2.2. SE Method. To compare the extraction efficiency, 15.0 g of the leaf powder LH was extracted by the SE method with 350 mL of ethyl acetate, extraction temperature of 80–85°C, and extraction time of 6 h. The collected extracts were concentrated under reduced pressure to partly remove solvents by using a vacuum evaporator at a temperature of 35°C to obtain crude extract marked as CLE.

2.2.3. SC-CO₂ Extraction Method. 15.0 g of the leaf powder LH was filtered and extracted by SC-CO₂ apparatus with the cosolvent ethanol under operational conditions including ethanol concentration (14, 16, 18, and 20%, w/w), CO₂ flow rate (12, 14, 16, 18, and 20 g/min), extraction temperature (40, 45, 55, and 60°C), and pressure (100, 150, 200, and 250 bar). While one operational parameter was changed, other operational parameters were kept constant. The extract obtained under optimal extraction conditions using SC-CO₂ was marked as CLC.

2.2.4. Effect of Single Factors on TPC. In this study, the influences of four single operational parameters including ethanol concentration, CO₂ flow rate, extraction temperature, and pressure were determined according to the TPC and antioxidant activity of the extracts obtained from single-factor experiments.

2.2.5. TPC Determination. TPC was determined based on the Folin–Ciocalteu method [12]. Folin–Ciocalteu's reagent was used to oxidize the samples, and the absorbance measurement was read at 760 nm after 60 min. Gallic acid was used as a standard to create calibration, and TPC was expressed as gallic acid equivalent per gram of dry weight of the Vietnamese *C. fragrans* extract.

2.3. The Antioxidant Activity Investigation of the Extracts. The extracts obtained from each single-factor experiment were tested for antioxidant activity by ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium) radical cation decolorization assay [18]. The ABTS radical cation (ABTS+) could be generated by mixing the 2.45 mM potassium persulfate solution and 7 mM ABTS solution with the ratio of 1:1. This reaction mixture was then kept in the dark at room temperature for 12–16 h before use. The absorbance of the sample was read at 734 nm. The Trolox was used as a standard in the antioxidant activity experiments to generate the calibration curve, and the antioxidant activity of the extract was expressed as $\mu\text{mol TE}$ (Trolox equivalent) per gram of dry weight of the Vietnamese *C. fragrans* extract. The percentage ABTS inhibition was calculated using the following equation:

$$\text{ABTS inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100\%, \quad (1)$$

where A_1 is the absorbance of the sample reaction solution and A_0 is the absorbance of the free radical solution. IC_{50} value was the inhibitory concentration of antioxidant needed to decrease the initial radical by 50%. The IC_{50} values were obtained from the standard curve, and a lower IC_{50} value represents a stronger ABTS scavenging capacity.

3. Results and Discussion

3.1. Effect of Single Factors on the TPC

3.1.1. Ethanol Concentration. Figure 1 indicates the effect of ethanol concentration on the TPC and antioxidant activity of the extracts. Experiments were conducted under extraction conditions including extraction temperature of 50°C, extraction time of 60 min, CO_2 flow rate of 14 g/min, and pressure of 200 bar. As can be seen from Figure 1, TPC increased from 35.54 mg GAE/g to 37.56 mg GAE/g with an increase in ethanol concentration between 10 and 14% and went down to 30.58 mg GAE/g when ethanol concentration surpassed over 14%. According to preliminary studies, a nonpolar CO_2 compound could be modified with a polar solvent such as methanol or ethanol to horn extraction yield. Although methanol might be the most efficient solvent used, it is more toxic compared to ethanol [19]. It was also reported that using ethanol as a binary solvent could lead to an increase in the TPC because of the alteration of its polarity. Besides, the cell permeability would be enhanced by this solvent as ethanol could have an impact on the phospholipid bilayer of the cell membrane [16]. Additionally, kinds of polyphenols extracted by ethanol, as is known from several

studies, are strongly dependent on ethanol concentration [20–22]. The antioxidant activity of the extracts experienced an upward trend and reached its maximum value of 181.58 $\mu\text{M TE/g}$ at an ethanol concentration of 14%. However, as the ethanol concentration continued to rise above 14%, antioxidant activity fell down rapidly. This could be articulated by the strong relationship between ethanol concentration and free radical properties of the crude extract due to the hydroxyl groups of polyphenols [23]. In accordance with other studies, it is revealed that the antioxidant activity of polyphenols has been strongly relevant to its structures [4]. Hence, higher ethanol concentration could be used to improve the effective dissolution of polar polyphenols. However, too high ethanol concentration may lead to dissolving byproduct compounds, resulting in the decrease of antioxidant capacities. Ethanol concentration changes cause solvent physical properties such as dynamic viscosity, density, and dielectric constant modification, influencing the phenolic compound extraction based on previous studies [24]. Therefore, 14% was chosen as an optimal ethanol concentration for further experiments.

3.1.2. CO_2 Flow Rate. Figure 2 presents the effect of the CO_2 flow rate on the TPC and antioxidant activity of the extract. To investigate the impact of the CO_2 flow rate, polyphenolics were extracted using 10% ethanol concentration at 50°C for 120 min with pressure of 200 bar. From Figure 2, it can be seen that TPC increased while rising CO_2 flow rate and gained the highest value of 65.58 mg GAE/g at 20 g/min. In terms of antioxidant properties, the antioxidant activity of the extracts grew up gradually and peaked at 192.58 $\mu\text{M TE/g}$ as the highest point with CO_2 flow rate value of 20 g/min. An increase in TPC could be elucidated by an elevation in the quantities of ethanol and CO_2 compounds presenting in samples, leading to strong interactions between solvents and target compounds. Additionally, adequate extraction time would positively contribute to the growth of TPC because it is relevant to mass transfer kinetics [17, 25]. The results would be in agreement with reported research [26]. Hence, the CO_2 flow rate of 20 g/min was selected as the optimum.

3.1.3. Extraction Temperature. Figure 3 shows the effect of temperature on the TPC and antioxidant activity of the extract. Polyphenols were extracted under extraction conditions including CO_2 flow rate of 14 g/min, extraction time of 120 min, ethanol concentration of 10%, and pressure of 200 bar.

It can be seen that increasing the extraction temperature from 40 to 45°C generally increases TPC and antioxidant activity of the extracts with the maximum values of 43.95 mg GAE/g and 180.5 $\mu\text{M TE/g}$, respectively. When the extraction temperature was over 45°C, a slight decrease in the TPC and antioxidant activity of the extracts could be observed. This phenomenon could be explained due to the fact that as increasing temperature until 45°C, the TPC elevated because of an increase in diffusion and desorption [19]. Moreover, raising temperature could be ascribed to some aspects such as permeability of cell walls, solubility of targeted

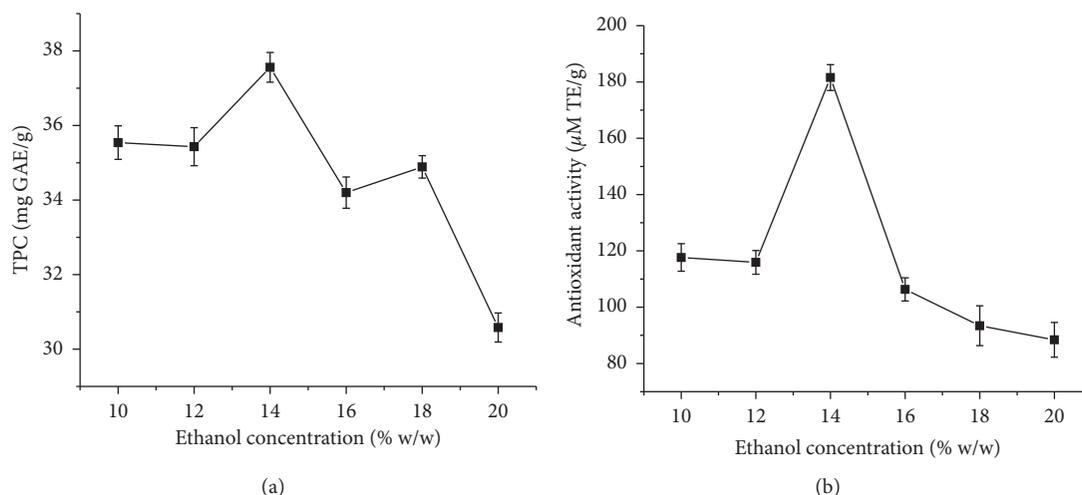


FIGURE 1: Effect of ethanol concentration (% w/w) on the (a) TPC (mg GAE/g) and (b) antioxidant activity ($\mu\text{M TE/g}$) of the extract.

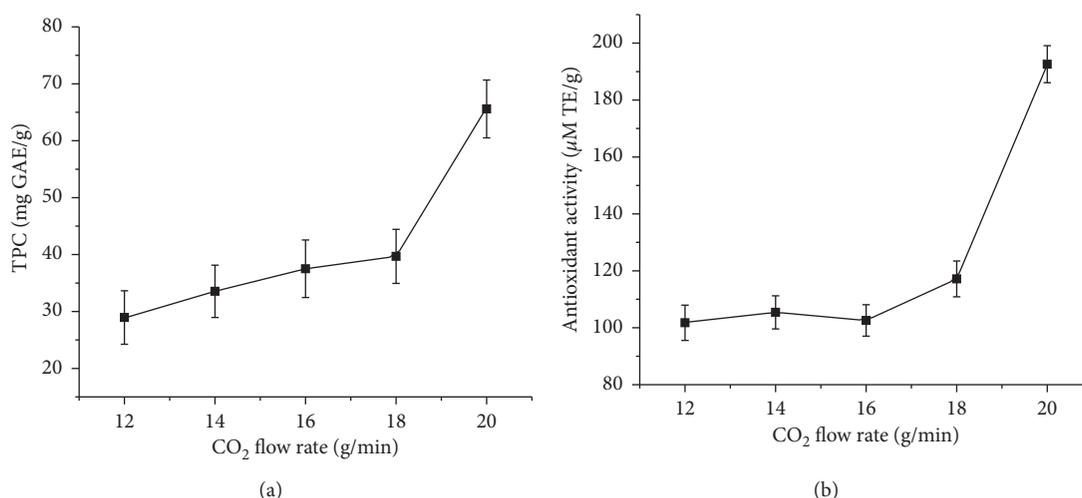


FIGURE 2: Effect of the CO₂ flow rate (g/min) on the (a) TPC (mg GAE/g) and (b) antioxidant activity ($\mu\text{M TE/g}$) of the extract.

compounds, and mass transfer phenomena [16]. It is cited that polyphenols such as anthocyanins are thermally unstable and easily hydrolyzed or oxidized. Hence, it could rapidly degrade by high temperature. In addition, the process of polyphenol hydroxylation and oxidization might induce a decrease in its antioxidant activity of the extracts [27]. The enhancement of temperature has directly exerted a detrimental impact on reducing the density of the modified supercritical CO₂, contributing to a decrease of the solubility [28]. This result could be found familiar with other research [29, 30]. Thus, the extraction temperature of 45°C was selected to employ for further research.

3.1.4. Pressure. Figure 4 describes the effect of pressure on the TPC and antioxidant activity of the extract. The influence of pressure on the TPC and antioxidant activity was studied at 100, 150, 200, and 250 bars with CO₂ flow rate of 14 g/min, extraction time of 60 min, ethanol concentration of 10%, and

extraction temperature of 50°C. As shown in Figure 4, the TPC and antioxidant activity of the extracts were enhanced and obtained its highest content of 33.55 mg GAE/g and 105.41 $\mu\text{M TE/g}$, respectively, upon increasing pressure to 200 bar, after which a gradual decline in the TPC and antioxidant activity of the extracts could be observed. It is reported that the main important mechanism could be used to explain for this would be the fluid density, which changes the solubility of the solute [31]. Although the density boosts the solvating power of CO₂ while increasing pressure, higher pressure could lead to the low mass transfer kinetics due to the low dispersion coefficient of supercritical CO₂ as falling diffusivity, negative influence on the highly porous extraction bed, and diminishing in the interaction between solute and solvent.

Thus, extraction efficiency and antioxidant activity decreased sharply [28, 32]. From the previous results, this study might be in consonance with other research [30, 33]. As a result, 200 bar would be set as an optimal pressure.

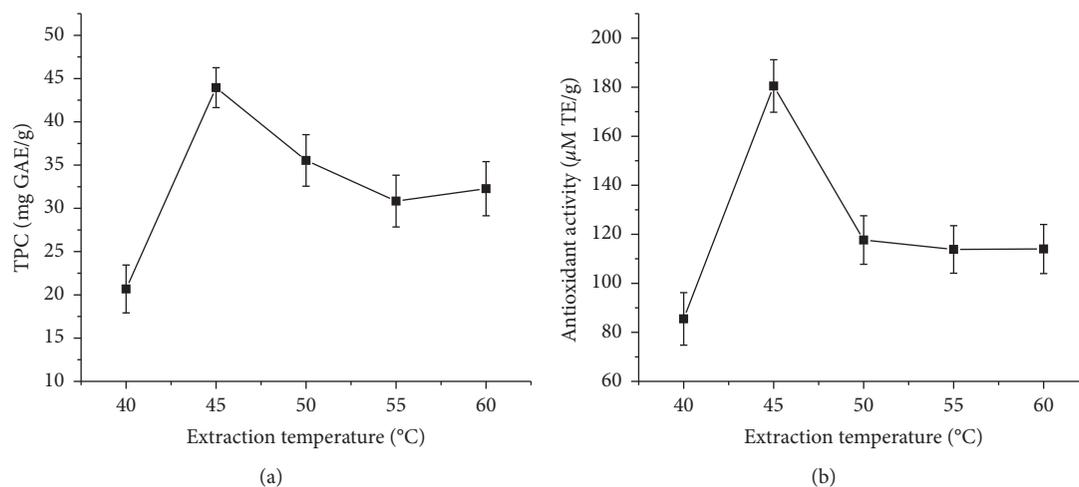


FIGURE 3: Effect of extraction temperature (°C) on the (a) TPC (mg GAE/g) and (b) antioxidant activity ($\mu\text{M TE/g}$) of the extract.

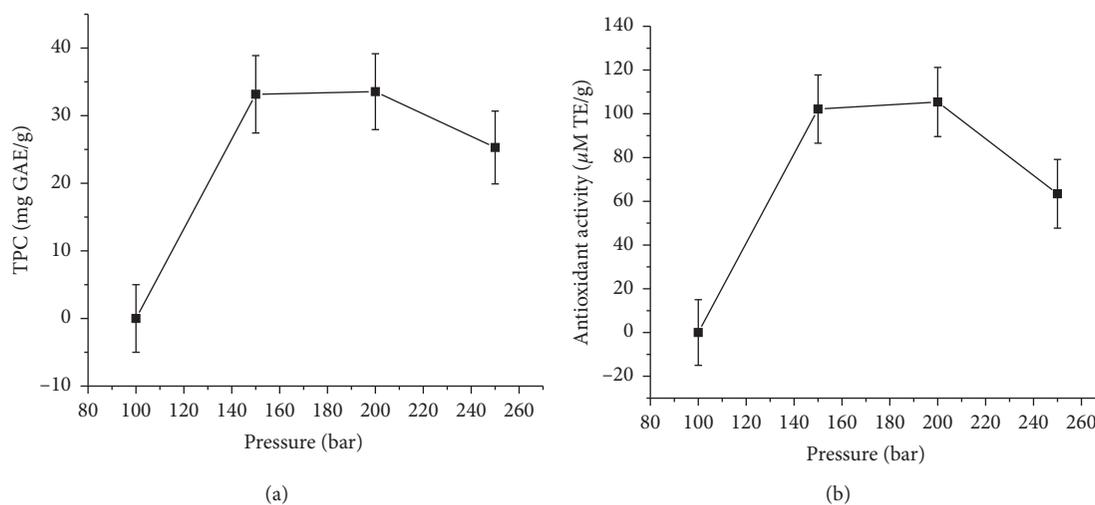


FIGURE 4: Effect of pressure (bar) on the (a) TPC (mg GAE/g) and (b) antioxidant activity ($\mu\text{M TE/g}$) of the extract.

3.2. SE Method. Table 1 describes the comparison of the TPC and antioxidant activity of the extracts obtained by the SC-CO₂ and SE method under conditions of using 350 ml ethyl acetate and temperature ranging from 80 to 85°C during 6 hours. The result showed that the TPC and the IC₅₀ value obtained using SC-CO₂ method were of 87.42 ± 1.33 mg/g and 243.83 ± 5.30 $\mu\text{M/g}$, respectively, while that obtained using the SE method were of 85.34 ± 4.27 mg/g, 84.60 mg/g, and 236.33 ± 7.66 $\mu\text{M/g}$, respectively. It is cited that among these conventional techniques, SE has been widely used for polyphenol extraction because employing higher temperature leads to higher mass transfer kinetics as well as continuous change in the transfer equilibrium by the new contact between fresh solvents and solid particles [10]. However, the method could have several restrictions such as long time extraction and volatile as well as flammable organic solvents consumption. These disadvantages might be

TABLE 1: Comparison of the TPC and antioxidant activity of the extracts obtained by the SC-CO₂ and SE method.

The extract	TPC (mg GAE/g)	Antioxidant activity ($\mu\text{M TE/g}$)
SC-CO ₂ extract	87.42 ± 1.33	243.83 ± 5.30
SE extract	85.34 ± 4.27	236.33 ± 7.66

altered by SC-CO₂, which could generate an improvement in extraction yields and antioxidant activity [11]. In application scale, with limitation on the amount of materials used, SE could not be scalable compared to SC-CO₂ as SC-CO₂ may be suitable for the large quantity of mass production without contaminating products with solvent residues [34]. This result is consistent with reported studies [3, 35, 36].

4. Conclusions

From Vietnamese *C. Fragrans*, based on the highest TPC and antioxidant activity of the extract obtained from each single-factor experiment, the extraction conditions determined with the application of SC-CO₂ method using ethanol as a cosolvent were ethanol concentration of 14%, CO₂ flow rate of 20 g/min, extraction temperature of 45°C, and pressure of 200 bar. Under these conditions, the extract was compared with that obtained under the SE method in terms of TPC and antioxidant activity. The results showed that the TPC and the antioxidant activity with IC₅₀ values of the extract obtained using SC-CO₂ method were of 87.42 ± 1.33 mg/g and 243.83 ± 5.30 μM TE/g, respectively, while that obtained using the SE method were of 85.34 ± 4.27 mg/g and 236.33 ± 7.66 μM TE/g, respectively. In terms of polyphenol composition determination by HPLC method for isolation and purification of polyphenol compounds, further research would be conducted in the next study. Therefore, SC-CO₂ method could be considered as a potential efficient extraction method in comparison with the conventional SE method.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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