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Research Article

Antioxidant and Antibacterial Assays on *Polygonum minus* Extracts: Different Extraction Methods

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The effect of solvent type and extraction method was investigated to study the antioxidant and antibacterial activity of *Polygonum minus*. Two extraction methods were used: a solvent extraction using Soxhlet apparatus and supercritical fluid extraction (SFE). The antioxidant capacity was evaluated using the ferric reducing/antioxidant power (FRAP) assay and the free radical-scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The highest polyphenol content was obtained from the medium polarity methanol extract of the leaf portion (645.60 ± 166.68 gallic acid equivalents/100 g (GAEs/100 g)). It also showed the highest antioxidant power for FRAP and DPPH radical inhibition and exhibited the largest inhibition zone in antibacterial activity on *Bacillus subtilis* (Gram+), *Staphylococcus aureus* (Gram+), and *Escherichia coli* (Gram−). The phase behavior and aldehyde profiles were further investigated using SFE with different cosolvents. The results indicated that a 50% ethanol-water cosolvent yielded the best aldehyde profiles in the presence of decanal, undecanal, and dodecanal.

1. Introduction

Natural bioactive compounds from plant sources especially herb plants have been investigated in recent years. Herb plants are also well known to be associated with many medicinal properties. In Malaysia, *Polygonum minus*, known as kesum, is a local herb plant that has been used widely as spice and condiment. Because of the natural aliphatic aldehydes present, *P. minus* is one of the herbs identified as having a significant potential as a source of essential oils, especially in the fragrance industry, because of its richness of C10 and C12 aldehydes [1]. Previous research investigating *P. minus* has focused mostly on the chemical screening and identification and isolation of its components and biological assay studies [2–4]. Noriham has also performed tests of antioxidant and antibacterial activity on *P. minus* and *Melicopelunu ankenda* extracts to examine the storage quality of chicken sausage [5]. A clear relationship between phenolic content and antioxidant capability of the extract (FRAP and DPPH value) has been established in this research where these two values are directly proportional to the phenolic contents. Therefore, it can be said that FRAP value and DPPH inhibition were dependent on the phenolic compound content of the plant because these two assays emphasized the antioxidant capability to reduce the involved radicals (ferric ion and DPPH free radical).

Seyoum et al. [6] proposed that the biological activity (including antiallergic, anti-inflammatory, antioxidant, antivirus, and anticarcinogenic properties, among others) was due to the presence of flavonoids. Flavonoids are a large family of polyphenolic components synthesized by plants. They are able to protect the biological system because of their antioxidant capability and their capacity to transfer the electron and free radical [7]. Flavonoid compounds also showed an inhibitory effect toward many types of viruses because the flavonoid compounds contain lipophilic

metabolites that can disturb the structure and function of the membrane of a microorganism. Flavonoids from *P. minus* have been reported by Urones et al. [8], who isolated two new flavones and identified 6,7-methylene-dioxy-5,3,4,5-tetramethoxyflavone and flavonol (6,7-4,5-dimethylenedioxy-3,5,3-trimethoxyflavone) from the ether extract of *P. minus*. Several minor components, such as hydrocarbons, monoterpenes, esters, fatty acids, and sesquiterpenes, have also been identified in this research.

Research on new chemical materials from plants should be a priority in the present and future efforts toward continuous conservation of biodiversity. The extraction of essential oil components using solvents at high pressure, or supercritical fluids, has received much attention in the past several years, especially in food and pharmaceutical and cosmetic industries, because it presents an alternative to conventional processes such as organic solvent extraction and steam distillation [1]. Supercritical fluid extraction (SFE) is a promising separation method for producing a high quality of natural essential oils because of the high selectivity of the nontoxic supercritical carbon dioxide solvent (SC-CO_2) employed. SFE can preserve the natural properties of the sample and produce a better extract by preventing thermal degradation. SFE has the capability to extract aromatic and higher molecular weight aldehyde compounds [9]. Besides, carbon dioxide can be used in combination with cosolvent (water and/or an alcohol) to form a gas-expanded solvent to extract desirable polar compounds such as phenolic. The alcohol-water cosolvent gave an interesting finding that polar solvents are able to enhance the yield of SFE extracts since fractionation between the less polar compounds and more polar compounds was possible [10].

The use of SFE in the extraction of antioxidant compounds has increased because the cosolvent composition in SFE extraction had a great influence on extract yield and composition, in terms of total phenolic compounds, total flavonoids, and antioxidant activity [11]. Besides, the study on the effect of the choice of solvents on the extraction of active components from *P. minus* is lacking. The study of the solvent effects on the extraction of active components from herbs is very important for the screening and selection of the solvent for the extraction, fractionation, and purification steps in the herbal processing. By understanding the solvent properties, component (solute) properties and solvent–solute interaction, rapid fractionation, and isolation of the desired components can be achieved [10]. Therefore, the objectives of this research were to identify and determine the biological activity, as well as the content of the bioactive compounds, of different types of *Polygonum minus* extracts through the bioassay method, to study the pressure and temperature effects of supercritical fluid extraction and to identify the best cosolvent for SFE using aldehyde profiles.

2. Materials and Methods

2.1. Plant Preparation. Fresh *Polygonum minus* samples were obtained from Ulu Yam, Selangor. The fresh samples were cleaned and washed using running tap water and then divided into two parts: leaf and whole plant. The samples were dried using an oven (Sheldon Manufacturing, Inc., FX2-2, USA) at 40°C and then ground for approximately 2-3 minutes using a grinder (*Multifunction disintegrator* SY-04, Golden Bull) before any further processing.

2.2. Chemicals and Reagents. The chemicals used for the extraction process were methanol (99.9% purity, Merck, Germany), *n*-hexane (99% purity, Sigma-Aldrich, Germany), distilled water, ethanol (95% purity, Merck, Germany), acetone (Sigma-Aldrich, Germany), dichloromethane (Sigma-Aldrich, Germany), and carbon dioxide (99.9% purity, NIG Gases, Malaysia). For the bioassay tests, the chemicals and reagents used were gallic acid (99% purity, Merck, Germany), Folin-Ciocalteu phenol reagent (Merck, Germany), sodium carbonate (Na_2CO_3, 99% purity, Merck, Germany), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (99% purity, Sigma-Aldrich, Switzerland), hydrochloric acid 37% (Merck, Germany), sodium acetate ($C_2H_3NaO_2$, 99% purity, Friendemann Schmidt, Australia), acetic acid (CH_3COOH, 99.9% purity, Sigma-Aldrich, Germany), ferric chloride ($FeCl_3{\cdot}6H_2O$, 99% purity, Friendemann Schmidt, Australia), ferrous sulfate ($FeSO_4{\cdot}7H_2O$, 99.5% purity, Friendemann Schmidt, Australia), and 1,1-diphenyl-2-picrylhydrazyl (≥85% purity, Sigma-Aldrich, USA). All chemicals and reagents used in the study were analytical grade.

2.3. Extraction Methods. Two different extraction methods were used in this study: solvent extraction by Soxhlet and high pressure extraction method by supercritical fluid extraction (SFE). Although Soxhlet and SFE are basically two different extraction methods, the comparison between these two methods was made to determine the efficient extraction of bioactive compounds from *P. minus*.

2.3.1. Soxhlet Extraction. Dried and ground samples for both parts of plant (10 g) were extracted using the Soxhlet apparatus that consists of a heater (Toshniwal, India), solvent flask, sample chamber, and condenser. The solvents (200 mL of *n*-hexane, methanol, or water) were used in the Soxhlet extractor, and the extraction was performed for 4 hours. The collected extract solutions were then evaporated using a vacuum rotary evaporator (Yamato Scientific Co., Ltd., RE 600, Japan) to yield a viscous mass. The crude extracts were weighed and diluted before being stored at 0–4°C for further analysis. The extraction yield was calculated using the following equation:

$$\text{Total extraction yield, } Y_T\,(\%) = \frac{\text{Mass of extract, } m_T}{\text{Mass of sample, } F} \times 100\%. \quad (1)$$

2.3.2. Supercritical Fluid Extraction (SFE). The samples were also extracted using a supercritical fluid extraction (SFE) system as shown in Figure 1. Supercritical carbon dioxide (SC-CO_2) has been used as the extraction solvent to extract 5 g of the leaf part of *P. minus* sample. A combination of

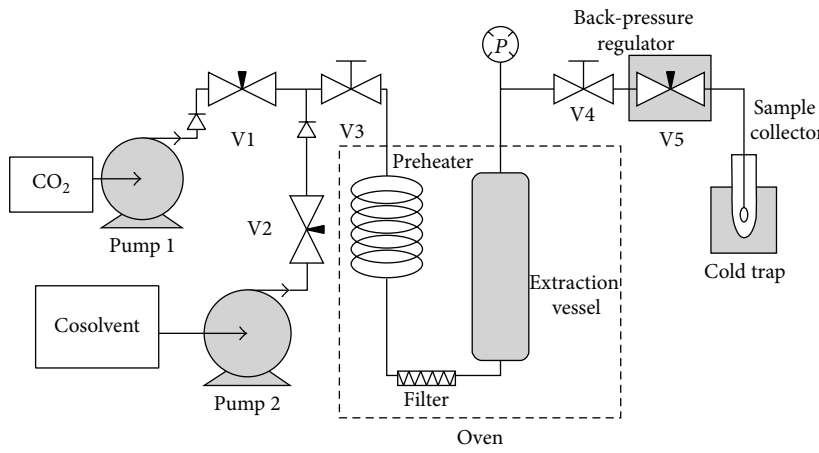

Figure 1: Schematic diagram of SFE system [10].

a half-hour static extraction was performed followed by a dynamic extraction at a solvent flow rate of 3 mL/min for 4 hours. The extract fractions were collected every 30 minutes during the dynamic extraction. The parameters investigated were pressure (150, 200, and 250 bars) and temperature (40 and 50°C). All extracts were then placed at room temperature conditions before weighing to determine the extract yields. The solubility and density of supercritical fluid can be easily manipulated by changing the pressure and temperature [12]. Therefore, both pressure and temperature were chosen as process parameter while other parameters (solvent flow rate and extraction time) were kept constant.

2.3.3. Cosolvent Selection. In this case, a static extraction using the SFE system was conducted. A Jergusen vessel (extraction vessel) was placed in a water bath, with the temperature controlled by a heater. The sample (2.0 g) was placed in the extraction vessel, and then 15% (v/v) cosolvent was added before extraction process started. During the extraction period, the pressure in the extraction vessel was maintained by pumping in CO_2 at a flow rate of 1–3 mL/min. Observations on phase change and the volume change of cosolvent were recorded. The extract was collected in a collector filled with *n*-hexane. All the extracts were dried using a freeze dryer at a temperature of –100°C for 4–6 hours and then weighed to obtain the final mass.

In this cosolvent study, several types of cosolvents (namely, water, 50% ethanol, 80% methanol, 70% acetone, and dichloromethane (DCM)) were used based on the measurement of polarity. The experiments were conducted at a temperature of 40°C and various pressures (80, 90, 100, 120, and 150 bars). The weighed extracts were diluted in *n*-hexane before further analysis using gas chromatography (GC) to determine which cosolvent showed a better aldehyde profile.

2.3.4. Gas Chromatography Analysis. The GC technique [2] was conducted using a Shimadzu GC system (Model 17A with FID, Japan). The column type was nonpolar DPX 1 with 0.25 mm ID × 50 m. The carrier gas used was helium and the gas flow rate was 1.3 mL/min. Temperature for detector was 280°C, while temperature for injector was 250°C. The oven temperature was increased from 70 to 200°C at 4°C/min. Identification of the aldehyde was achieved by comparison with retention times of standards.

2.4. Determination of Total Phenolic Content (TPC). The total phenolic content of the *P. minus* extracts was determined using the Folin-Ciocalteu reagent (FC) as described by Singleton and Rossi [13]. Properly diluted *P. minus* extract solution (20 μL) was mixed with 100 μL of FC reagent in the dark. The reagent was prediluted 10 times with distilled water. After the reagent stood for 3–8 minutes at room temperature, 80 μL of sodium carbonate solution (7.5% w/v) was added. The solutions were mixed and allowed to stand in the dark for 2 hours at room temperature for the reaction to occur. The absorbance at 765 nm was measured. The results were expressed on a fresh weight basis as mg gallic acid equivalents/100 g of sample.

2.5. Ferric Reducing/Antioxidant Power (FRAP) Assay. The FRAP assay was performed according to a modified method described by Benzie and Strain [14]. FRAP reagent was freshly prepared by mixing 5 mL 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution (10 mM) in 40 mM hydrochloric acid solution with 5 mL $FeCl_3 \cdot 6H_2O$ solution (20 mM) and 50 mL acetate buffer solution (0.3 M, pH 3.6) and incubated at 37°C after the mixing. Properly diluted *P. minus* extract (50 μL) was mixed with 1.5 mL of FRAP reagent under dark conditions. The absorbance at 593 nm of 200 μL of the mixture was determined against a blank. FRAP values were expressed on a fresh weight basis as micromoles of ferrous equivalent Fe (II) per gram of sample.

2.6. DPPH Free Radical-Scavenging Assay. The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by de Ancos et al. [15]. Diluted extract (20 μL) was mixed with 80 μL of methanol and 200 μL of

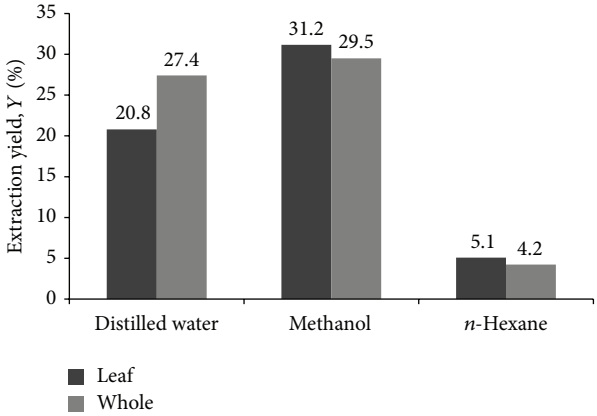

Figure 2: Total extraction yield in Soxhlet extraction of *P. minus* using different solvents and plant parts.

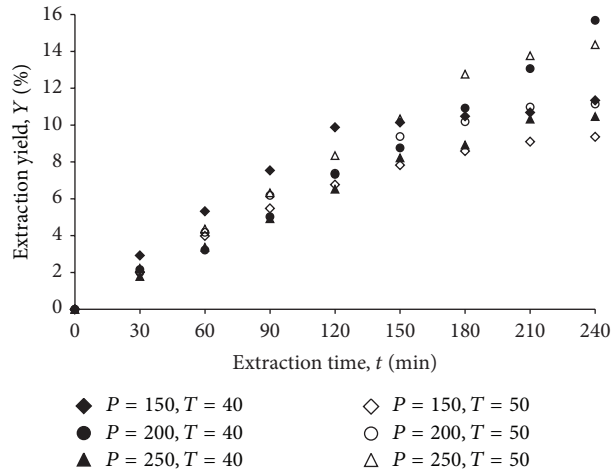

Figure 3: Overall extraction curves in SFE of *P. minus* at different temperatures and pressures.

0.1 mM DPPH. The mixture was kept in the dark for 30 minutes before the absorbance at 515 nm was measured against a control solution of methanol and DPPH without extracts. The results were expressed as percentage of the DPPH radical. The percentage of the DPPH radical was calculated according to following equation:

$$\%\text{inhibition of DPPH} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100, \quad (2)$$

where A_{control} is the absorbance of DPPH without extract, while A_{sample} is the absorbance of the extracts.

2.7. Antibacterial Assay Using Disc Diffusion Method. The antibacterial assay was conducted using the disc diffusion method described by Mackeen et al. [16] for the three selected bacteria (*Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli*). Cultured bacteria (0.1 mL) were inoculated uniformly on nutrient agar. Six pieces of Whatman number 1 filter paper discs (6 mm diameter) that had already been loaded with the extract were placed on the inoculated agar. The plates were inverted and incubated for 24 hours at 30°C. Clear inhibition zones around the discs indicated the presence of antibacterial activity. The strength of activity was classified as strong for inhibition zone diameters ≥ 15 mm, moderate for diameters ranging from 10.0 to 14.5 mm, and weak for diameters < 10 mm.

3. Results and Discussion

3.1. Soxhlet Extraction. Figure 2 shows the percentage yield of *P. minus* extract that was obtained using Soxhlet extraction. The results show that the percentage yield of *P. minus* extracts differed when different solvents were used. Methanol extraction produced the highest yield of extract (31.17%), followed by distilled water extraction. The lowest yield of extract was shown by the *n*-hexane extracts because solvent extractions are influenced by the extraction capability. The extraction capability depends on the solvent chemical structure and its polarity, and these factors influence the extraction yield [17]. A study by Noriham [5] showed that *P. minus* extract using the maceration method with distilled water as the solvent gave the highest yield of extract (5.00%) compared to methanol (2.41%). This difference was possibly due to the extraction method itself. For the maceration method, the samples were mostly extracted at room temperature. For the Soxhlet extraction method, the temperature used differed for the different solvents because Soxhlet extraction requires the evaporated and condensed solvent to be at the solvent boiling point.

From the results obtained, the leaf portion of the plant gave the higher yield of extract compared to the whole plant because different parts of the same plant may synthesize and accumulate different compounds or different amounts of a particular compound because of the differential gene expression, which affects the biological properties of the plant extracts produced [18, 19]. The leaf part of *P. minus* contains a large amount of a particular compound compared to the other parts of the plant, resulting in a higher extract yield for the leaf part. In addition to solvent polarity, plant part, and extraction method, other factors affecting the percentage of yield are sample particle size, temperature, and the volume ratio of sample to solvent [20].

3.2. Supercritical Fluid Extraction (SFE) Using Carbon Dioxide. Essential oils were extracted from *P. minus* using SFE at pressures of 150, 200, and 250 bars and at temperatures of 40°C and 50°C. The cumulative percentage of extract yield at different pressures and temperatures is presented as a graph in Figure 3. The operating parameters at 200 bars and 40°C were found to produce the highest yield (15.68%), while the lowest yield (9.36%) occurred at 150 bars and 50°C. The difference in extract yields was due to a combination of factors: pressure and temperature affect density and the solvent power of CO_2 as well as solute volatility [21].

3.2.1. Effect of Pressure and Temperature. The extraction yield at different pressure and temperature was shown in Figure 3.

The extract yield was found to increase with increasing pressure at constant temperature. At a temperature of 40°C, the extract yield increases reached maximum at 200 bars and then decreased when the pressure was over 200 bars. At 50°C, the extract yield increased with increasing the pressure until a pressure of 250 bars was reached.

The differences in extract yield with changes in pressure were caused by two factors. First, an increase in pressure caused the density of the supercritical carbon dioxide solvent (SC-CO_2) to increase and thus increased the plant component solubility and extract yield at both 40°C and 50°C. The second factor is that increasing the pressure will reduce the diffusivity of SC-CO_2, causing the extract yield to be reduced. For pressures over 200 bars, the effect of low solvent diffusivity was dominant and reduced the yield of extract, as shown at 250 bars and 40°C. The pressure effect of SFE on *P. minus* at 50°C was consistent with other studies [21–23]. A study by Döker et al. [23] of SFE extract yield for sesame oil at three different temperatures showed that the extract yield increased with increasing pressures from 250 to 350 bars. This study also reported that the time needed to achieve the maximum extract yield was shortened with increasing pressure.

From Figure 3, at constant pressures of 150 and 200 bars, it is shown that the yield decreased with increasing the temperature. At a pressure of 250 bars, the extract yield increased with increasing the temperature. Temperature also showed a complex and inconsistent effect on extraction near the critical point. However, the resulting graph showed that increasing the temperature will decrease the density of SC-CO_2 and thus decrease the solubility of *P. minus* in SC-CO_2. A temperature rise from 40°C to 50°C at pressures of 150 and 200 bars decreased the extract yield because of the reduction in solvent density. This phenomenon could be inverted at higher extraction pressures where the temperature increase can increase the solute vapor pressure and thus increase the extract yield. The temperature effect on the extraction rate under these specific extraction conditions does not appear to be as intense as the effect of pressure. The effect of pressure can be explained by the differential increment in the solvent density [24]. This enhancement of extraction yield is observed through increasing temperatures in the range of 40°C to 50°C at 250 bars, where the higher solute solubility due to increased vapor pressure overcomes the effect of the solvent density decrease [25].

The effect of pressure and temperature on the solubility of the extracts also showed the same trend as shown in Figure 4. The same solubility effect has been reported for other essential oils obtained through SFE [23, 24, 26]. A study of the application of SFE for fatty acids from trout fish powder by Nei et al. [22] showed that a temperature rise over 53°C decreased the extract yield due to the density reduction of SC-CO_2. Other than the pressure and temperature effects, the yield of SFE extract was also affected by other factors, such as solvent flow rate and the porosity effect. However, in this study, the solvent flow rate and the porosity effect did not affect the yield of extract because the constant flow rate (3 mL/min) and the same porosity (same sample for each parameter) were used during the experiments.

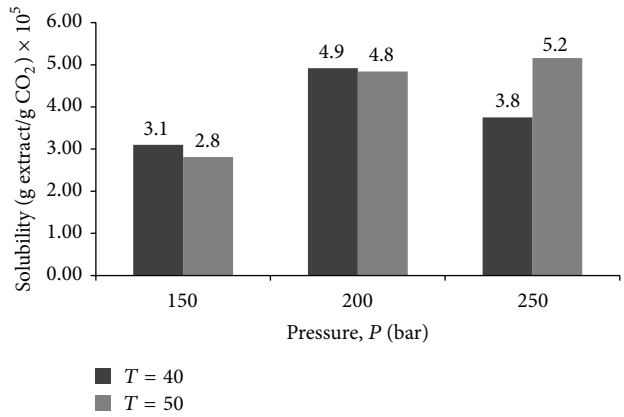

FIGURE 4: Solubility of *P. minus* in SC-CO_2 at different pressures and temperatures.

Extensive reviews on the SFE of essential oils from various plant sources have been prepared by several researchers. Most of the studies showed that the quality of the essential oils extracted using SFE is better than steam distillation and solvent extraction [9, 27, 28]. SFE using a cosolvent is better than Soxhlet extraction because SFE can yield more extract, perform a faster extraction, and produce a pure extract due to the use of a nontoxic carbon dioxide solvent. The addition of a small amount of liquid cosolvent as a modifier can significantly enhance the extraction efficiency and reduce the extraction time [29]. However, the results obtained in this study for the extract yield from Soxhlet extraction were higher than those from the SFE extraction because the SFE in this study did not use a cosolvent to enhance the extraction efficiency; instead, only the nonpolar solvent CO_2 was used. SFE is different from Soxhlet extraction that uses a polar solvent, such as distilled water and methanol. The composition of *P. minus* appears to be higher in polar constituents.

3.3. Phenolic Content Analysis and Antioxidant Capacity. The total phenolic content in the extracts of *P. minus* is shown in Table 1. Methanol extracts showed the highest phenolic content (645.6 ± 166.7 mg GAEs/100 g) compared to other extracts. The total phenolic content for each solvent was different because of the different solvent polarities and solvating strengths [30]. The solvent polarity is very important in increasing the solubility of phenolic compounds [31]. *n*-Hexane and CO_2 are nonpolar solvents and produce lower phenolic content than methanol and distilled water. The phenolic compound content is different for the leaf part and the whole plant. Zainol et al. [32] found that different parts of the same plant affected the total phenolic content of *Centella asiatica* (pegaga) where the highest content was in the leaf part, followed by the root part, and the lowest phenolic content was found in petiole part. A study by Heidar [33] on the antioxidant enzyme activities in leaves, stem, and roots of *Sorghum* (*Sorghum bicolor* L.) showed that the upper and middle leaves were the most sensitive organs to oxidative damage compared to the other parts of the plant, and lipid

Table 1: Antioxidant activity in *P. minus* extracts.

Extract	Part	Phenolic content (mg GAE/100 g)	FRAP value (μmol Fe (II)/g)	DPPH inhibition (%)
Distilled water	Leaf	472.2 ± 75.4	491.5 ± 61.6	59.8 ± 3.1
	Whole	482.2 ± 14.1	479.6 ± 58.2	50.3 ± 5.9
Methanol	Leaf	645.6 ± 166.7	633.3 ± 13.4	90.4 ± 0.4
	Whole	626.1 ± 22.2	591.4 ± 13.0	86.6 ± 2.5
n-Hexane	Leaf	48.7 ± 1.8	235.9 ± 17.1	1.3 ± 0.8
	Whole	51.9 ± 2.2	249.8 ± 3.5	7.7 ± 1.2
SFE	Leaf[1]	39.9 ± 1.1	265.9 ± 7.3	5.3 ± 1.7
	Whole[1]	40.1 ± 1.8	256.5 ± 2.2	14.3 ± 1.6

[1]Carbon dioxide at 150 bars and temperature of 40°C.

Table 2: Inhibition diameter of bacteria (including filter paper).

Extract	Bacteria	Inhibition diameter ± standard deviation* (mm)		
		Leaf	Overall	Control**
Distilled water	BS	8 ± 0.5	—	—
	SA	9 ± 0.5	—	—
	EC	9 ± 0.00	—	—
Methanol	BS	12 ± 1.5	11 ± 0.6	—
	SA	12 ± 0.6	11 ± 1.0	—
	EC	12 ± 0.6	11 ± 0.6	—

BS is *Bacillus subtilis*, SA is *Staphylococcus aureus,* and EC is *Escherichia coli*.
*Deviation for 3 times of repetition.
**Negative control that used dilution solvent without the extract.

peroxidation (LPO) levels were particularly high in the upper leaves.

The antioxidant power of the different *P. minus* extracts was studied using the ferric reducing/antioxidant power (FRAP) test and the DPPH free radical inhibition test. Table 3 shows the FRAP values and percentage of DPPH inhibition for all extracts. The antioxidant power is related to the phenolic antioxidant activity [34]. The test results showed that the antioxidant capability is directly proportional to the total phenolic content. The results are different in the case of distilled water for the leaf and the whole part of plant as well as in the case of SFE and *n*-hexane extract. However, the difference is not obvious and it can be assumed as within experimental error. All the samples showed antioxidant power in the four types of solvents that were used. The FRAP values obtained varied for different solvents because of the difference in solvent polarities. The FRAP values for the *n*-hexane and SFE extracts were nearly the same because both are nonpolar solvents. The SFE extract with minimum parameter value (150 bars and 40°C) was chosen to be compared with *n*-hexane extract that was extracted at atmospheric pressure. The SFE extract appears to offer an advantage over the *n*-hexane extract using Soxhlet extraction because SFE produced a higher yield of extract. The FRAP values also showed that the phenolic content is positively correlated with the antioxidant capability of the plant because the *P. minus* extracts were apparently able to donate an electron to the reactive radical and change that radical to a stable and nonreactive species [5].

A positive correlation exists between the antioxidant activity and the reducing capability of the extracts. The FRAP value and the percentage of DPPH inhibition show the same trend: the greater the extract reducing power, the greater the antioxidant activity. This similar trend has also been reported in banana, pineapple, and guava plants [35]. The relationship between extract reducing power and antioxidant activity may be due to the same reaction mechanism for both FRAP and DPPH assays. These two methods are concerned with the capability of the antioxidant to reduce the involved radicals (ferric ion and DPPH free radical). A direct relationship between the total phenolic content and the antioxidant capabilities of the extracts (both FRAP and DPPH values) also exists. The existence of this relationship demonstrates that the phenolic compounds are the main components contributing to the antioxidant activity of the plant.

3.4. Antibacterial Activity. The antibacterial activity of *P. minus* extracts was tested on three bacteria (*Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli*); the results are shown in Table 2. Only the methanol and distilled water extracts showed antibacterial activity. Methanol extracts for the leaf and the whole plant showed moderate antibacterial activity (10 to 14.5 mm inhibition zone) for all of the bacteria tested while the distilled water extract showed weak antibacterial activity (8 to 9 mm inhibition zone). The difference in bacterial inhibition is possibly due to the effect of different compounds present in the different solvent extracts. This study also demonstrated that the *n*-hexane and SFE extracts

Table 3: Cosolvent effects on ternary mixture in carbon dioxide.

Cosolvent	Volume changes of liquid phase (mL)	Number of liquid phases	Final color of liquid phase
None	3.4–3.5	1	Clear
Water	0.1–0.2	1	Brown
Aqueous ethanol (50% v/v)	0.2	1	Brown
Aqueous methanol (80% v/v)	1.0–3.0	1	Green
Aqueous acetone (70% v/v)	4.4–4.6	2	Clear (top) Green (bottom)
Dichloromethane (DCM)	1.0–1.4	2	Clear (top) Brown (bottom)

did not show any antibacterial activity because the diffusion method is not appropriate when the agents to be studied are not soluble in water; the essential oils and nonpolar extracts are commonly extracted by nonpolar solvents, such as chloroform, petroleum ether, and *n*-hexane [5]. The negative control that used only dilution solvent without the extract also showed no antibacterial activity.

The bacterial inhibition may also be due to the presence of phytochemical components such as polyphenols. Noriham [5] reported that polyphenols presented in *P. minus* (alkaloid, triterpene/steroid, flavonoid, and saponin) have been detected in the *P. minus* extract as well. The biochemical properties of polyphenols, such as flavonoids, attract the attention of many biologists. Polydoro et al. [36] also reported that flavonoids act as an inhibitor toward the superoxide anion and the hydroxy and peroxy radicals to inhibit the key enzyme in mitochondrion respiration.

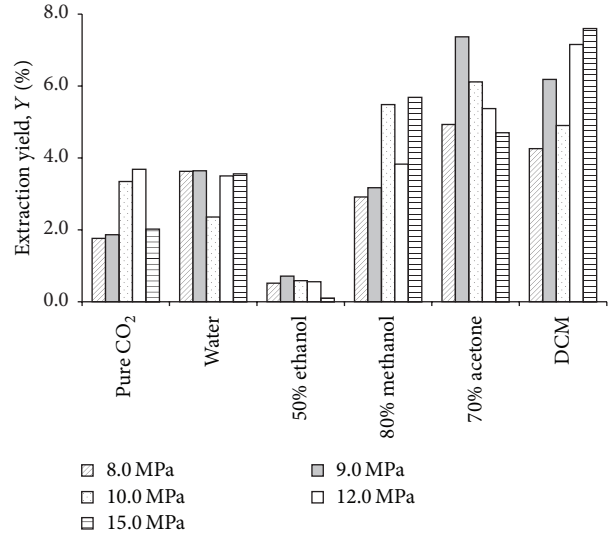

Figure 5: Yield of extract using different cosolvents at different pressures.

3.5. Cosolvent Selection and Aldehyde Profiles

3.5.1. Cosolvent Effect on Mixture of Ternary Phase. The presence of a cosolvent in supercritical carbon dioxide (SC-CO_2) affects the extract yield of *P. minus* at a temperature of 40°C and pressures ranging from 80 to 150 bars. Table 3 shows the effects of different pressures at constant temperature on the phase and volume changes of the cosolvent (liquid phase). The presence of the liquid phase demonstrated that the critical point of the fluid mixture had not yet been achieved. This effect can be observed by using pure CO_2 (without cosolvent). When CO_2 was fed at 60 bars, a liquid layer was formed that completely changed back to the gas phase at pressures of approximately 76 bars (the critical pressure was achieved). The aqueous methanol cosolvent (80% methanol (%v/v)) showed the highest volume expansion, demonstrating that 80% methanol was more soluble in SC-CO_2. The liquid phase shows a color change from clear to green, showing that some of the components from the plant have been dissolved in the solvent mixture. An aqueous solvent mixture consisting of 50% v/v of ethanol and water did not show any obvious volume change.

Less polar cosolvents (such as aqueous acetone (70% v/v) and DCM) produced a second liquid layer. This second liquid layer was formed because the presence of the cosolvent increased the critical point of the solvent mixture. Because the CO_2 did not dissolve in the cosolvent, part of the CO_2 changed to a liquid under the critical point of the mixture, and there was no cosolvent expansion volume. When the pressure increased and the critical point was achieved, liquid CO_2 changed completely to the supercritical phase. Although the largest liquid volume changes were obtained when 70% acetone and DCM were used, the presence of a second layer was undesirable in the extraction process because the second layer would disturb the phase equilibrium (the supercritical fluid component) and decrease the extraction efficiency. Volume expansion demonstrated the changes in the properties of the supercritical fluid mixture. Appropriate cosolvents can be added to the CO_2 to extract more polar components in the essential oil extraction process or to separate nondissolved components in the study of gas antisolvent (GAS). GAS is a process that is based on the super saturation of a liquid solution, by the dissolution of a near critical or supercritical fluid. The dissolved gas creates an antisolvent effect, which results in the precipitation of the solid solute [37].

3.5.2. Cosolvent Effect on Extract Yield. Figure 5 shows the yield of extract that was obtained using different cosolvents with increasing pressure. The cosolvent with the lower polarity produced a higher yield of extract. Gas chromatographic

Table 4: Aldehyde components detection in *P. minus* extract using gas chromatography.

Cosolvent	Yield* (%)	Aldehyde components		
		Decanal (t = 10.5 min)	Undecanal (t = 13.1 min)	Dodecanal (t = 15.7 min)
None	1.8–3.7	/	—	—
Water	2.4–3.6	/	/	—
Aqueous ethanol (50% v/v)	0.1–0.7	/	/	/
Aqueous methanol (80% v/v)	2.9–5.7	/	—	/
Aqueous acetone (70% v/v)	4.7–7.4	/	—	/
Dichloromethane (DCM)	4.3–7.6	—	—	—

*Average yield at different pressure.

(GC) analysis was performed to identify extracted aldehyde components. The earliest eluting component that was detected was decanal followed by undecanal and dodecanal with retention times of 10.5 min, 13.1 min, and 15.7 min, respectively. The result for aldehyde detection for different cosolvents was shown in Table 4. Decanal was identified in all the extracts except for the SFE analysis with DCM being the cosolvent. Undecanal was identified in water and 50% ethanol, while dodecanal was extracted using a cosolvent mixture of water and organic solvent (50% ethanol, 80% methanol, and 70% acetone). The extract yields obtained by SFE were higher compared to the 0.3-0.4% yields reported by Yaacob [38]. However, the high extract yields did not necessarily contain high selectivity of desirable aldehyde component. Heavier or more polar components could have been extracted together with essential oils using SFE, especially if the cosolvent was miscible in CO_2, but the mixture was still in the subcritical state.

The study using 15% v/v cosolvent percentage and results exhibited three different behaviors in the chromatographic analysis: selectivity for the light components, selectivity for the heavy components, and a lack of selectivity (both light and heavy components were extracted). At low pressure, SFE with a cosolvent of 50% ethanol showed selectivity for the lighter components at a pressure less than 100 bars and was not selective at pressures more than 100 bars. Other cosolvents were not selective at any of the different pressures that were used. The cosolvent effect on the yield of lemongrass (*Cymbopogon citratus* Stapf.) extract has been compared with extractions using steam distillation and solvent extraction using 10%, 20%, and 30% *n*-hexane, acetone, and methanol as cosolvents [39]. According to these researchers, the extract obtained using 10% *n*-hexane cosolvent resembles the extract from steam distillation. The extract using 30% *n*-hexane was the same as the extraction with 10% *n*-hexane cosolvent. Solvent extraction using *n*-hexane and 20% acetone extracted the components that were not extracted by either concentration of *n*-hexane cosolvent. The extraction performed using 10% methanol as the cosolvent was not selective. The findings and observations of this study show that the cosolvent can increase the CO_2 polarity or can act as the main solvent if the content is sufficiently high, where component selectivity will be low.

The role of water-alcohol solvent mixture in the extraction of fractions has been observed by previous studies. Seabra et al. [11] have performed the fractionated high pressure extractions from the elderberry pomace. High antioxidant activity anthocyanin-rich extracts were successfully obtained from elderberry pomace using CO_2 and diverse CO_2/EtOH/H_2O mixtures in a fractionated high pressure extraction methodology. The CO_2/EtOH/H_2O solvent composition had a great influence on extract yield and composition, in terms of total phenolic compounds, total flavonoids, anthocyanins, and rutin. The presence of EtOH and H_2O was important to promote the extraction of anthocyanins, even if their presence was not directly related to the extract's antioxidant activity. Park et al. [40] have employed supercritical carbon dioxide (SC-CO_2) coupled with a cosolvent (ethanol and water) on the decaffeination of green tea. The study showed, by varying the extraction conditions, changes not only in the amount of caffeine, but also in the quantities of the principal bioactive components of green tea, including catechins, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC).

A study on selective fractionation of carbohydrate complex mixtures by supercritical extraction with CO_2 and different cosolvents has been performed by Montañés et al. [41]. From the study, a general conclusion has been derived; that is, supercritical conditions (temperature, pressure, and amount of polar cosolvent dissolved in the SC-CO_2 solvent) affect recoveries, but not selectivity, which is mainly influenced by the cosolvent composition. It also reported that, under optimal conditions, satisfactory recoveries and high purity of the ketosugars, tagatose, or lactulose were achieved.

Therefore, SFE technique using water-alcohol solvent mixture has the potential to extract the high purity of aldehydes from *P. minus*. Aldehyde components are intermediate in polarity, and the SFE process needs the presence of a suitable cosolvent to extract these compounds. Although not all low polarity compounds were removed, the first CO_2 extraction step was important to concentrate the phenolic and other polar compounds for the subsequent extraction step. In this study, the cosolvent mixture of 50% ethanol (CO_2-ethanol-water) was the best cosolvent because this cosolvent mixture extracted all three aldehydes (decanal, undecanal, and dodecanal), as compared to the other cosolvents, as shown in Figure 6. Optimization of the type and composition of the cosolvents is crucial to determine the selectivity of the

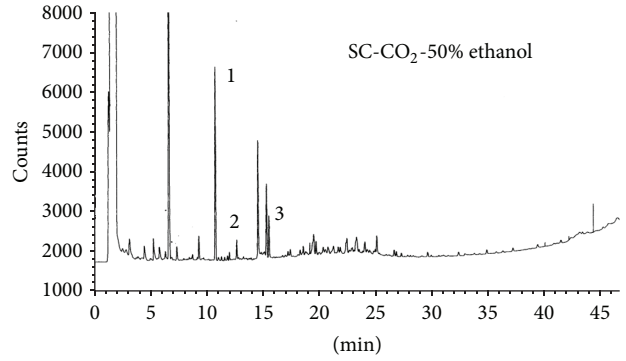

Figure 6: Peak of aldehyde components extracted from cosolvent mixture of 50% ethanol in SC-CO_2 (1: decanal, 2: undecanal, and 3: dodecanal).

system for aldehyde components or other useful components from this plant.

4. Conclusions

The biological activity of plant extracts depends on the type of solvent used, and methanol was shown to be the best solvent to extract the phenolic compounds because the methanol extract showed the best antioxidant and antibacterial properties compared to the other extracts. A positive relationship between the phenolic content and the antioxidant capacity can be seen from this study: the higher the phenolic content of the plant, the higher the FRAP value and DPPH inhibition. This study showed that *P. minus* has potential as a natural source of antioxidants in the food and pharmaceutical industries. The Soxhlet extraction showed that methanol was the best solvent for obtaining a higher extract yield. The best operating parameter from the range of parameters that have been studied for SFE extractions of *P. minus* is a pressure of 200 bars and a temperature of 40°C, resulting in a 15.68% yield. Although this percentage is lower than the yield of the methanol extract resulting from the Soxhlet extraction, if the SFE system uses polar cosolvents, the yield can be improved in the future. From the study of cosolvent selection, a mixture consisting of the 50% ethanol cosolvent system showed the best performance for extracting the desired aldehyde compounds because the ethanol cosolvent system was able to extract all three aldehydes (decanal, dodecanal, and undecanal) compared to the other cosolvents. Therefore, this result could be a guide in order to determine the best cosolvent in future studies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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