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A study of essential oil extraction and antioxidant activity of patchouli (*Pogostemon cablin*) using supercritical carbon dioxide

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Abstract

This study investigates the extraction of patchouli oil using supercritical carbon dioxide (SC-CO_2). The patchouli plants utilized in this study were obtained from Indonesia. The effect of pressure on yield, antioxidant activity and total phenolic content of the extracts was investigated at operating pressures ranging from 100 – 200 bar. The extract yield, antioxidant activity (evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay) and total phenolic content were compared against conventional steam distillation. SC-CO_2 extraction obtained extract yields and total phenols in the range of 3.41 to 3.95% and 1.869 to 2.756 mg GAE/g respectively, which were higher than steam distillation (1.13% and 1.677 mg GAE/g). Furthermore, the SC-CO_2 extracts exhibited IC_{50} (effective concentration to reduce 50% of DPPH free radicals) values in the range of 0.581 mg/ml to 1.538 mg/ml, which were better than the IC_{50} value exhibited by steam distilled patchouli oil (> 4 mg/ml).

Introduction

Pogostemon cablin (patchouli) is an aromatic herb that is extensively cultivated in Brazil, China and Indonesia for its essential oil. The essential oil of patchouli plant is known to have great business potential in the international market due to its unique flavour, fragrance and biological activities [1]. In the perfumes and cosmetics industry, patchouli oil is a vital ingredient in exotic perfumes offering a rich and spicy fragrance. Its fixative properties provides a strong lasting character to perfumes when blended with other essential oils. It is also used as a perfume in its own right [2]. Furthermore, the fixative properties of patchouli oil assists in limiting evaporation and promoting firmness, allowing it to be valuable in the manufacturing of soaps, scents, body lotions and detergents. In aromatherapy, it is used to soothe nerves, control appetite, and relieve depression and stress. Moreover, patchouli oil is on the FDA's (Food and Drug Administration) list of substances approved for human consumption as a natural additive for food flavouring [3]. In food industries, patchouli oil is extensively used as a flavour ingredient in major food products including alcoholic and non-alcoholic beverages [4]. Very low concentration (2 mg/kg) of this oil is used to flavour foods, beverages, candy and baked products [5].

Indonesia is the largest producer of patchouli oil, accounting for over 80 percent of its total annual production [6]. Patchouli oil extraction in Indonesia is generally carried out using conventional methods which limits their production capacity [7]. Conventional steam distillation of dried patchouli leaves have drawbacks such as long extraction time, low yield and adverse effects of high temperature [7,8]. High temperature is detrimental to heat sensitive compounds present in essential oil as it may cause a chemical alteration, resulting in a different flavour and fragrance profile. For instance, fatty aliphatic aldehydes, terpenic hydrocarbons, as well as terpenoid aldehydes, in citrus oils are thermally unstable and are readily oxidized by atmospheric oxygen which gives rise to undesirable formation of malodorous carboxylic acids [9]. In this regard, the application of supercritical fluids is of considerable interest. SC-CO_2 extraction involves mild operating temperature, while being organic solvent free, is especially beneficial in cosmetics, foods, and pharmaceutical products where there are more stringent requirements.

Limited studies were conducted on SC-CO_2 extraction of patchouli oil. For instance, Donelian et al. [10] studied the yield and chemical composition of essential oil obtained by SC-CO_2 extraction from patchouli leaves cultivated in Brazil. Liu et al. [11] studied the yield of concrete and oil obtained by SC-CO_2 extraction from patchouli leaves and stems from China. In this work, the objective is to study SC-CO_2 extraction of essential oil from patchouli plant (Indonesia), to analyse the effect of pressure on extract yield, antioxidant activity and total phenolic content and to compare extraction results with conventional steam distillation.

Materials and methods

Materials

Dried patchouli (*Pogostemon cablin*) plant with a moisture content of 10.60 ± 0.07%, measured using a halogen heating moisture analyser (MOC63u UniBloc, Shimadzu), was obtained from Lam Seng Hang Co. Pte Ltd (Indonesia). The leaves and stems were separated initially followed by grinding in an electric blender. The ground material was then passed through a 0.6 mm stainless steel sieve. Samples were packed and stored at room temperature until utilisation. SC-CO_2 extraction experiments were carried out with a mixture comprising of 1:1 ratio of ground leaves and stems. Liquid CO_2 with a purity of 99.5% was obtained from Air Liquide Singapore Pte Ltd. Ethanol 96% (technical grade) was obtained from Quality Reagent Chemical. Methanol (analytical grade) was obtained from Fischer Scientific Pte Ltd (Singapore). Anhydrous sodium carbonate, sodium sulphate and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma-Aldrich Pte Ltd (Singapore). Folin Ciocalteu's phenol reagent and gallic acid were procured from Merck Pte Ltd (Singapore) and used as obtained.

Supercritical CO_2 extraction

SC-CO_2 extractions were carried out using a customized supercritical fluid extractor with CO_2 recycle system (Model SFE 1000 System, Waters Corporation, USA). Figure 1 shows a schematic diagram of the process. Ground samples were loaded and packed with glass beads. The loaded sample was heated until the desired temperature was reached. Liquid CO_2 was pressurized by a cooled liquid pump to the desired extraction pressure, followed by a preheating stage where it was heated to the extraction temperature. The pressure of the extraction vessel was controlled by an automated backpressure regulator (ABPR). The pressure of the collection vessel was maintained below critical pressure by a manual backpressure regulator (MBPR), for the recycling of CO_2. At this stage, the CO_2 reverts to its gaseous state and the plant extracts precipitate at the bottom of the vessel. The temperature in the extraction and collection vessels were maintained using band heaters. The plant extracts were collected from the collection vessel at the end of each run. After collection, the pipeline of the system was purged with CO_2 to recover residues. Small amounts of extracts that remained within the walls of the collection vessel were rinsed down with ethanol before evaporating using a rotary evaporator (Heidolph, Germany). The extracts were weighed and stored in a refrigerator for further analysis. The extract yield obtained from each experiment were calculated using the following equation:

$$\text{Extract yield (\%)} = \frac{\text{Mass of extract (g)}}{\text{Mass of sample (g)}} \times 100\% \quad (1)$$

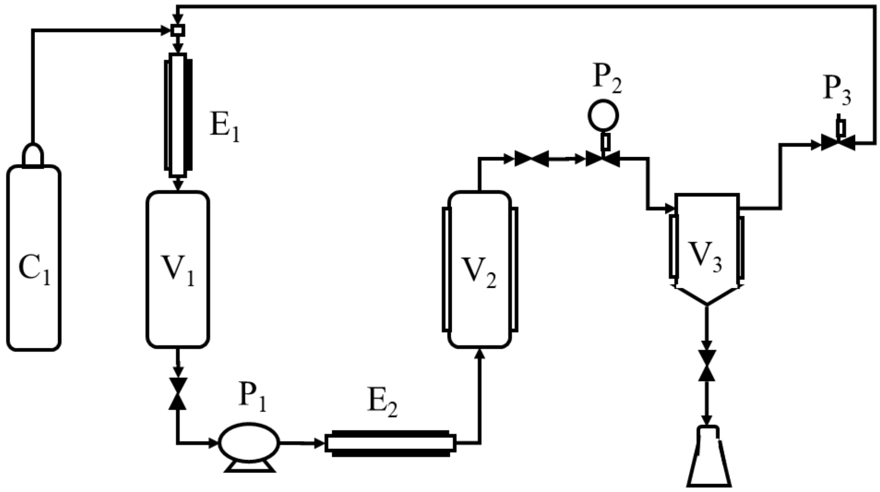

Figure 1: Supercritical fluid extraction setup C_1: Compressed CO_2 cylinder; E_1: Condenser; E_2: Electric preheater; P_1: High pressure liquid pump; P_2: Automated backpressure regulator; P_3: Manual backpressure regulator V_1: CO2 recycler; V_2: Extraction vessel; V_3: Collection vessel

Steam distillation

100 g of dried patchouli leaves were placed in a biomass flask and subjected to steam distillation for 5 h. Patchouli oil was separated from water using a separatory funnel. Residual water content was removed from patchouli oil by drying with anhydrous sodium sulphate. The dehydrated patchouli essential oil was weighed and stored in a refrigerator for further analysis.

Antioxidant activity analysis

Antioxidant activity was analysed by free radical scavenging activity of the patchouli extract towards 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [12,13]. A range of concentration from 0.25 to 4 mg/ml of the plant extracts were prepared with methanol. Plant extract solutions (2 ml each) of the different concentrations were mixed with 3 ml of freshly prepared 0.004% (w/v) DPPH methanol solution and shaken well before incubating in dark and ambient conditions for 30 minutes. The absorbance of the sample was then analysed and recorded at 517 nm using an ultraviolet and visible (UV-Vis) spectrophotometer (UV-1800, Shimadzu). The percentage of radical scavenging activity was calculated using the following equation:

$$I\% = \frac{A_b - A_s}{A_b} \times 100 \quad (2)$$

Where A_b and A_s are the absorbance values of the DPPH blank solution and tested samples respectively. Lower absorbance values of sample indicated higher DPPH radical scavenging activity. IC_{50} value (mg/ml) is the effective concentration of essential oil at which DPPH radicals were scavenged by 50%. This was estimated by interpolation and linear regression.

Total phenolic content analysis

Total phenolic content was analysed using the Folin Ciocalteu method with some modifications [14]. 200 µl of properly diluted extract (10% w/v in methanol) or a standard solution of varying concentrations was mixed with 400 µl of Folin Ciocalteu's phenol reagent. The solution was diluted to a total volume of 4.6 ml and thoroughly mixed. After incubating for 10 min at room temperature, 1 ml of 20% sodium carbonate (Na_2CO_3) solution was added to the solution and then instantly mixed, centrifuged and incubated for 2 h. The absorbance was measured at 760 nm using an UV-Vis spectrophotometer. Gallic acid was used as the standard and the total phenolic content of samples were expressed in milligram gallic acid equivalent per gram of extract (mg GAE/g).

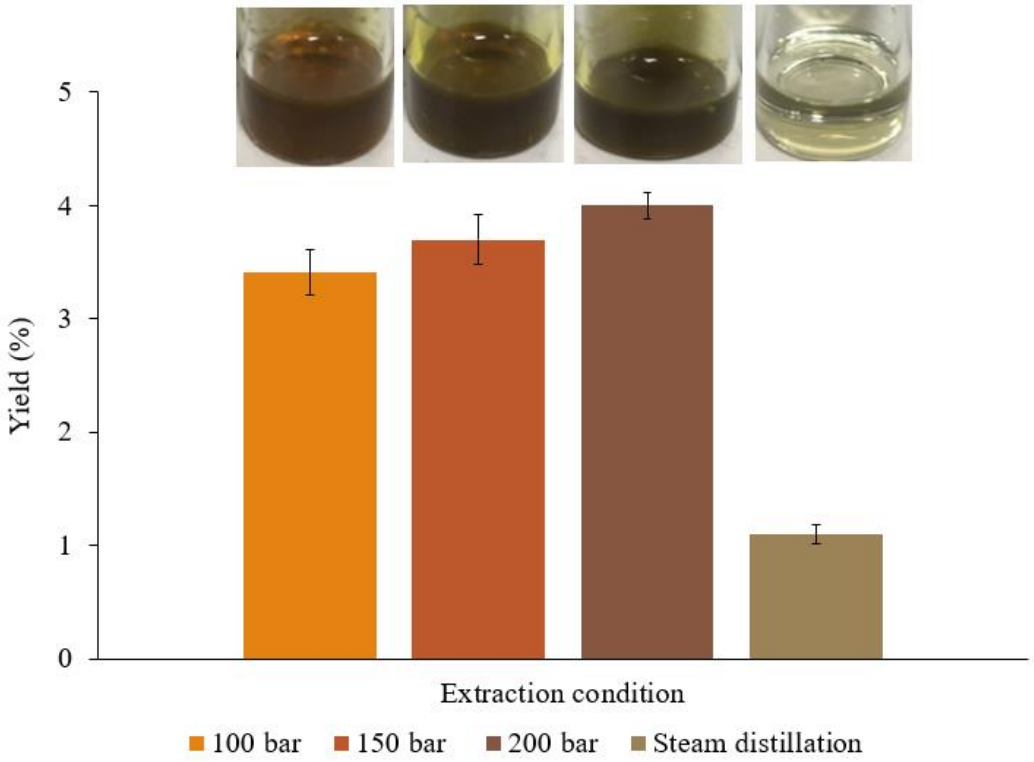

Figure 2: Extract profile and yield under different pressures of SC-CO_2 extraction and by steam distillation

Results and discussion

Extract yield

SC-CO_2 extractions were conducted at pressures ranging from 100 – 200 bar. The extractions were fixed at 40°C. Figure 2 shows the extract yield under different conditions of SC-CO_2 extraction and comparison with extraction by steam distillation. An increase in pressure resulted in higher yield, which was attributed to an increase in SC-CO_2 density and solvation power at higher pressure. Moreover, in this study, the extract yield by SC-CO_2 extraction is clearly higher than that obtained by steam distillation which is consistent with results reported in similar work [10,12,15]. Steam distillation lacks the capability to recover non-volatile compounds. For instance, sclareol, an important component of clary sage, is usually recovered in only very small quantities in steam distillation due to its very high boiling point [9]. On the other hand, the selective nature of SC-CO_2 extraction allows it to extract a wide range of compounds by fine-tuning operating conditions and altering the properties of SC-CO_2. As the density of SC-CO_2 increased, more non-volatiles were extracted. Therefore, the ability to extract non-volatile compounds enables SC-CO_2 extraction to achieve a higher extract yield than steam distillation. However, this also meant that undesirable non-volatile compounds such as cuticular waxes were co-extracted in SC-CO_2 extraction [16]. In addition to extract yield, Figure 2 shows the profiles of the extracted patchouli oils from steam distillation and SC-CO_2 extraction at different pressures. Despite having higher yields, the dark brown colour extracts observed from SC-CO_2 extraction indicated some co-extraction of heavier, non-volatile compounds as compared to the pale yellow volatile oil obtained from steam distillation [17]. Furthermore, the colour of the extract became more intense as SC-CO_2 extraction pressure increased due to the presence of more non-volatile compounds [18].

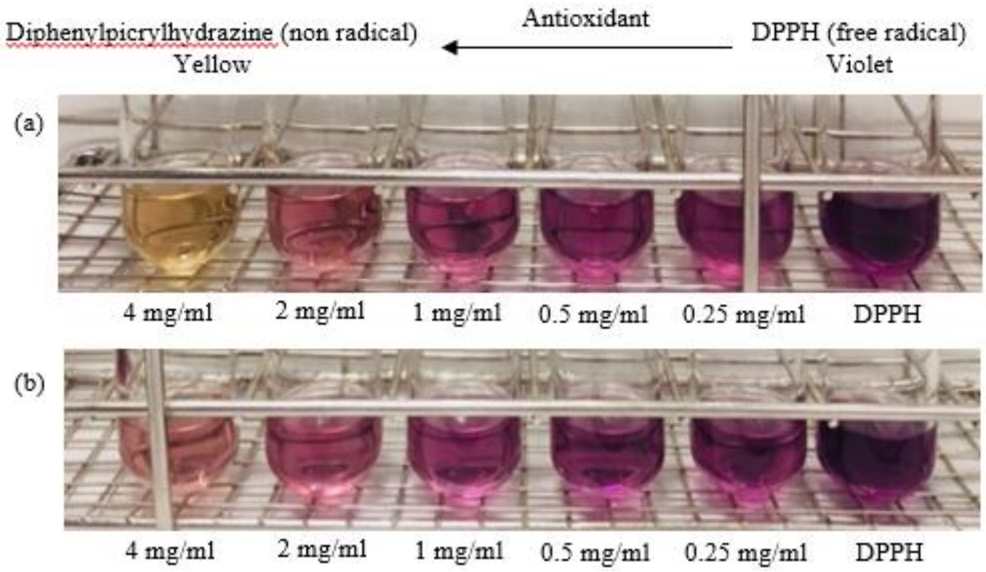

Figure 3: Extract solution at different concentrations (0.25 – 4 mg/ml) mixed with DPPH (SC-CO_2 extraction pressure: (a) 200 bar and (b) 150 bar)

Antioxidant activity and total phenolic content

Antioxidant activity of extracts were evaluated by a DPPH radical-scavenging assay that predicted the ability to quench free radicals. The radical scavenging activity was determined by measuring the degree of absorbance quenching for varying sample concentrations. Figures 3(a) and (b) show the degree of absorbance quenching at different sample concentrations obtained from SC-CO_2 extraction at 200 bar and 150 bar respectively.

Table 1 displays the IC_{50} values and total phenols under different pressures of SC-CO_2 extraction and by steam distillation. Experimental results showed IC_{50} values ranging from 0.581 – 1.538 mg/ml and total phenols ranging from 1.869 – 2.756 mg GAE/g under different pressures of SC-CO_2 extraction. Lower IC_{50} values indicated better antioxidant activity. Table 1 shows that SC-CO_2 extraction resulted in better antioxidant activity and more total phenols than steam distillation (> 4 mg/ml and 1.677 mg GAE/g). This highlights the capability of SC-CO_2 extraction of recovering non-volatile, polar bioactive compounds as opposed to steam distillation, which is only able to extract volatile oils. Therefore, the higher yields attained in SC-CO_2 extraction can be further explained by the co-extraction of bioactive compounds on top of the volatile compounds generally obtained by steam distillation. Steam distilled patchouli oil have lower antioxidant activity due to low content of active components. During steam distillation of patchouli oil, the antioxidant activity is partially lost. Steam distilled oils contain mostly volatile compounds, which have generally low antioxidant activity and can be limitedly applied in food industry as authentic antioxidants [19]. The characteristics of the target antioxidant compounds must be considered in the selection of extraction method. The active components extracted from plants depend on heat, light, oxygen, and a number of other factors. The prolonged exposure of the patchouli oil to heat and light in steam distillation had degraded these active compounds and caused major deterioration to the antioxidant activity [12,19,20]. SC-CO_2 extraction was performed in a closed extraction vessel without the presence of light and oxygen, therefore this minimized the degradation reactions of active compounds. Furthermore, SC-CO_2 extraction was performed at mild temperatures as compared to steam distillation and therefore degradation of heat sensitive compounds was reduced.

Table 1: IC_{50} values and total phenols obtained from extracts under different pressures of SC-CO2 extraction and by steam distillation

Extraction	IC_{50} (mg/ml)	Total phenols (mg GAE/g)
100 bar	1.538 ± 0.175	1.869 ± 0.059
150 bar	0.676 ± 0.035	2.281 ± 0.097
200 bar	0.581 ± 0.036	2.756 ± 0.086
Steam distillation	> 4	1.677 ± 0.068

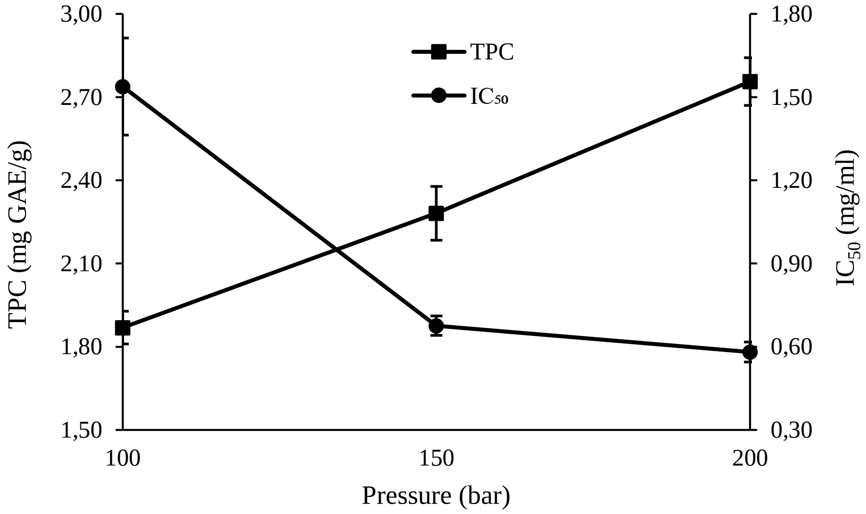

Figure 4: Effect of pressure on antioxidant activity and total phenols obtained by SC-CO_2 extraction (TPC = total phenolic content)

Figure 4 shows the main effect plot of pressure on the antioxidant activity and total phenols obtained by SC-CO_2 extraction. It was observed that the total phenols increased at higher pressures. Similar to extract yield, the observation was due to the improved density of SC-CO_2 at higher pressures, which improved the solvation power of the solvent. As the solvent density increased at higher pressures, the distance between the molecules decreased. Therefore, interaction between solutes and CO_2 increased, permitting higher specific extraction of polar active compounds [12]. In addition, Figure 4 shows that a rise in pressure displayed positive effect on the antioxidant activity of the extract. This is reflected by the enriched extraction of polyphenols, amongst other bioactive compounds that may be present, at higher pressures as these compounds contribute to antioxidant activity [13,21].

Conclusion

SC-CO_2 extraction of essential oil from Indonesian based patchouli plant was studied and compared against conventional steam distillation. The effect of pressure on extract yield, antioxidant activity and total phenolic content was investigated. SC-CO_2 extraction offers a selective extraction which can be targeted towards recovering compounds of interest by changing operating parameters. The extract yield, antioxidant activity and total phenols were all improved at higher pressures. SC-CO_2 extraction displayed higher yields of extract and phenols, with better antioxidant activities as compared to steam distillation. Due to its solvent free and low temperature processing nature, enhanced yield and bioactive compounds with therapeutic benefits, SC-CO_2 extraction can be a better alternative to conventional methods for application. Further studies on the optimization of the process parameters and techno-economic evaluation of the SC-CO_2 extraction process for patchouli oil will be carried out for large-scale production.

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