Comparison of Supercritical fluid extraction using CO_2 on patchouli leaves (*Pogestemon Cablin) Benth* essential oil and Soxhlet

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Abstract

Supercritical fluid extraction (SFE) of essential oil from dried Patchouli leaves (*Pogestemon Cablin*) using CO_2 was reported in this work. Effect of pressure and effect of temperature towards yielding of patchouli essential oil in correlation towards quality of oil has been study. Process parameter was pursuing out under two different temperatures (40 ^{O}C and 80 ^{O}C) as well with three stages of pressure (100 bar, 200 bar and 300 bar). Result demonstrated that the extraction yield was increased as pressure increase from 1.13 % at 100 bar to 1.34 % at 200 Bar ultimately increased to 1.44 % at 300 Bar. For the temperature effect, the yield was decreased as the temperature increases, from 1.13 % at 40 ^{O}C to 0.24 % at 80 ^{O}C. For comparison purposes, patchouli leaves were extracted by Soxhlet extraction using absolute EtOH. Result showed that Sohxlet extraction provided a better yield (12 times better at 17.21 %) than supercritical fluid extraction, nevertheless the quality of the Patchouli essential oil was poor, 1 times lower with only 34.912 % patchoulol composition than supercritical fluid extraction .

Keywords: *Patchouli Essential oil; Supercritical Fluid Extraction; Soxhlet Extraction; Patchoulol*

1. Introduction

Patchouli is one of the commercial plants that well known in manufacturing of essential oil. This potential essential oil included Patchouli oil that has been dominant the world market by 800 ton essential oil product per year [1]. Patchouli plant has been cultivated around Asian country such as India, Indonesia, Malaysia and Philippines since Asian region is commonly well-known as the land of aromatic plant' due to its geology that provide favorable and suitable environment such as climatic condition in growth and development of plant [2]. This well development of Patchouli cultivation being done to address high demand of Patchouli oil fulfill basic needs of everyday life

Chemical composition of Patchouli essential oil can be described as a complex compound of $C_{15}H_2O_6$ since it was made up with over 24 different hydrocarbon susquiterpenes [3]. The major constituent of Patchouli essential oil is susquiterpenes Patchoulol which act as the primary component that regulate aroma of patchouli oil [4]. The strong and long-lasting aroma present in Patchouli oil serve an advantage to be used in fragrant industry. Other main constituent such as α-Guaiene, α-Patchoulene and β-Patchoulen also has tendency in regulating aroma of patchouli oil [5]. Patchouli oil has been scientifically A part of that, Patchouli alcohol that is the major constituent of Patchouli oil is having capability as anti-inflammatory. Patchoulol compound reacts by stimulate responds of inflammatory system to excreting inflammatory agent such as serotonin, prostaglandins, leukotrienes and nitric oxide direct from damage cells or near infected site [6]. Moreover, pogostone that is recognized as one of the chemical constituents presents in Patchouli oil possess as anti-aging activity. The pogostone compounds has an ability to prevent cell damages from continuous expose towards solar ultraviolet, making this constituent is favorable in cosmetics industry – often used as UV blockage lotion [7].

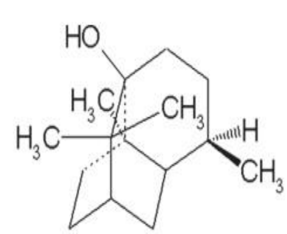

Figure 1: major constituent of susquiterpenes Patchoulol in Patchouli essential oil.

Nowadays, Soxhlet extraction is one of the extraction methods present in extracting Patchouli essential oil with low operating cost and simplicity operation [8]. However, this technique always use high temperature (at boiling point of solvent) which can cause degradation of thermally labile compound present in patchouli essential oil. Furthermore, Soxhlet extraction results to poor oil quality due to loss of some valuable compounds from the essential oil. Supercritical fluid extraction using supercritical carbon dioxide (scCO_2) is a modern technology for the extraction of essential oil at mild operating condition as CO_2 exhibit unique characteristics of having low critical temperature and pressure. Supercritical fluid extraction has been regarded as environmentally friendly extraction method as the scCO_2 is non-toxic, non-explosive and cheap solvent, compared to Soxhlet method which use organic solvent such as ethanol as solvent of extraction. Extraction using scCO_2 enhance high selective extracts without denaturation of valuable component at high temperature compare to soxhlet Extraction operating at high temperature above boiling point of solvent used [9, 10]. Moreover, using scCO_2 as extraction solvent in supercritical fluid extraction indicate to provide high quality of essential oil extracted since solvent-free promoted in this extraction method. In this study, variation in the yield and quality of patchouli essential oil obtained using supercritical fluid extraction under different pressure (100, 200 and 300 bar) and temperature (40 ^{O}C and 80 ^{O}C) and soxhlet extraction will be determined.

2. Material and methods

2.1 *Plants material*

Patchouli plants, *Pogestemon P. Cablin* were purchased from local herbs market in Indonesia. Patchouli leaves collected first are separated from its branches and cores. Patchouli then being pulverized and sieve into 300 mm-400 mm of size. Final product of plant sample were the kept in dark place and stored in the room temperature to avoid UV-radiation from denaturing component in Patchouli plant.

2.3 Sohxlet Extraction

Approximately 10 g sample placed in a thimble and transferred to a Soxhlet extractor. The extractor was filled with 250 ml of pure ethanol and heated under reflux for 6.5 hours through a water bath. The extraction was performed at water bath under temperature of 78.73 ^{O}C (b.p of ethanol). Extraction performed in triplicate and the total extraction yield was obtained by the mean value of the extracted oil mass divided by the mass of the raw material used, on dry weight base.

$$Yield\% = \frac{\text{Weight of dry oil}}{\text{Weight of dry sample}} \text{ x } 100$$

The solution then was dry through rotary evaporator with unit operation of RE-52 and being adjusted to 2 ml of solution in order to ensure an extract are complete dry from recovered solution. The extract then stored at -4 ^{O}C under the dark condition in order to avoid UV-ray denature the composition present in the extract essential oil.

2.4 Supercritical Fluid Extraction

The supercritical fluid extraction (SFE) experiments were carried out using an SFT 100. 10 g of Patchouli leaves were being used and being loaded into 50 ml SC-CO_2 extraction vessel. Glass wool has been used at both ends of the extractor to stop entrainment of the substrate being weight first. The extraction pressure will manually being controlled with an air-booster pump at pressure of 100, 200 and 300 bar meanwhile the temperature of the air bath (convection oven) containing the extraction vessel set constant at two different temperature of 40 ^{O}C and 80 ^{O}C. Supercritical fluid extraction started as soon as the desired pressure and temperature had been reached. Flow rate of the expanded gas CO2 (under atmosphere pressure and room temperature of 20 ^{O}C) was set at 20 L/min in all runs. Dynamic extraction time was set up to 20 min where for each 5 min sample obtained were weighted for yield calculation. Extraction yield was estimated as follows:

$$Yield\% = \frac{\text{Weight of oil extracted}}{\text{Weight of dry sample}} \text{ x } 100$$

2.6 Gas Chromat0hgraphy - Mass Spectrometer (GC-MS)

Stock essential oil solution obtain from the previous supercritical fluid extraction was being solubilized with ethanol first and filter through 0.45 μm Nano filter before performing a GC-MS analysis. Unit operation Agilent 6890 was used to perform GC-MS analysis. Firstly, compound of essential oil was separated through 30 m x 0.25 mm diameter of capillary column coated with 0.25μm film. Helium gas was used as a gas carrier which has been set at flow rate of 1ml/min. Both injector and detector temperature of GC-MS was set at 250 ^{O}C. The oven temperature was set at a range of 60-300 ^{O}C with a flow rate at 6 ^{0}C/min and the final temperature are being held constant in period of 10 min via the transfer line of 250 ^{O}C. The electron impact mass spectrum of GC-MS also was set constant at the acceleration of 70eV. Manual injection in GC-MS analysis was being performed via the split mode respects to split ratio of 20:1. Then, as the results of GC-MS analysis, sesquiterpenes was identified by comparing

Table 3.1: $ScCO_2$ density and apparent oil solubility

Comparative	$scCO_2$ Density (kg/m^3)	Oil solubility (g dry extract/ kg CO_2)	Total Patchoulol composition (%)	Average yield (%)
40 ^OC/ 100bar	628.6	0.30	20.71%	1.13 ± 0.35
40 ^OC/ 200bar	839.8	0.39	32.39%	1.34 ± 0.24
40 ^OC/ 300bar	909.0	0.71	37.56%	1.44 ± 0.25
80 ^OC /400bar	221.6	0.22	36.51%	0.24 ± 0.02
SE	-	-	34.91%	17.21 ± 0.24

relative retention times and the mass spectra to the standards [1].

3. Results and Discussion

Effect of pressure and temperature on extraction of Patchouli essential oil is the main objective of the study. Effect of different pressure and different temperature are summarized in table 3.1: pressure at 100 bar, 200 bar and 300 bar and temperature of 40 ^OC and 80 ^OC respects to resulted yield %. Each of the sample extraction was pursue last up till 80 min and each sampling was taken every 5 min of extraction time. The results were presented as yield % (weight dry oil, g/ weight feed material, g) against pressure (100 bar, 200 bar and 300 bar) at Figure 3.0 and temperature (40 ^OC and 80 ^OC) at Figure 3.1 to demonstrate results of findings.

3.1.1 Effect of pressure

From Figure 3.1 it can be observed at constant temperature of 40 ^OC and different pressure of 100, 200 and 300 bar, the curves showed a similar trend, which exhibit two main stages. The first main stage is a linear stage where extractions yield increase rapidly until a certain extraction period. At this stage constant extraction rate occur in linear form due to the oil are accessible through the cell rupture that cause by grinding the sample are extracted easily by scCO2 used as solvent in supercritical fluid extraction (e.g. at min 25 for extraction that occur under the pressure of 300 bar and temperature of 40 ^OC). Otherwise, the extraction rate will be limited by the diffusion of oil to the surface of particle where the oil cell will not ruptured to the extent [11]. From the figure, linear stage at pressure of 300 bar indicates to be the shortest up only to 25 min. This is due to higher pressure enhance a great oil accessibility through the rupture cell as well promote a low extraction time compare to extraction process occur under low pressure at 200 (30 min) and 100 bar (55 min), respectively. Then, extraction rate are entering a constant phase section. This stage is called a transition period where extraction rate are predicted to decrease rapidly, continues at slow extraction rate which resulting in constant graph behavior since accessibility of oil through rupture cell reach a maximum rate.

Pressure influenced a significant effect in yield of Patchouli essential oil. As shown in Table 3.1, average percentage Patchouli essential oil recovered at 300 Bar indicate a highest percentage at 1.44% followed by percentage recovered at 200 and 100 Bar which is 1.34% and 1.13%. This is due to increasing in pressure led to a higher fluid density of scCO2 inducing a greater solubilization through a cell wall [4]. Therefore, an increasing in operation pressure resulted in increasing of yield patchouli essential oil as well with promoting a short extraction time. On the other hand, effect of pressure towards quality of Patchouli essential oil are measure based on the percentage composition of Patchoulol present as main sesquiterpenes, Based on previous study, high pressure is not recommended for supercritical fluid extraction usage due to the extraction of undesirable component such as cuticular waxes that will reducing the quality of essential oil [4]. In order to evaluate quality of Patchouli essential oil obtained influenced under a different pressure of 100, 200 and 300 bar, Gas Chromatography - Mass Spectrometer (GC-MS) analysis were performed to determined qualitative and quantitative composition present in patchouli essential oil. As shown in table 4, percentage composition of Patchoulol obtained at 300 bar observed to be the highest at 37.56% compared to percentage composition of Patchoulol at 200 bar and 100 bar respectively at 20.71% and 32.39% as well with other valuable component in Patchouli essential oil. Therefore, pressures are accounted towards quality of Patchouli essential oil. Increasing in pressure from 100 to 300 bar was not associated with decreasing in quality of Patchouli essential oil. Otherwise, enhance towards increases of Patchoulol sesquiterpenes concentration present in Patchouli essential oil

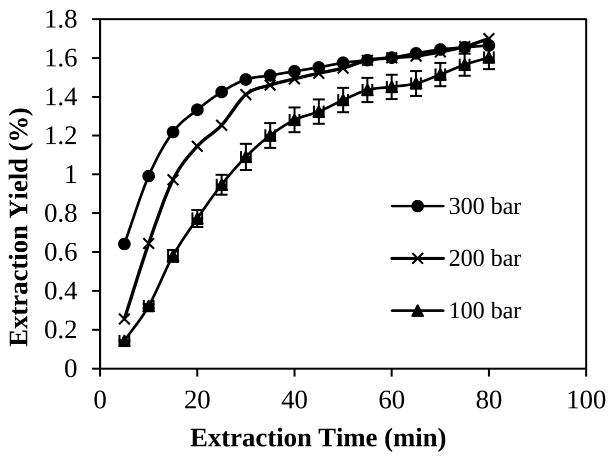

Figure 3.1: Yield% versus extraction time influenced under different pressure of 100, 200 and 300 bar at constant temperature of 40 ^{O}C

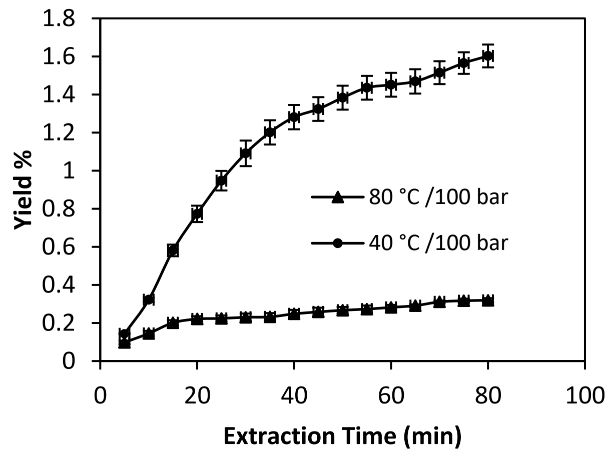

Figure 3.2: Yield% versus extraction time influenced under different temperature of 40 ^{O}C and 80 ^{O}C at constant pressure of 100 bar

3.1.2 Effect of temperature

Figure 3.2 shows that elevated temperature of 40 ^{O}C and 80 ^{O}C the curve indicate different trends. As temperature increase to 80 ^{O}C under a constant pressure of 100 bar, yielding percentage increase slowly until 80 min of extraction time compare to extraction rate at 40 ^{O}C which increasing rapidly in linear phase before entering a transition phase. This is due to increasing in temperature lead to decreasing in fluid density of scCO2 and a former promoting a poor fluid solubilization through cell wall enhance a low yield of Patchouli essential oil. Therefore, under a constant pressure as operating temperature increase from 40 ^{O}C to 80 ^{O}C, average yield of Patchouli essential oil are decrease from 1.13% to 0.24%.

Moreover, temperature promoted a great influenced towards selectivity of valuable component present in patchouli essential oil. As shown in Table 3.2 percentage of Patchoulol concentration increase as temperature increase causes an opposite. From Table 3.2, Patchouli concentration present at temperature of 40 ^{O}C identified to be lower than Patchoulol concentration present at 80 ^{O}C. This variation of peak areas occur might be causes from decreasing of density and solubility of solvent latter causes and increases in vapor pressure [12]. Therefore, in this study, increasing of main of patchoulol attribute from increasing in vapor pressure. Moreover, from increasing of temperature could also lead to faster molecular desorption and diffusion as well with efficiency of extraction process [13].

3.3 Supercritical fluid extraction and Soxhlet extraction

A comparison study has been made between

Table 3.2: Compound identified in Patchouli essential oil

Compound	**Comparative chemical composition**				
	40 ^{O}C/ 100 bar	**40 ^{O}C/ 200 bar**	**40 ^{O}C/ 300 bar**	**80 ^{O}C/ 100 bar**	**SE**
β-Patchoulene	1.29	1.47	1.12	1.44	0.87
Caryophylene	2.01	2.16	1.98	2.14	0.95
α-guaiene	23.36	17.66	29.00	20.75	2.05
β-Guaiene	12.11	12.26	11.77	11.69	6.51
α-Patchoulene	4.51	4.64	4.57	4.51	2.99
Caryophylene Oxide	2.71	2.97	3.93	3.00	2.86
linalool	1.51	1.34	1.71	1.84	1.29
Heptatriacontan-1-ol	1.53	0.89	1.37	1.71	1.95
Patchouli alcohol	**20.71**	**32.39**	**37.56**	**36.51**	**34.91**
β-Cholestanol	0.57	0.80	-	0.88	2.17
δ-Elemene	-	-	-	-	2.38

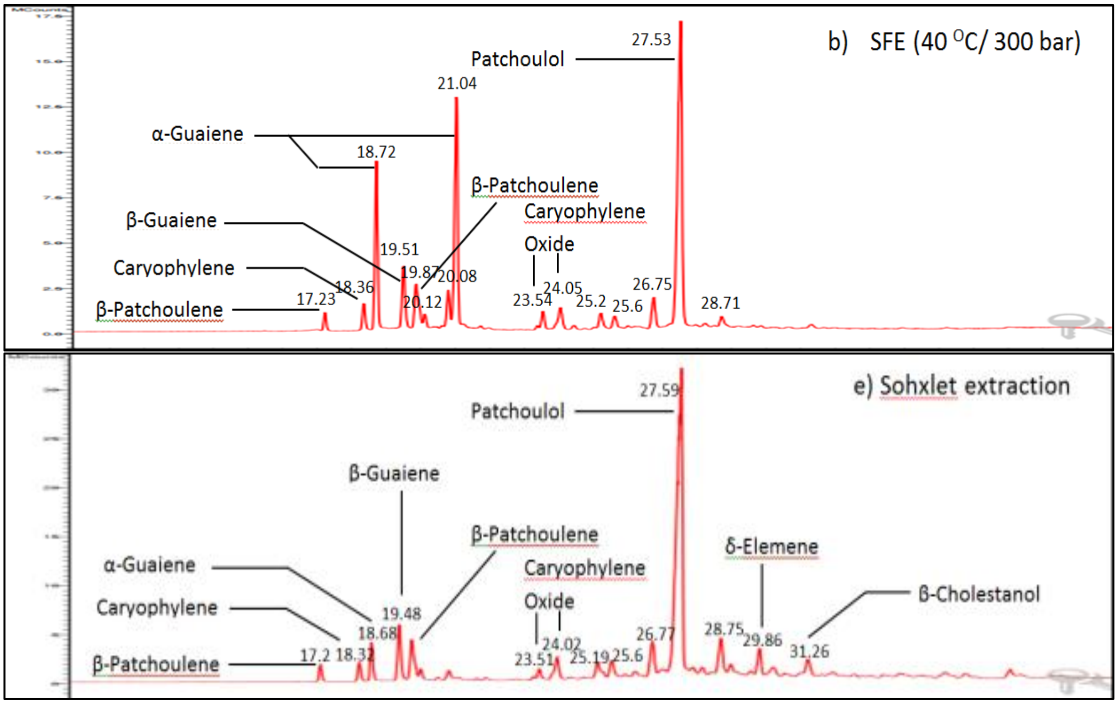

Figure 4.2: Patchoulol composition in GC chromatograms of Pogestemon Cablin extracts at (a) 40 ^{O}C/ 300 bar (b) Soxhlet Extraction

Supercritical fluid extraction and Soxhlet extraction in extracting patchouli essential oil since both exhibit a similarities in working principle, enable extraction solvent to be used above of its characteristics boiling point meanwhile at same time still remain a solvent in a liquid phase providing a better kinetics of extraction in the process [14]. To compare this supercritical fluid extraction and Soxhlet extraction technique, yield of patchouli essential oil and quality of patchouli essential oil obtained has been accounted as measurement factor. Figure 3.3 summarized a yield percentage different between supercritical fluid extraction and Soxhlet extraction. from the figure, Soxhlet extraction indicates producing higher yield of Patchouli essential oil at 17.21% in relation to the oil obtained by supercritical fluid extraction which producing a low yield of Patchouli essential at 1.44 % accounted from the highest oil obtain from supercritical fluid extraction under the pressure of 300 bar and temperature of 40 ^{O}C.

Nevertheless, through Table 3.1, it was also verified that soxhlet extraction method produces a lower quality of patchouli essential oil, this is due to patchoulol as majorsesquiterpenes compound present are indicated to be much low at 34.91% compare to supercritical fluid extraction which producing a better quality of Patchouli essential oil at a patchoulol percentage of 37.56%. Total no of main component present in patchouli essential oil using both extraction techniques indicate has no signification.

However, other main susquiterpenes such as α-Guaiene, β-Guaiene and α-Patchoulene are extracting better using supercritical fluid extraction technique the same as found by Donellialan [4]. As can be seen on Figure 4.2, peak areas of other major component in Pogestemon Cablin extract achieved by using supercritical fluid extraction at pressure of 300 bar and temperature of 40 ^{O}C are larger than using soxhlet extraction. This is due to localized heating apply in Sohxlet extraction lead to denaturation and degradation of valuable component present in Patchouli essential oil [15].

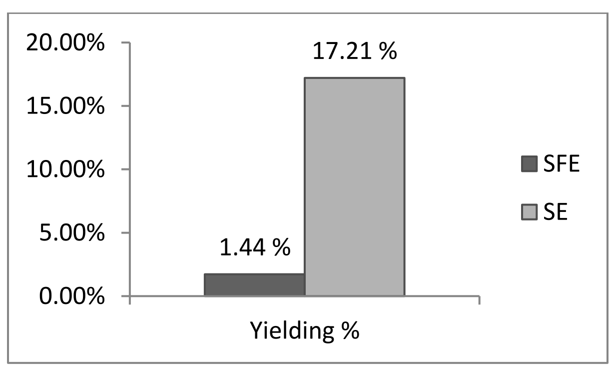

Figure 3.2: Comparison on average yield % obtain by Supercritical fluid extraction and Soxhlet extraction

4. Conclusion

Extraction of pachouli essential oil using Soxhlet extraction provide the best yield (17.21 %) compare to supercritical fluid extraction at 40 ^{O}C/ 100bar (1.13 %), 40 ^{O}C/ 200 bar (1.34 %), 40 ^{O}C/ 300 bar (1.44 %) and 80 ^{O}C/ 100 bar (0.24 %). In relation towards quality of patchouli essential oil obtain, patchoulol percentage in patchouli essential oil composition indicated the best by using Supercritical Fluid Extraction using $scCO_2$ at 37.56 % under pressure of 300 bar and temperature of 40 ^{O}C. However, patchoulol composition obtain by using Soxhlet extraction much lower than extracted using $scCO_2$ at 80 ^{O}C/ 100 bar (36.51 %) and 400 ^{O}C/ 300 bar (37.56 %) but higher then supercritical fluid extraction perform at 40 ^{O}C/ 100 bar (20.71%) and 40 ^{O}C/ 200 bar (32.39 %).

Therefore, in terms of yield percentage of patchouli essential oil obtained, Soxhlet extraction shows a higher yield than using supercritical fluid extraction. Meanwhile, in measuring quality of Patchouli essential, supercritical fluid extraction using $scCO_2$ shows a higher percentage of patchoulol recover. The best condition in operating Supercritical fluid extraction in this study was under high pressure at 300 bar and temperature at 40 ^{O}C.

5. References

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