International Journal of Engineering & Technology, 7 (4.18) (2018) 65-70

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Supercritical Carbon Dioxide Extraction of *Leucaena leucocephala* Pod– Oil Yield & Component Identification

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Abstract

Relatively previous researches on *Leucaena Leucocephala* (*L.leucocephala*) were undergone by using traditional method such as hydro-distillation and soxhlet extraction methods which have their own disadvantages such as long extraction time and non-solvent free oil extract. Thus, a clean extraction method of supercritical fluid extraction (SFE) method using supercritical carbon dioxide (SC-CO_2) with ethanol as co-solvent was proposed for the extraction of *L.leucocephala* pods to produce oil with pharmaceutical components. The SC-CO_2 extraction of *L.leucocephala* was studied by using measurement of yield of oil extracted and component identification. The extraction was conducted at constant CO_2 flowrate of 24mL/min and constant ethanol flowrate of 2.4mL/min at extraction time of 60 minutes within a range of temperature (40°C, 50°C and 60°C) and range of pressure (3000, 4000, 5000, 6000, 7000 psi). The component in *L.leucocephala* oil with the highest oil yield was determined using Gas Chromotography-Mass Spectrometry (GC-MS). The highest extraction oil yield of 4.92% (g oil/ g sample) was obtained at pressure 6000 psi and temperature 60°C. The component in the oil extract with the highest oil yield was determined based on the major components identified comprised of pentanoic acid, phosphonic acid, ecgonine, oleic acid and octadecadienoic acid.

Keywords: *Component; Leucaena Leucocephala pods; oil yield; supercritical carbon dioxide (SC-CO_2); Supercritical Fluid Extraction (SFE)*

1. Introduction

This Petai Belalang or its scientific name *Leucaena Leucocephala* (*L.leucocephala*) is a fast growing trees and belongs to a family of *Fabaceae*. The tree is naturalized at Southern Mexico and North Central America and has been spreaded in tropical and sub-tropical locations [1]. The parts of the plants including roots, leaves, stem bark and seeds are highly used to the human beings and animals [2]. *L. leucocephala* has been well known with high of nutritional content and similar to the one of alfalfa compounds[3]. *L. leucocephala* produce a gum which being used in ice-cream production, cosmetic and pharmaceutical industry. Furthermore, the seeds of *L. leucocephala* contains 6-7 % of oil and highly content of unsaturated linoleic acid and oleic fatty acid [4]. The different parts of *L. leucocephala* plant are used for medicine purpose because of the rich source of proteins containing 3% tannin, carotene, leucenal and 3-4% flavanol glycosider [5]. In addition, the bioactivity studies have revealed its anthelmintic, antibacterial and antidiabetic activities [6].

According to [7] *L. leucocephala* leaf extract demonstrated that the presence of components that contribute in pharmacological activity comprises of antioxidant, anticancer, cancer-preventive, diuretic, antiandrogenic, antimicrobial, antitumor, pesticide, nematicide, intecticide, antiarthic, and antiacne [7]. The chemical components in *L. leucocephala* such as Oleic acid, pentanoic acid, octadecanienoic acid was identified by GC-MS contribute to the pharmacological activities [7].

soxhlet extraction has been widely used and well known as solvent extraction [8]. Previous study reported some drawbacks of solvent extraction mainly it consumes large amount of hazardous and low boiling point organic solvent such as methanol, ethanol, hexane, pentane, dichloromethane, acetone and chloroform which dangerous to environment and human beings [8], [9]. Hydrodistillation method is another conventional method to extract desired substance from plant materials. This technique is different compared to the soxhlet extraction method as it boils water and use the steam to extract essential oil from the plant [10]. The mixture of vapour and oil condenses and will be separated once the distillate enter in the separator. The use of steam from boiling water, it is safer than other organic solvent which is hazardous. A part from that, there are few of drawbacks proven by previous study. The extraction method is expensive compared to soxhlet and SFE method during the production of essential oil in large scale [11], [12]. Other than that, the use of high temperature might damaging components of extracted essential oil and loss of highly volatile components [13]. Further recommendations have been considered due to the use of high temperature and pressure, thus hydrodistillation method is not suitable for extraction.

Therefore, conventional method such as SFE can replace the traditional extraction method because it is clean and safe technology to conserve the high value of components in extract essential oil [14]. Moreover, supercritical fluid is suggested due to its high oil yield and high quality compared to hydro distillation and soxhlet extraction is also known as a clean technology and the value compound can be extracted naturally pure without any organic solvent pre-

sent [15]. Furthermore, SFE is an alternatives way in reducing the consumption of organic solvent while increasing the oil yield [16]. The objectives of this research is to explore the possibility of SC-CO_2 method for extraction of oil from *L. leucocephala* pod and to determine the best operating pressure and temperature for highest oil yield extract. Hence, the identification of bioactive component in the highest oil extract was done by using Gas Chromatography Mass Spectrometer (GCMS).

2. Materials and Methods

2.1. Materials

The pods of *L leucocephala* used on this experiment was obtained nearby in Permatang Pauh, Pulau Pinang. The pods were cleared neatly with distilled water to remove any dirt and impurities on the surface of the pods. 100 g of pods was cut into slice with the length is about 1 to 2 cm follow by drying in oven for 7 hours to reduce moisture content to 10 % of its weight. The dried sample pods were then grinded by using Grinder Medical Industry 2 HP Buyamag Inc. made to obtain powdered *L. leucocephala* pods.

2.2. Moisture Content Determination

The moisture content determination for ginger rhizome was specified based on Palm Oil Research Institute of Malaysia (PORIM) test method. Dry glass dish was weighted until it achieved constant weight [17]. 10 g of sliced pod was weighted in dry glass dish and undergo oven drying at 40°C for 7 hours. Moisture content expressed in percentage of the equivalent ratio of water content with the total mass of the sample was calculated using equation (1).

$$Moisture\ content, \% = \frac{m_1 - m_2}{m_1 - m_0} \quad (1)$$

Where; m_0= mass of the dish (g)
m_1= mass of the dish with sample before drying (g)
m_2= mass of the dish with sample after drying (g)

2.3. Percentage of Solid Oil Yield Determination

To determine the extraction yield, weight of the collection vials will be measured before and after SFE process. The extraction product was weighed using the analytical balance model Mettler Toledo AB204-S to ginger solid oil yield determination. The extraction yield is defined as the mass of the extracted solid oil particle divide by the mass of the ground sample loaded in the extraction column. The solid oil yield was determined using (2).

$$Yield\ (\%) = \frac{mass\ of\ solid\ oil\ particle\ (g)}{Initial\ ginger\ sample\ (g)} \quad (2)$$

2.4. Particle Size Determination

Preliminary study was done to determine the best size of particles to extract oil in order to obtain the highest oil yield. The dried L. *leucocephala* pods were grinded using mechanical Herb Grinder Medical Industry 2 HP from Buyamag Inc. The grinded pods were sieved by shaker Model Retsch AS 200 to three different size which are 125 µm, 250 µm and 500 µm. Every each of pods sample based on the size was weighed at 10 g to be extracted by SC-CO_2. The best particle size of L. *leucocephala* pods was determined based on the percentage of oil yield. The percentage of oil yield can be obtained by calculating using (1). The process of SC-CO_2 will be repeated in triplicate for every each of particle size sample pods. The data presented will be given based on the average and best particle size will be chosen at highest percentage of oil yielded.

2.5. Extraction Time Selection

Preliminary study was done to determine the effect of extraction time to the percentage of oil yield. Extraction time is important parameter that need to be optimized to ensure complete extraction was occurred [14]. The range of time were experimented at 10 minutes to 80 minutes. The oil yield for every extraction will be obtained by using (2) and the process of SC-CO_2 was repeated in triplicate for each of the time which were 10, 20, 30, 40, 50, 60, 70 and 80 minutes. The date presented will be given based on the average values.

2.6. Supercritical Carbon Dioxide Extraction

SC-CO_2 extraction of L. *leucocephala* pods was carried out by using Thar SFC Process (USA) at temperature of 40°C, 50°C, and 60°C and the pressure was at 3000 psi, 4000 psi, 5000 psi, 6000 psi and 7000 psi. Fig. 1 shows the model of SFE Model Thar SFC, which prepared in Faculty of Chemical Engineering Laboratory, UiTM Pulau Pinang. The mass of 10 g of each sample will be loaded. The SFC equipment was designed to operate at maximum temperature of 90 °C and maximum pressure of 7800 psi .The parameters such as pressure, temperature, time of extraction, percentage of co-solvent used and flowrate of SC-CO_2 identified to determine the efficiency of yield extracted.

Fig. 1: SFE model Thar SFC

The temperature and pressure for the extraction will be set up and 10 g of each L. *leucocephala* sample will be placed into cotton bag. The sample in the cotton bag will be inserted into extraction vessel. Make sure the cotton bag will not touch with the top of the extraction vessel before sealing the top tight. On or off valve (MV1), CO_2 cylinder valve and co-solvent release valve (MV4) will be fully opened, and the other valve such as CO_2 bleed valve (MV2), drain valve (MV3) will be fully closed along the process of extraction. When the temperature achieved to the optimum value, the CO_2 pump will run continuously into the vessel of extraction at constant flow rate of 24 ml/min. All the pump will be stopped and valve MV1 will be closed when extraction time was reached. The extracted oil will be collected by using a beaker by opening valve MV3 slowly until the pressure drop to 0 psi. Valve MV1 and MV2 will be opened to release CO_2. Valve MV2 will be closed and the connection of MV1 valve to the extraction vessel top will be disconnected and the sample will be taken out. The procedure of the extraction process will be repeated in triplicate

and data presented will be given based on the average value at desired temperature and pressure.

2.7. Component Identification Using GCMS

The oil sample was diluted with a solvent at ratio of 1:100 in order to analyze the components present in the oil extracted. The 0.03 g of oil sample was diluted with 3 ml methanol and was placed in 2 ml glass vial provided with septa. The *L. leucocephala* oil extracted from SC-CO_2 will be analyzed by using Perkin Elmer Clarus 680 Gas Chromatography with Mass Spectrometry model Perkin Elmer Clarus 600T. The GC-MS equipped with a non-polar Perkin Elmer Elite 5 ms column with thickness of 1 µm, length of 30 m and 0.25 mm diameter. The temperature of the column was maintain and set at 50 °C for 2 minutes. The temperature will be increased to 150 °C at the rate of 10 °C min^{-1} and will be hold for 20 minutes. The helium which was the gas carrier at rate of 1.1 mL min^{-1} with split ratio of 1:20 and 1 µm injection volume of sample [18]. National Institute of Standard Technology (NIST) database will be used to identify the components exists in the extracted oil [19].

3. Result and Discussion

3.1. Effect of Particle Size and Extraction Time Selection

The pressure and temperature of SC-CO_2 extraction of *L. leucocephala* oil were constant at 6000 psi and 50°C. The flowrate of CO_2 is set to be 24 mL/min. Three sizes of sample were prepared which are 500µm, 250µm and 150µm of *L. leococephala* pods used in the preliminary analysis. Fig. 2 shows effect of different particle size and extraction time for 3 differents particle size at constant temperature of 50°C and pressure of 6000 psi. The most suitable particle size was 125µm which has given the highest oil yield which is 3.89%. Based on the previous study done by [20] if the particle size is reduced, more oil will be extracted as more oil cells were broken apart [20]. From Fig. 2, it show that after 60 minutes, the extraction yield is constant and the graph show steady line. So in this study, particle size of 125µm and maximum 60 minutes of extraction time was chosen.

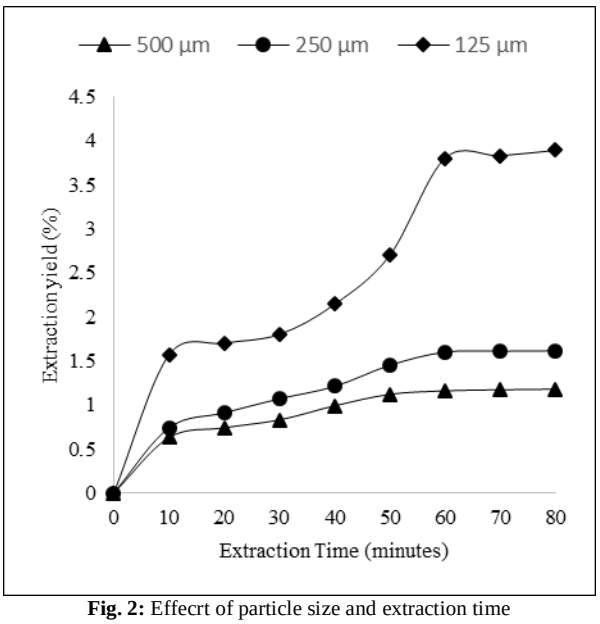

Fig. 2: Effecrt of particle size and extraction time

3.2 Effect of Pressure on Extraction Yield

In this study, the effect of different extraction pressure of 3000, 4000, 5000, 6000 and 7000 psi and different temperature of 40, 50 and 60°C on oil yield were investigated. The oil extracted was collected and recorded at extraction time of 10, 20, 30, 40, 50 and 60 minutes. As mentioned by [21] , the time for extraction of SFE would take less than 60 minutes compared to traditional method such as soxhlet extraction which took 6 to 48 hours to complete the extraction process [21].

Fig. 3, Fig. 4 and Fig. 5 shows the effect of different extraction pressure at constant extraction temperature of 40°C, 50°C and 60°C, respectively. In overall oil yield extracted increased as time increased. The highest oil yield, 4.92 % was obtained at pressure of 6000psi at constant temperature of 60°C. The lowest oil yield. 0.1% was obtained at pressure of 4000psi at extraction temperature of 40°C. In this work, effect of pressure shows significant impact on the extraction yield. Extraction yield increased significantly with increasing of pressure at constant extraction temperature. By an increase of pressure at the constant temperature might increase the dissolving power of SC-CO_2. Increasing the extraction pressure, higher solute solubility produced, hence resulted in higher amounts of extraction yield [15].

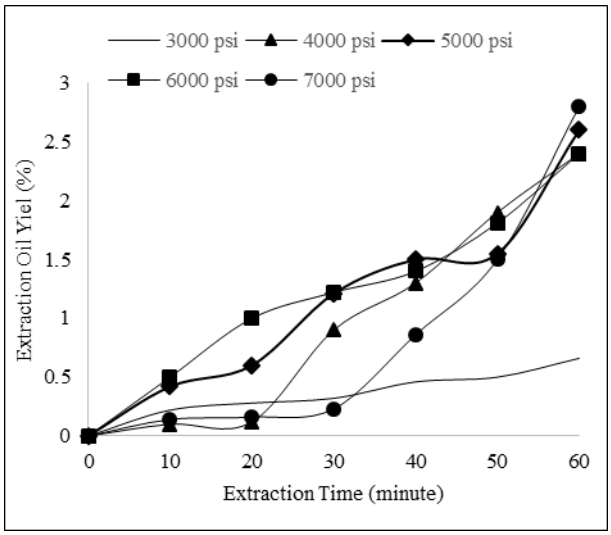

Fig. 3: Effect of different extraction pressure at constant temperature of 40°C on extraction yield

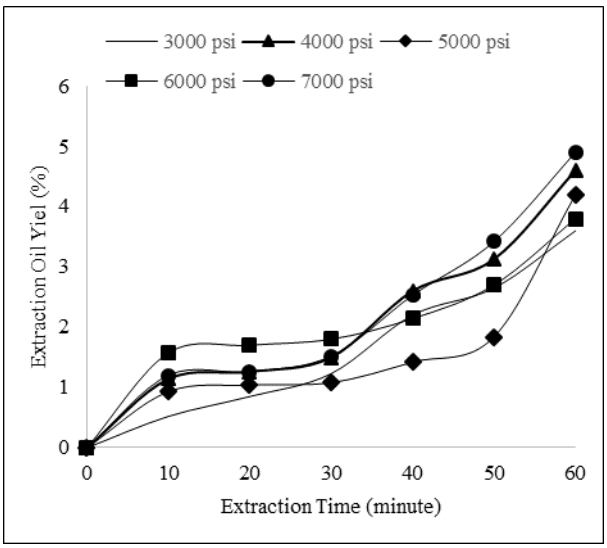

Fig. 4: Effect of different extraction pressure at constant temperature of 50°C on extraction yield

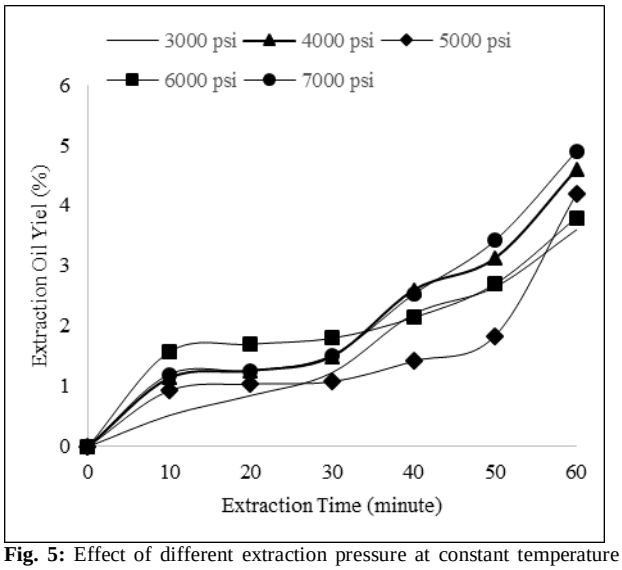

Fig. 5: Effect of different extraction pressure at constant temperature of 60°C on extraction yield

3.3. Effect of Temperature on Extraction Yield

In this study, the effect of different extraction temperature of 40, 50 and 60°C and different pressure of 3000, 4000, 5000, 6000 and 7000 psi on oil yield were investigated. The oil extracted was collected at extraction time of 10, 20, 30, 40, 50 and 60 minutes. Fig. 6, Fig. 7, Fig. 8, Fig. 9 and Fig. 10 shows the effect of different extraction temperature at constant extraction pressure 3000, 4000, 5000, 6000 and 7000 psi, respectively on oil yield. The highest oil yield, 4.92 % was obtained at temperature of 60°C at constant pressure of 6000psi. For Fig. 6, at constant pressure of 3000 psi, the relationship between temperature and extraction yield was observed to be increase as temperature increase. But increasing temperature from 50°C and 60°C as shown in Fig. 7, Fig. 8, Fig. 9 and Fig. 10 for constant extraction pressure of 4000, 5000, 6000 and 7000psi, the extraction yield reduced. It may be caused by factors changing of temperature at constant pressure is more complicated compare to change of pressure at constant temperature [22]. Increase the temperature might reduce the supercritical CO_2 solvating power, hence reduce density of solutes might reduce extraction yield.

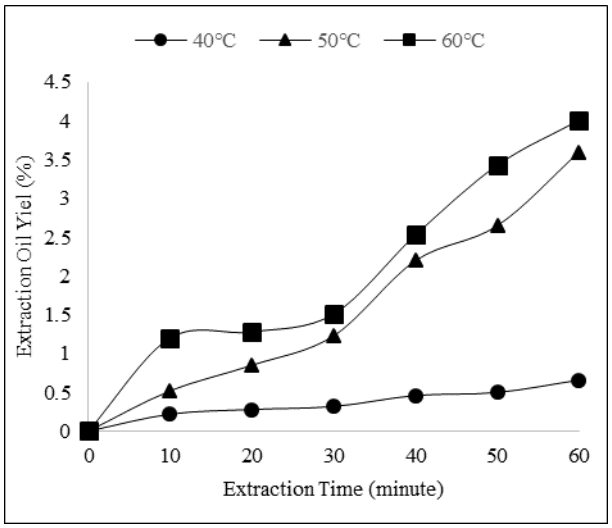

Fig. 6: Effect of different extraction temperature at constant pressure of 3000 psi on extraction yield

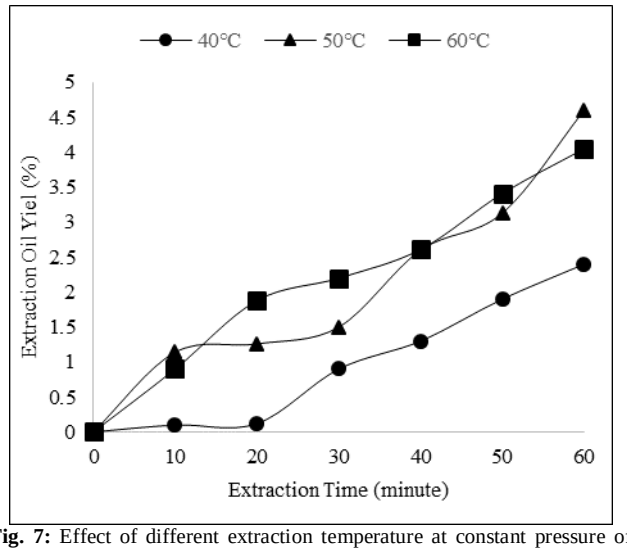

Fig. 7: Effect of different extraction temperature at constant pressure of 4000 psi on extraction yield

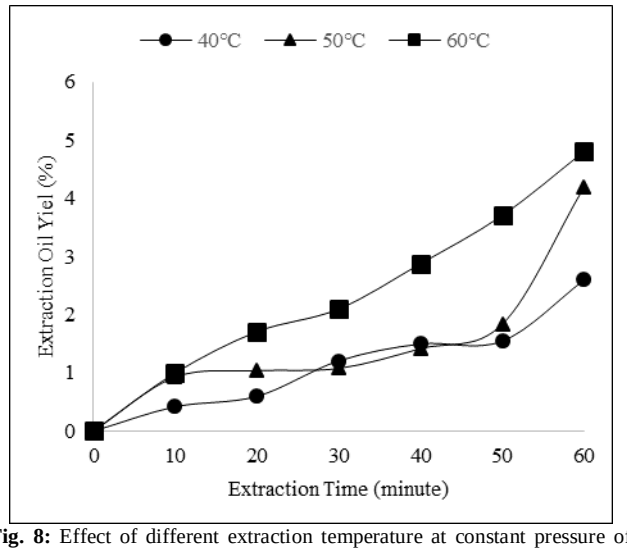

Fig. 8: Effect of different extraction temperature at constant pressure of 5000 psi on extraction yield

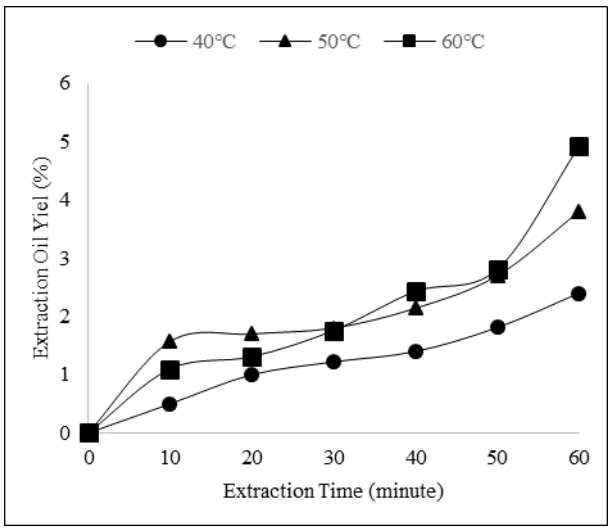

Fig. 9: Effect of different extraction temperature at constant pressure of 6000 psi on extraction yield

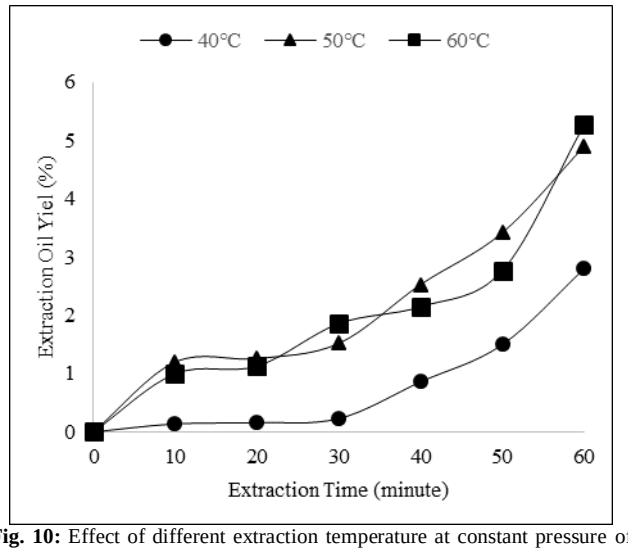

Fig. 10: Effect of different extraction temperature at constant pressure of 7000 psi on extraction yield

3.4. Component Identification

Fig. 11 shows the peak which had been identified based on the components by GC-MS. The component identified are tabulated in Table 1 with name of the components, retention time and its peak are of each of the component. Based on the peak, components were found were pentanoic acid, phosphonic acid, ecgonine, oleic acid and octadecadienoic acid. The other components were not detected because of the component exist in the other parts of L. *Leucocephala* such as at leaves. [7].

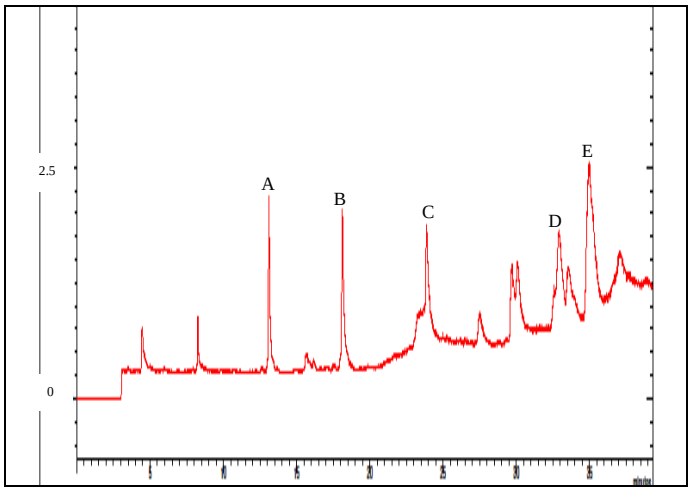

Fig. 11: Chemical components of L. *leucocephala* oil extracted from SC-CO_2 at 60°C and 5000 psi

Table 1: Main Components in Oil Extracted from *L. Leucocephala* Pods

Symbol	Components Identified	Retention time (min)	Peak area (%)
A	Pentanoic acid	13.6491	2.132
B	Phosphonic acid	18.1396	2.067
C	Ecogonine	23.9397	2.267
D	Oleic acid	32.8778	7.441
E	Octadecanienoic acid	34.8640	4.657

4. Conclusion

The objective of the research was to determine the highest oil yield and component identification of *L. Leucocephala* pods oil extracted by SC-CO_2 and identified by GC-MS. The effect of temperature and pressure were done at temperature range of 40°C, 50°C and 60°C and at 3000, 4000, 5000, 6000 and 7000 psi. Preliminary study was done to obtain the best drying time was 7 hours of drying to ensure the moisture content is left to be less than 10%. The best particle size of the sample to be extracted was 125µm which results with the highest oil yield compared to 250µm and 500µm. The highest oil yield, 4.92 % was obtained at temperature of 60°C at constant pressure of 6000 psi for 60 minutes extraction times. Based on the component identified by GC-MS, pentanoic acid, phosphonic acid, ecgonine, oleic acid and octadecadienoic acid were found in the study. The five major components were studied to have contribution in pharmacological activities such as antioxidant, anticancer and anticancer, Furthermore, the extraction method of *L. Leucocephala* oil using SC-CO_2 to be clean method which can replace the usage of volatile and hazardous solvent.

Acknowledgement

The authors are grateful for financial support provided by Institute of Quality and Knowledge Advancement (InQka), Universiti Teknologi MARA (UiTM) and UiTM.

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