Potential Evaluation of *Pelargonium Radula* Leaves Extract Using Supercritical Fluid Carbon Dioxide for Mosquito Repellent Skin Product

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***Abstract*— Supercritical Fluid Extraction (SFE) is a modern technique that is widely used when it comes to the extraction purposes. Recently, the SFE in *P. radula* leaves contains a lot of benefits towards human being because it possesses as a therapy in diabetes treatment, antimicrobial activity, pharmaceutical industries, cosmetics and as well as perfumery industries. This study focused on the highest oil yield that could be used as a repellent product toward mosquito bites. Many researchers used conventional technique such as hydro-distillation and solvent extraction to extract valuable component in oil formed itself. Unfortunately, these techniques have several disadvantages such as long extraction time, produce toxic residual and high usage of solvent. SFE has short extraction time, inexpensive, non-toxic and non-flammable. The extraction was conducted using temperature of 40, 45, 50, 55 and 60ºC and the pressure used were 1450, 2180, 2900, 3630, 4350, 5080 and 5800psi at constant flowrate of 24ml/min for 70 minutes extraction time. In this study, the best operating condition for the highest oil yield were 60ºC and 5800psi which results 1.7474%. Gas Chromatography-Mass Spectrometry (GC-MS) show that *P. radula* leaves contained geraniol and citronellol while for Fourier Transform-Infrared Spectroscopy (FT-IR), it consists of methyl (CH_2) group, methylene ($-CH_3$) group, methyl ($-CH_3$) and bend methyl (C-H) group. Besides, the best dosage of *P. radula* essential oil was 1%.**

***Keywords*— Geranium oil, Mosquitoes, P. radula leaves, SFE-CO_2.**

I. Introduction

Pelargonium species (*P. radula*) is a plant that originates from South Africa and a bushy plant up to 75cm in height. It is also known as Geraniceae family. It can be shrubs with strong aroma. The ancestors called it as “pokok halau nyamuk” specifically in Malaysia [1]. In recent years, many countries and areas in Asia have been experiencing diseases that transmitted from mosquitoes such as dengue, filariasis, malaria and Japanese encephalitis activity. In fact, there were 3 million people each year killed by malaria disease including one child per 30 seconds. In Malaysia, dengue cases are rising from year to year such as in 2012, there were 9,607 dengue cases that caused 20 deaths compare to in 2011 which is 7,963 cases with 12 cases [2]. Apart from that, synthetic repellents such as N,N-diethyl-meta-toluamide (DEET) have been used widely to protect humans from mosquitoes. However, from previous research, when DEET is applied on skin, it caused an oily and burning sensation to some DEET users [3]. From previous studies, DEET is based product that used frequently for mosquito repellent. But DEET have several effects towards human skin especially children skinIn contrast, the extraction of geranium oil from *Pelargonium* sp. revealed a significant anti-inflammatory activity as it contains citronellol, geraniol, and citronellyl formate as major components and other esters. In this research, geranium oil extraction from the Pelargonium Radula leaves is being focusing and further studying as well as the active ingredient in the geranium oil for mosquito repellent formulation skin product via SFE method. SFE is defined as a process to separate one component from another component by using supercritical fluids as the extracting solvent. Carbon Dioxide (CO_2) is considered to be used since extraction conditions for supercritical CO_2 are above the critical temperature and pressure which 31°C and 1073.28 psi respectively [4]. This is inert gas is easily removed from the extract besides non-flammable, non-toxic, with easy to follow supercritical conditions [5]. The CO_2 solvent also could be recycled or discharge to the atmosphere [6].

Generally, previous researchers used traditional method such as steam distillation and solvent extraction for essential oil extraction. However, the retention time of the extraction process for both method was longer which is 8 to 10 hours for steam distillation and 2 days for solvent extraction [7]. As for steam distillation method, it requires high temperature to complete the extraction process. In contrast, SFE method is an effective method as it is environmentally, non-toxic, non-flammable and inexpensive [8]. It also used short extraction time compare to the traditional method.

Basically, the information of geranium oil extracts was studied and focused as well as its chemical composition on the sample and the potency of geranium extracts on insect repellency activities. Previous studies showed that Gas chromatography and GC-MS analysis of geranium oil revealed the components that found were geraniol, linalool, methone, citronellol and geranyl acetate but the major constituent was geraniol (28.51%) [7]. As eloquently stated by Chen & Viljoen (2010), geraniol had significantly more repellent activity rather than citronellol and linalool [9]. This proved that geraniol was effective compare to the others.

The main objectives of this research are to determine the best operating condition for the highest oil yield by using sup ercritical carbon dioxide within the specified range of temperature and pressure and at constant CO_2 flowrate, to identify the best dosage of *P. radula* essential oil to be applied in lotion production toward mosquito bites, and to assess repellent activity on the mosquito bites of essential oil of *P. radula* extract. Up to date, there is no documented data were found on study SFE method for extraction of *P. radula* leaves and also the effect of yield of the oil extract from the sample that could be applied for mosquito repellent skin product

II. METHODOLOGY

A. Material Preparation

The *P. radula* leaves will be separated from the plant, then it will be cut and cleaned up with distilled water to remove impurities and dirt. An amount of 6g of sample leaves will be dried in oven to remove moisture content until it achieves below 10%. This is to ease

the CO_2 solvent to extract the samples without any distraction of water molecule and it is also for storage purpose. After that, the dried sample will be grinded using mechanical grinder (042241-12 series) through a laboratory sieve plate (Endecotts) of 150µm, 250µm, and 500µm of pore size to determine the *P. radula* leave powder and then, the dried sample powder were stored in refrigerator at 4°C for next use.

B. Moisture Content Determination

A study of moisture content is very important to gain the suitable drying time for removing moisture content from *P. radula* leave sample. The moisture content determination for *P. radula* was specified based on the Palm Oil Research Institute of Malaysia (PORIM) test method [10]. In general, the moisture content is mean the quality of water that present in the moist sample itself. First, the mass of petri dish is weighted. Then, the mass of sample is weighted. After that, mass of petri dish with the *P. radula* leave sample were dried in oven to remove moisture content about 2 hours with drying temperature of 40°C. The moisture content then was determined as percentage of ratio of water content in *P. radula* leave sample with total mass of the *P. radula* leave sample as shown as in the (1):

$$\text{Moiture content} = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100 \quad (1)$$

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).

C. Supercritical Fluid Extraction (SFE)

6g of grinded geranium leaves will be inserted in sachet. Then, the sample was charged into extractor, closed and placed into the equipment. Before the cover is close, the seal on top of an extraction vessel was sealed tightly. The system was pressurized to the extraction set pressure. The stabilization of pressure and temperature took 20-30 min. During the extraction time, dynamic valve will be opened and the restrictor valve was tightly closed. In this experiment, the pressure and temperature oscillations was ±2°C and ±5psi. The operating conditions used in the SFE experiments are temperature at 40°C, 45°C, 50°C, 55°C and 60°C while for pressure 1450psi, 2180psi, 2900psi, 3630psi, 4350psi, 5080psi, and 5800psi. Each experiment was run using new geranium leaves.

The CO_2 pump was run to feed high pressure liquid CO_2 after the extraction temperature obtained its desire value. The liquid CO_2 is then converted into supercritical condition when it is pump into extraction vessel. The gas CO_2 was pressurised with high pressure pump at flow rate of 24mL/min. After reaching 70 minutes of extraction time, the restrictor valve will be opened quickly to depressurise the supercritical solution for separation of solute from solvent. Besides, the depressurise CO_2 will then transformed into gas form and discharged into ambient conditions. The CO_2 pump will continuously actuate in order to achieve the desired pressure set point. The extract product usually will be collected in collection vial. The extraction yield is obtained and calculated based on the weight of collection vials before and after process. The extraction yield will be obtained and calculated based on the weight of collection vials before and after process. The oil yield was determined using equation (2):

$$\text{Oil Yield (\%)} = \frac{\text{weight of collection vials before process}}{\text{weight of collection vials after process}} \times 100$$

The procedure of extraction process was repeated three times under desired operating conditions and the date were given based on the average values. Figure 1 shows the diagram of SFE-CO2 apparatus.

Fig 1: The SFE-CO2 Apparatus.

D. Gas Chromatography-Mass Spectrometry (GC-MS)

Analysis will be carried out to perform analysis of GC-MS on sample using gas chromatography (Varian 450-GC) attached to a mass spectrometer (Varian 240-GC). The amount of 0.05g sample of *P. radula* leaves oil was collected in collection bottle from SFE-CO_2 process. Then the sample is diluted with 1mL of hexane. The sample is placed in a glass vial provided with the septa. This analysis will be conducted at Faculty of Chemical Engineering. GC-MS consists capillary column (30m long, 0,25mm internal diameter, 0.25µm film thickness). The flowrate of the carrier gas helium (99.999%) is set up to 1.0mL/min with split ratio of 1:10 injector temperature of 280°C. The analytical condition with sample injection (1µlL). The temperature programming varied from 80°C to 300°C at 6°C/min with oven temperature of 80°C. National Institutes of Standards and Technology (NIST) mass spectral program will be referred to detect component of sample based on mass spectra survey.

E. Fourier Transform-Infrared Spectroscopy (FT-IR)

The sample undergoes FT-IR analysis. The basic components of FT-IR spectrometer are sample compartment, source, interferometer, amplifier, detector A/D convertor, and a computer. In addition, FTIR analysis could be used to analyse and confirm the functional group of the sample. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined [11].0.05g sample of *P. radula* leaves oil collected in collection bottle from SFE-CO_2 process will be diluted with 1mL of hexane. The top plate of the equipment was cleaned up by using acetone. The sample was drop slowly onto hole of a top plate. The sample was loaded in FT-IR spectroscope (Model: Spectrum One, Serial No.: T4630), with a scan range from 400 to 4000cm^{-1} with a resolution of 4 cm^{-1}. The data of FT-IR analysis is shown based on wavelength that present on each peak of the graph from the computer.

F. Repellency Assessment

Mass Rearing:

The *Aedes aegypti* strain used in the experiment was collected as eggs. The *Aedes aegypti* colony is maintained under controlled conditions at 27°C, 80% relative humidity photoperiod of 14:10 h (light: darkness). The light power and photoperiod will influence the advancement in the existence cycle of the mosquitoes. The eggs will be collected on moist filter paper. A small bowel is lined with a 3 wides strip of filter paper. Then, the container is placed in a cage of adults and the egg collecting container is left in the cage for 48h. The bowel is then removed and any excess water will be drained out of the bowel. The egg paper is allowed within 24h in the cage and then it is removed and air dried for 4 days. It is stored in a large sealed plastic container [12].

The eggs were hatched in deoxygenated and the temperature of water is around 27°C. The larvae hatch in 6-12h. A plastic tray (20cm x 15cm x 5cm) is satisfactory to rear 1500 larvae. Water in rearing containers will be refreshed everyday by removing some quantity of

water from rearing tray and replaced it with fresh water. The purpose of refreshing the water in rearing containers was to block scum or layer formation on the water surface. After that, pupae will be collected from day 7 to 15 and placed it onto emergence cage whey they emerged. When all of them were emerged, the cover is replaced on the plastic container [12].

Repellency Test:
Animal test subjects were most preferable and suitable rather than human test due to ethical issue. The method used was a screened cage method [13]. The screened cage consists of 40cm3 aluminium-frame cage with a screened top and back, metal bottom, a front stockinette sleeve for access and clear acrylic sides (for viewing). Treatment consists of 1%, 5%, 10%, 15% and 20% of extract oil *P. radula* will be applied to the mice skin at the rate of 1mL/650cm2 of skin surface area. There were 20 of mosquitoes will be inserted on each cage for repellency test purpose. Before that, the mice's fur will be shaved deliberately. Then, the treated mice skin will be inserted into the mice cage. The number of mosquitoes that land in and bite the skin in 10 minutes is observed and recorded. The observation is repeated with different concentration of extract oil *P. radula* every 10 minutes. After the observations have been made, the best dosage or concentration of the extract oil *P. radula* will be identified [13]. Figure 2 shows the repellency assessment on laboratory mouse at insectarium laboratory, Faculty of Health and Science, UiTM.

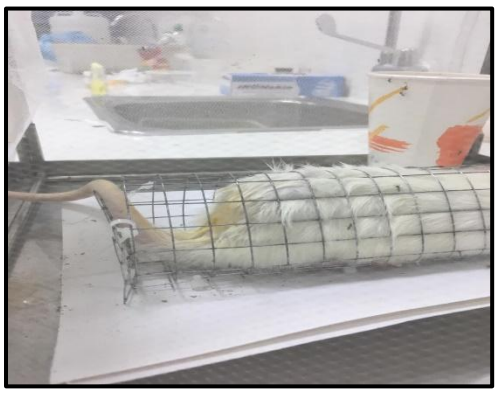

Fig 2: Repellency assessment apparatus.

III. RESULTS AND DISCUSSION

A. Preliminary Study for Best Extraction Particle Sizes

Three samples with different sizes which are 150µm, 250µm, and 500µm were undergo extraction process. There were only 6g of sample that could fitted in extraction chamber. Therefore, 6g of the *P. radula* leaves powder were put into a cotton bag and placed into the extraction chamber. Three different sizes sample were extracted within 70 minutes of extraction time and the oil yield extract were recorded for every 10 minutes. Each sample were run at 60°C with pressure 1450psi, 2180psi, 2900psi, 3630psi, 4350psi, 5080psi, and 5800psi. Figure 3 shows the preliminary study to determine the best particle size that produce the highest extraction yield. From Figure 1, the best particle size that produce highest yield is 150µm which is 1.0450%. The smaller size results in highest yield because of the higher surface area to volume ratio where it enhanced the contact between the solvent molecules and plant material during the extraction process [14]. Thus, it produced highest yield compare to 250µm and 500µm.

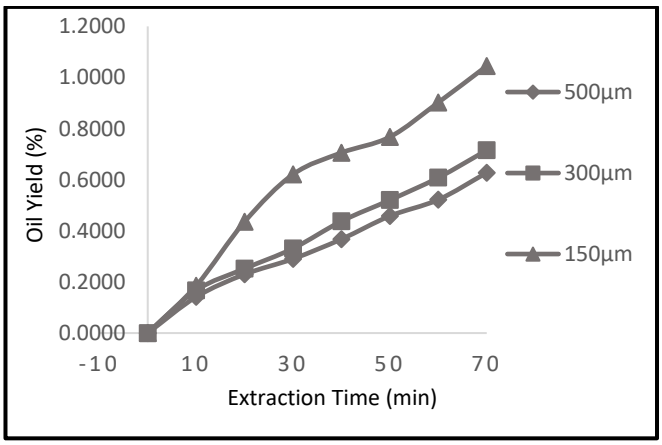

Fig. 3: Preliminary study for best extractions particle sizes.

B. Preliminary Study for Best Extraction Time

According to Figure 4, it shows the preliminary study for best extraction time at constant temperature of 40°C. From Figure 3, the best extraction time could be seen clearly at the minutes of 70 where the extraction yield of all the parameters was constant. In this case, the initial phase (A) of the extraction is represented by the dispersion coefficient of the solute between the thick liquid stage and the sample matrix [15]. Thus, it will give a way to a diffusion controlled process in the next stages (B) of the extraction. Previous study done by Paula B. et al,. (2007) run the experiment of extraction process of green leaves pelargonium sp. and the extraction time is 60 minutes[16]. However, there were slightly different of extraction since there is no documented data on the extraction of *P. radula* via SFE method. Therefore, for the next SFE method on other parameter will be run using 150µm [16].

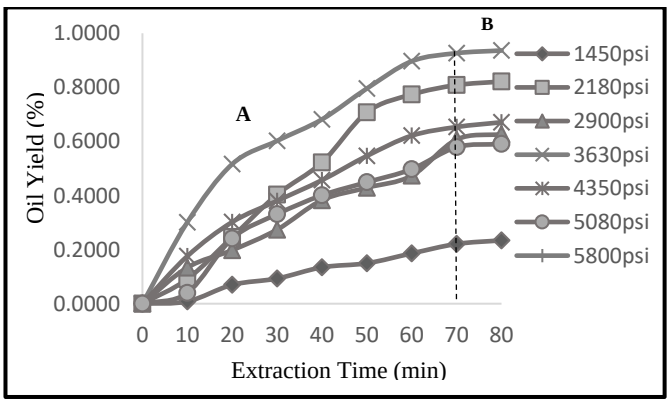

Fig. 4: Preliminary study for best extraction time

C. Effect of Moisture Content

In this research study, the percentage of moisture content of the sample which is the P. radula leaves were determined and calculated by using equation (1). The percentage of moisture content were reached below 10% which is around 9.7%. The duration of the drying process should not be taken too long as to avoid that there is no volatile compound lost. As stated by the previous researchers that the moisture content of the sample above 18% will give negative effect toward the extraction efficiency. The moisture content will affect the solubility and mass transfer kinetic during the extraction process by reducing the SFE and sample contact. This is because the moisture will act as a barrier that could avoid the sample from contacting with the CO_2 during the extraction [17]. Therefore, the total moisture content of the dry P. radula leaves should be determined before conducting the extraction process. Since the total moisture content in this study is below 10%, hence there is no effect of the moisture content of sample during extraction process.

D. Effect of Pressure on Oil Yield

In this work, the effect of different extraction pressure on oil yield (%) were studied by using varies pressure which are 1450, 2180, 2900, 3630, 4350, 5080, and 5800psi while holding the constant temperature of 40, 45, 50, 55 and 60°C. 70 minutes of the extraction time was running constantly. The result on effect of different pressure on yield extract with constant temperature are shown in the Figure 5. In this study, the results are based on the total solute of extract yield per 6g sample pelargonium leaves powder.

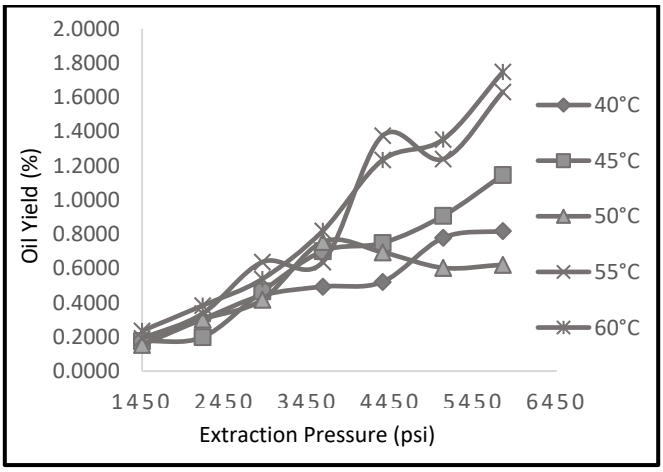

Fig. 5: Effect of Different Pressure on Oil Yield at constant temperature, and constant CO_2 flowrate (24ml/min) at 70 minutes extraction time.

Based on Figure 5, it indicates that the highest oil yield extraction is at 60°C with 5800psi which recorded 1.7474%. This shows that higher in pressure gave higher percentage of extraction oil yield. Higher pressure will increase solubility as well as the fluid density [4]. Therefore, to obtain quantitative recoveries of essential oil, higher pressures are necessary [18]. Thus, highest oil yield is produced.

It is then followed by temperature at 55°C with 5800psi which recorded 1.6311%. As can be observed from the Figure 4, the oil yield decreases after from 2900psi to 3900psi. In my opinion, this is might be the extraction is not working and suitable at this operating condition due to retrograde behaviour that take place in this region. According to retrograde behaviour, when the pressure was increased at constant temperature, it will reduce the solubility power of the solvent [10]. Therefore, small oil yield will be produced.

When further increase in pressure, the oil yields start to increase except for temperature of 50°C where the oil yield decreases from 3630psi to 5800psi. K. Khaw et al. (2017) stated that an increase in pressure may cause the solid matrix to compact and the void fraction leads to unfavourable extraction outcomes [19]. In my opinion, the best operating condition is above 50°C in order to have high recovery of oil particle as done by a researcher of Paula et al (2006) and E.I. Ponomoreva & E.I. Monohova (2017). They conclude that at temperature 40°C and 60°C can extract highest yield with low operating pressure [15,19]. Meanwhile at constant temperature of 60°C, the oil yield is high at pressure of 5080psi while for the constant temperature of 40°C and 50°C, the oil yield extraction is kept decreasing. In this study, we can observe that after 5080psi, the oil yield is increased. This shows that the best operating pressure is at 60°C and 5800psi. This result is might be the root cause of the increased dissolving power of supercritical CO_2 due to increase density cause by an increase of pressure at the constant temperature. The previous researchers prove that at operating condition of 60°C and 7250psi where they claimed that it is the best condition for producing highest oil yield [18].

At pressure of 1450psi to 4350psi, the oil yield increased for each of operating temperature as shown as Figure 5, except for operating temperature of 50°C and 55°C. In my opinion, increase in pressure will increase the solvent density and viscosity. Thus, it reduced the capability of the fluid to penetrate the raw material and interact with extractable components. This effect might have counteracted the increase in the fluid density and reducing the oil yield [20,21]. At pressure of 4350psi to 5080psi, the oil yield starts to decrease from temperatures of 50 to 55°C with 0.6030% and 1.2387% respectively. When increasing pressure to a certain point might reduce the diffusivity of the SFE solvent and result in a reduced contact with pores in the raw material. Therefore, it has potential in reducing the solute dissolution [19]. In general, from the Figure 5. by increasing the temperature from 40 to 60°C, the extraction yield is also increasing. Meanwhile at operating pressure 5800psi, the total extraction yield recorded is the highest compared to 1450, 2180, 2900, 3630, 4350 and 5080psi.

E. Effect of Temperature on Oil Yield (%)

In this study, the effect of temperature on oil particle yield (%) will be further studied and discussed using varies temperature which are 40, 45, 50, 55 and 60°C. The operating pressure is considered as a constant variable which are 1450, 2180, 2900, 3630, 4350, 5080, and 5800psi. As usual, the extraction time will be constant for 70 minutes for each parameter running. The graph of effect different temperature on oil yield extract with constant pressure were shown as Figure 6.

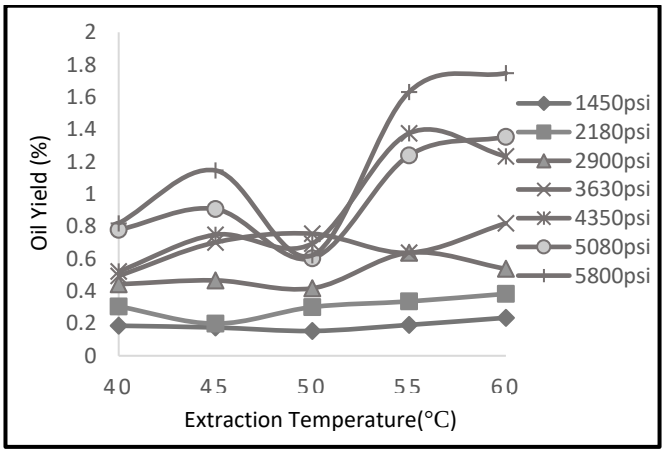

Fig. 6: Effect of Different Temperature on Oil Yield at constant pressure, and constant CO_2 flowrate (24ml/min) at 70 minutes extraction time.

Based on the Figure 6, it shows that at 45, 55 and 60°C possess the highest extraction yield recorded which are 1.1459%, 1.6311% and 1.7474% respectively at constant pressure of 5800psi. Besides that, the second highest yield extract was at constant 5080psi. It can observe that the operating temperature of 45, 55 and 60°C with constant operating pressure of 5800psi is the best condition to be used and applied in production of mosquito repellent skin product. It shows that as the pressure increases, the oil particle yield also increases. In addition, when the pressure increased, the fluid density and the solubility of the solute increased [19]. This is proven that the oil yield extract increased from 40 to 60°C. Another example is the extraction oil from Amelia et al (2005), who performed SFE studies on Pelargonium bare roots, reported high oil yield (2.53%) at high pressure with operating conditions of 40°C, 4351psi and 40 minutes [21]. In addition, in some cases, an increase in pressure may cause the solid matrix to compact and the void fraction leads to unfavourable extraction outcomes [19].

At operating temperature of 40°C to 45°C with constant operating pressure of 2180psi, the oil yield extract starts to decrease which is 0.3049% to 0.1988% respectively. However, as pressure increase from 1450psi to 2180psi, the extraction yield from temperature of 45 to 60°C increase. As eloquently stated by the previous researchers, a higher temperature resulted in higher yields, contrary to cross over of density effects. The authors reasoned that increases in kinetic energy from increasing temperature were directly proportional to the rate of diffusion of CO_2 within the raw plant material [19]. This is proven that our trend is quite same as what Khaw et al (2017) mentioned on their articles that oil yield is directly proportional to operating temperature.

Moreover, at constant pressure of 1450psi with operating temperature of 50°C, the oil yield extract is the smallest compare to the others. The trend starts to decrease from 40°C to 50°C and increases from 50°C to 60°C. In my opinion, the operating temperature of 50°C is not a suitable and working temperature for extraction at low pressure. The fluctuation of the oil yield is might be due to factors of changing the temperature at constant pressure [22]. Changing in temperature is more sensitive rather than changing in pressure [23]. Therefore, it caused a significant change on *P. radula* solubility and it results in fluctuation of oil yield. This result is different from a study done by Omprakash H, et al (2012). They mentioned that the high amount of oil yield is recorded at temperature of 55°C with pressure of 2176psi [24]. This is might be due to the density flowrate of the CO_2 used which is around 5 to 20mL/min. Thus, flowrate of CO_2 gave a significant change in percentage of oil yield [25]. However, different sample might affect different optimum parameter to extract the selected compound [26].

F. Gas Chromatography-Mass Spectrometry (GC-MS)

The oil extract obtained from *P. radula* leaves was subjected to GCMS analysis. The composition in the chromatograms of Figure 7 noted that the peak is referred on what kind of component that has been detected of the oil extract. Sixty-one components were identified in a sample of the best operating condition which is 5800psi and 60°C. The most abundant components were Eudesmol (12.38%), Geraniol (11.6%), and Citronellol (7.59) as the main representatives. Eudesmol is an oxygenated sesquiterpene contained in medicinal or edible plants which brought significant increments in food intake in rats and elevated plasma ghrelin levels [27]. In addition, it is used in traditional medicine for the relief of hemorrhoids, dysentery, inflammation and cancer [28].

As stated from previous researcher, they found that the main component in the *Pelargonium* species were Oxygenated monoterpenes (74.2%), Geraniol (50.2%), and Citronellol (14.2%) as the main representatives [29]. Besides another researcher found that the oil contained citronellol (27%), geraniol (11%), citronellyl formate (7%), and 10-epi-γ-eudesmol (6%) as major constituents [30]. The main bioactive compounds which contain insecticidal properties as discussed in Chapter 2 are known as geraniol, citronellol, linalool, methone, and geranyl acetate. In this study, only geraniol and citronellol was presented in oil extract. In fact, these two components have been studied and claimed by a number of researchers to have an insecticidal activities or repellent value as discussed in section 2.2 and 2.3. The other three components mentioned was not detected. In my opinion, this is might be due to the origin *P. radula* since *Pelargonium* species is more than one species.

Furthermore, citronellol is a component that has been mentioned by Maia & Moore (2011) in their paper that this compound has insecticidal properties [31]. As mentioned by Amelia et.al,. (2005) in their work, geraniol, citronellol and linalool is proved to have insecticidal properties on repelling mosquitoes [21]. Besides, peppermint oil extract from *Mentha piperita* sp. is believed to have availability on insect repellent. [32]. They claimed that component inside the peppermint oil which is citronellol could against different type of adult mosquitoes. It same goes to lavender plant, researcher found on their study that the lavender oil has potential on repelling mosquitoes [33]. The identification component of lavender oil was linalool and citronellol [34]. For hydro-distillation method, researchers found that citronellol, 0-octen-1-01 and geraniol contained on oil extract of *Pelargoinum* leaves [35].

To be conclude, it could be clearly observed that the essential oil extract from *P. radula* leaves contained the geraniol and citronellol. These components were believed to have the potential on repellent activity [9]. In advanced, eudesmol could be used an anti-inflammatory agent for mosquito repellent skin product. Table 1 shows the oil extract identified compound using GC-MS anlysis.

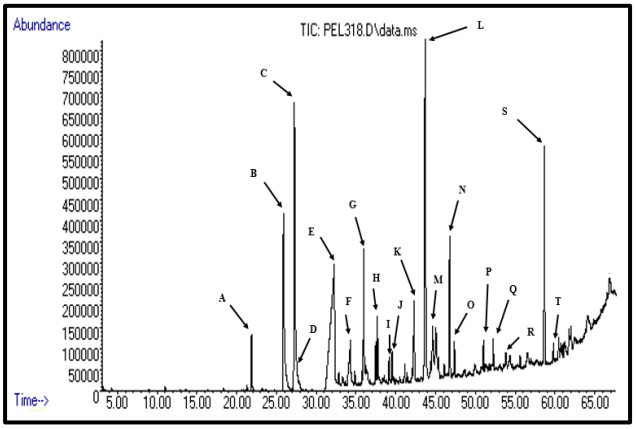

Fig. 7: GC/MS chromatograms of *P. radula* leaves oil extract.

Table 1:
Chemical composition of P. radula leaves oil extract at T :60°C and P:5800psi

Peak	Identified Compound	Chemical Formula	Retention Time (min)	MW (g/mol)	Area (%)
A	Methone	$C_{10}H_{18}O$	21.855	154.25	1.29
B	Citronellol	$C_{10}H_{20}O$	25.872	156.27	7.59
C	Geraniol	$C_{10}H_{18}O$	25.872	154.25	11.66
D	Farnesal	$C_{15}H_{26}O$	27.874	222.37	0.36
E	Carvenone	$C_{1o}H_{16}O_2$	32.275	152.23	0.32
F	Decanoic acid	$C_{10}H_{20}O_2$	34.312	172.26	3.05
G	Dauca-5,8-diene	$C_{15}H_{24}$	35.960	204.36	3.40
H	Germacrene D	$C_{15}H_{24}$	37.682	204.36	1.81
I	Cadinene <gamma>	$C_{15}H_{24}$	39.147	204.36	0.66
J	Cadinene <delta>	$C_{15}H_{24}$	39.536	204.36	0.77
K	Phenyl ethyl tiglate	$C_{13}H_{16}O_2$	42.277	204.27	2.48
L	Eudesmol	$C_{15}H_{26}O$	43.679	222.37	12.38
M	Selinene	$C_{15}H_{24}$	45.023	204.36	2.99
N	Geranyl tiglate	$C_{15}H_{24}O_2$	46.768	236.36	3.34
O	Neryl propanoate	$C_{13}H_{22}O_2$	47.352	210.32	0.93
P	Citronellyl acetate	$C_{12}H_{22}O_2$	51.104	198.31	0.77
Q	Geranyl butanoate	$C_{14}H_{24}O_2$	52.239	224.34	0.76
R	Methyl lactate	$C_4H_8O_3$	53.824	104.11	0.64
S	Citronellyl formate	$C_{11}H_{20}O_2$	58.670	184.28	5.04
T	Sesquilavandulol	$C_{15}H_{26}O$	59.803	222.37	0.47

G. Fourier Transform-Infrared Spectroscopy (FT-IR)

Figure 8 shows the peak that has been identified by FT-IR. There are six peaks been identified and listed in Table 2. Based on the peak,

components that most found are functional groups of the geranium oil extract from SFE process.

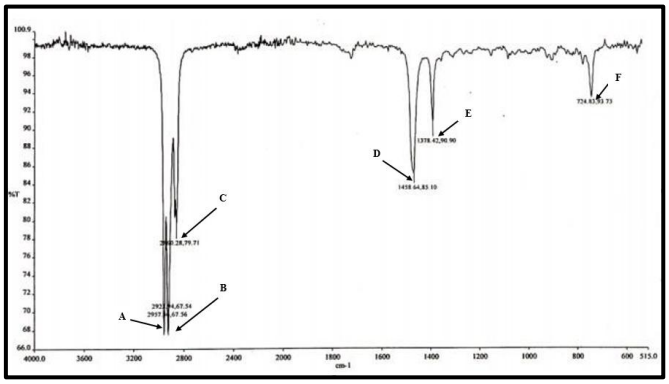

Fig.8: Functional Group of *P. radula* Leaves Extracted from SFE.

Table 2:
Functional Groups of P. radula Leaves Extracted from SFE

Functional Group	Group Frequency, cm^{-1}	
	Proposed Assignment	Geranium Oil Peak
Stretching (OH)	3367-3426	-
Asymmetric stretching, methyl (-CH_2)	1452-2962	A
Symmetric stretching, methylene (-CH_3)	2926-2933	B
Asymmetric stretching, methyl (-CH_3)	2872-2875	C
Stretching aldehyde (C=O)	2728	-
Stretching (C=O), aldehyde	1708-1730	-
Stretching (C=O), carbonyl	1713	-
Alkenyl (C=C)	1625-1671	
Asymmetric bend methyl (C-H)	1452-1453	D
Symmetric bend methyl (CH)	1376-1377	E
In-plane bending (O-H)	1267	-
Skeletal (C=C)	1174	-
Stretching, alcohol (C-O)	724-1058	F

Source: Geranium Oil Composition via FT-IR analysis [36].

Table 2 indicates the functional groups that were found on the geranium oil of *P. radula* leaves while Figure 5 shows the FT-IR result on the oil extract at operating conditions (T=1450psi, P=40°C). From Figure 5, it can be observed that the wavelength is 2957.46cm^{-1} which means the functional group is methyl (CH_2). It is proven that the methyl group was present in the geranium oil extract. Moreover, at peak of 2923.94cm^{-1} shows that functional group of methylene (-CH_3) also found in the geranium oil extract. At peak of 2860.28cm^{-1}, it can be observed that the functional group of methyl (-CH_3) is present in the oil extract. Besides that, the functional group of bend methyl (C-H) and bend methyl (CH) also present in the oil extract as shown as Figure 6. The peak of 724.83cm^{-1}show that alcohol group existed on this oil extract.

In addition, The main components of geranium essential oil are geraniol, citronellol and linalool [37]. In fact, the proportion of geranium oil can change depending on the region and other more specific factors. An interesting aspect is that these three compounds have some structural similarities. For example, all three compounds have a =C(CH_3)$_2$ group [36]. A CH_3 bending mode appears in the wavelength of 2860cm^{-1} except when the CH_3 group is directly attached to C=C, C≡C, C=O or an aromatic ring. In fact, the spectrum of geranium essential oil from Figure 8 shows an intense band at 2860cm^{-1}, which is assigned to a CH3 bending mode. Geraniol, citronellol and linalool have in their chemical structure a OH group. Hence, the band at 724.83cm^{-1} of geranium essential oil has been assigned to the C–O stretching mode.

H. Repellency Assessment

The leaf extract showed significant degree repellency. There were 100 of mosquitoes that have been used for mosquito repellency test and each 20 of them will be inserted into five different mosquito cages. Treatment consists of 1%, 5%, 10%, 15% and 20% of extract oil *P. radula* will be applied to the mice skin at the rate of 1mL/650cm2 of skin surface area. As a result, 1% of extract oil *P. radula* showed that the mosquitoes did not bite the mice even after 10 minutes. It is then followed by 5%, 10%, 15% and 20% of *P. radula* oil extract and it showed that the mosquitoes did not bite the mice. It proved that the geranium oil extract could repel 100% of the mosquitoes. Study done by Hamzeh et al., (2015) stated that 25, 50 and 75% concentration of *Pelargonium roseum* wild (*Geraniaceae*) essential oil extract were used and applied to repel the *Anopheles stephensi*. In this study, they claimed that 25% concentration was clearly less effective and 75% is the most effective [38]. In my opinion, the result is different due to different types of sample and repellency test used.

Apart from that, it proved that the *Pelargonium* oil extract can be used to repel the mosquitoes. It same goes to *P. radula* leaves oil extract from distillation method where it produced volatile oil. These volatile oil has stronger aroma where it is believed from the geraniol compound and this makes the geraniol need lower concentration to repel the mosquitoes [7]. Study done by Nurahayat Tabanca et.al., (2013), they claimed that geraniol and eudesmol compound that identified from less than 5% geranium leaves oil extract has a significant value on repelling tick and mosquitoes [30]. Thomas et.al., (2006) stated on their study that 1% of geranium oil has significant on repelling *Ixodes Ricinus* [39].

Moreover, the main component of geranium oil extract which are geraniol and citronellol have been indicated as potential agent in the treatment of inflammation and wound healing [40]. Therefore, the extract oil *P. radula* is proved as a main ingredient for repellent skin product as well as treatment of inflammation where it is suitable to all types of skin.

IV. Conclusion

SFE-CO_2 extractions of geranium (*P. radula* leaves) were carried out at operating pressures of 1450psi, 2180psi, 2900psi, 3630psi, 4350psi, 5080psi, and 5800psi, operating temperatures of 40°C, 45°C, 50°C, 55°C and 60°C, and constant CO_2 flow rates (24mL/min) in order to determine the optimum conditions for geranium oil extraction (the conditions under which the highest oil yield was obtained). At high pressure (5800psi), high temperature (60°C) and these conditions give the highest overall oil yield. Therefore, they were considered to be the optimum extraction conditions. The highest yield obtained under these conditions was 1.7474%. GC-MS and FT-IR analyses were carried out. For GC-MS analysis, the most abundant components were Eudesmol (12.38%), Geraniol (11.6%), and Citronellol (7.59) as the main representatives. While for FT-IR analysis, the wavelength showed the functional groups that exist on the geranium oil extract were methyl (CH_2), methylene (-CH_3), methyl (-CH_3) and bend methyl (C-H). 1% of *P. radula* oil extract showed that it could repelled 100% the mice from mosquito bites.

Acknowledgment

Huge thanks to my supervisor, Madam Nur Ain Binti Mohd Zainuddin for her fully guidance and encouragements. Then I would like to say thank you to my co-supervisor from FRIM, Saidatul Husni Binti Saidin for all the cooperation and helps. Special thanks

to Dr. Nazri Che Dom, senior lecturer from Faculty of Health and Science who involved on giving me an opportunity and guideline in order for me to complete my final year project. I would like also to thank my family and friend for all support. Last but not the least, thank you to technicians of Chemical Engineering Faculty who are very patient in order to fulfil my needs on the information of this final year project.

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