RESEARCH ARTICLE | DECEMBER 06 2018

Effect of CO_2 flow rate, co-solvent and pressure behavior to yield by supercritical CO_2 extraction of *Mariposa Christia Vespertilionis* leaves FREE

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AIP Conf. Proc. 2045, 020072 (2018)
https://doi.org/10.1063/1.5080885

Effect of CO_2 flow rate, co-solvent and pressure behavior to yield by supercritical CO_2 extraction of *Mariposa Christia Vespertilionis* leaves

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Abstract. *Mariposa Christia Vespertilionis* (MCV) is a plant which have being known as ‘red butterfly wing’ to local people in Malaysia. MCV has been known to be used as a traditional treatment for bundle of diseases such as tuberculosis, bronchitis, inflamed tonsils, colds, muscle weakness and poor blood circulation. Supercritical Fluid Extraction (SFE) is the technology equipment that can be used for the extraction of MCV leaves for its valuable compound. SFE is a process of separating inner matrix from outside matrix by extracting inner compound with CO_2 as the main solvent. SFE technology is widely used in extraction of herbal plant because of the environmental friendly process compared to the conventional extraction with eco-friendly co-solvent used like ethanol. The objectives of this study was to analyze the optimum parameter of CO_2 flow rate among selected parameter range (15g/min – 35g/min), to analyze the optimum parameter of co-solvent percentage among selected parameter range (5% - 25%) and to study the pressure behavior effect on extraction yield of MCV leaves using SFE-CO_2. The fixed variables in this study were temperature at 50^{o}C, particle size of 63µm, ethanol as the co-solvent and extraction time was fixed to 1 hour for all parameters. After SFE extraction process, the co-solvent was separated from the extract using Rotary Evaporator (ROVAP) with 76^{o}C temperature in order to obtain the final extraction yield of MCV leaves. At the end of the study, for the CO_2 flow rate, it was noticed that the extraction yield improved starting from 5 to 25 g/min and declined after that. Thus the optimum CO_2 flow rate was 25 g/min with extraction yield of 31.22%. For the co-solvent, the result of the extraction yield was directly relative to the percentage of the co-solvent. As the percentage of co-solvent increased, the extraction yield was also increased. The optimum extraction yield was 40.93% with 30% co-solvent. For the pressure behaviour, the higher number of pressure magnitude would give higher extraction yield. The optimum extraction yield was 39.47% with 9 pressure magnitudes.

INTRODUCTION

Mariposa Christia Vespertilionis (MCV) is the scientific name for Red Butterfly Wing plant [1]. It is called as Red Butterfly Wing due to its leaves resembling butterfly in a flight. MCV leaves are averagely between 2 to 4 inches wide [2]. According to the local MCV growers in Malaysia, this unusual species can grow in the range of 1.5 to 2.0 feet height starting from the seed planting in four-month period [2]. Furthermore, the leaf ripe for medicine can be harvested after four-month period from seed planting. MCV is an important medicinal plant with many benefit properties such as anticancer, anti-inflammatory and beneficial phytochemicals [3]. There are limited information sources revealed from scientific study about MCV from all possible scientific study and society. Actually, in Malaysia, MCV leaves had been used for traditional uses for curing some minor injuries [2]. There are phyto-chemistry and bioactivity in MCV plant especially from the leaves that contribute to traditional uses. MCV leaves were used for healing bone fracture while the whole plant part is used for treating tuberculosis and bites from

4th Electronic and Green Materials International Conference 2018 (EGM 2018)
AIP Conf. Proc. 2045, 020072-1–020072-7; https://doi.org/10.1063/1.5080885
Published by AIP Publishing. 978-0-7354-1771-7/$30.00

snake [4]. MCV plant also had been reported to be used for treating bronchitis, inflamed tonsils, colds, muscle weakness and also poor blood circulation [4]. All of these important compounds in the MCV leaves can be harvested or extracted using traditional or modern extraction technologies.

Extraction is a chemical process that can be defined as, acquiring something valuable inside a compound or things for specific purpose. In chemistry, it is a separation process which consists of two types of extractions which are liquid-liquid and solid-liquid. Each of them consists of individual benefit and criteria for extraction. Along with that, extraction seems to be existed with several techniques and had been developed with many new techniques for the past decade [5]. Several techniques for extraction are, Soxhlet, maceration, percolation, turbo (high speed mixing) and sonification which are traditional methods for solid-liquid extraction [5]. These techniques actually face with some problems which are time-consuming and require a large quantity of environment polluting solvent. Compare with the present and advance techniques which are supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and pressurized solvent extraction (PSE), they are much reliable with time-saving and efficient unconventional methods [5].

SFE method is becoming more popular to most researcher globally and well established SFE [6] shows more complete extraction technology compared to other methods which are classified as "traditional method" [7]. SFE can be explained as separating extract component from another phases or matrix whether in solid or liquid using supercritical fluid (SF) acts as a solvent. Mainly, the solvent used in SFE is carbon dioxide, CO_2 supported with other co-solvent compound depends on the extractant. SFE methods had been used frequently by researchers especially in extracting valuable oil or extract compound in herb plant [8]. Several authors agree that, SFE can demonstrate differences in desorption behaviors with fractioned SFE technique by increasing extraction pressure and temperature [9].

In this research, the SFE had been used as extraction process for MCV leaves, with CO_2 as the supercritical fluid. The intervention of ethanol as co-solvent had also been discussed further in this study. Liquid CO_2 exists only at pressure above 5.1 atm and clearly it shows that the triple point of carbon dioxide is approximately 518 kPa at -56.6^{o}C or 216.4K [10]. Amorphous glass-like solid was observed at high pressure between 1000 bar to 10000 bar which another form of CO_2 [10]. On the other hand, the supercritical fluid region shows the supercritical CO_2 behavior with low temperature range from 303.8K to 400K [10]. The relatively low temperature process and the stability of CO_2 allow most compound to be extracted with low damage or denaturing to the sample [11].

METHODOLOGY

Material and Chemical

In this study, the material that had been extracted was MCV leaves. Ethanol was used as a co-solvent or modifier to enhance the extraction yield in SFE unit. The purity of the ethanol used for this study was 96% composition.

Supercritical Carbon Dioxide Extraction

In SFE, the MCV leaves was prepared by cleansing it using deionized water to remove wax material and then dried it at direct sunlight for 4 hours. The leaves were then cut into small pieces prior to further drying in oven for 30°C overnight to ensure its moisture content in the range of 10% - 20%. Then, the dried leaves were grinded and sieved into 63μm. The SFE unit consisted of CO_2 gas, heat exchanger, high pressure pump, sample vessel, product vessel, chiller and automated back pressure regulator. The SFE model used in the Faculty of Chemical Engineering laboratory at Universiti Teknologi Mara (UiTM) Penang Branch was Thar SFC. This model could be loaded with a maximum volume sample of 100mL, maximum operating temperature and pressure of 90°C and 400bar respectively.

The MCV was weighed 5g for each sample by using analytical balance. Then, the sample was covered with white thin cloth. The covered sample was put into the sample vessel of the SFE equipment. The extraction process occurred in the SFE. The extracting variables or parameters such as CO_2 flow rate, co-solvent composition and pressure magnitude were varied and manipulated on the SFE for this study. The extracted sample was collected within one hour for each sample with 10-minute interval. The extracted sample was collected and then further processed in rotary evaporator in order to determine the yield.

Rotary Evaporator

Rotary evaporator was used for the next step in order to separate the ethanol content from the extracted sample. The model of this equipment was Butchi Rotavapor R-215. This step was to obtain the real mass of the extracted sample. The sample was weighed first before running into the rotary evaporator. The actual temperature was set to 76^{o}C and pressure 320mPa. After being separated from the ethanol, the extracted sample was weighed again to obtain the yield of MCV leaves.

Experimental Design

The experimental design for this study was based on selected parameters being studied as shown in Table 1 and Table 2 which listed out all the parameters involved.

TABLE 1. Scope of study

Parameters	**Units**	**Range of Parameters**				
CO_2 Flow rate	g/min	15	20	25	30	35
Co-Solvent	Percentage, %	5	15	20	25	30

TABLE 2. Pressure magnitude flow

	Range of Parameters									
Pressure	**Pressure magnitude flow from time 0-minute to 60-minute**									**Duration for each pressure magnitude (min)**
1-magnitude	375	-	-	-	-	-	-	-	-	60.00
3-magnitude	375	150	375	-	-	-	-	-	-	20.00
5-magnitude	375	150	375	150	375	-	-	-	-	12.00
7-magnitude	375	150	375	150	375	150	375	-	-	8.57
9-magnitude	375	150	375	150	375	150	375	150	375	6.67

RESULTS AND DISCUSSIONS

Extraction Oil Yield

There were three parameters in this study which were to identify the optimum CO_2 flow rate among the selected range of parameters (15g/min – 35g/min), to identify the optimum co-solvent percentage among the selected range of parameters (5g/min – 30g/min) and to study the pressure behavior to the extraction system (pressurized-depressurized). For pressure behavior study, the parameters were depending on the number (magnitude) of the pressure being increased or decreased for example when the pressure was started with 375bar, decreased to 150bar and increase back to 375bar, meant that this parameter was identified as three times pressure magnitude. Then the extracted yield after rotary evaporator separation process can be calculated using equation 1. The result for overall study is presented on Table 3.

$$\text{Yield, y (\%)} = \text{((final weight-initial weight))/(sample weight)} \times 100 \quad (1)$$

TABLE 3. MCV Leaves Extracted Yield Results

Fixed Variable: Temperature = 50°C; Sample Size = 63μm; Extraction Time = 1-hour; Sample Initial Weight = 5g					
Parameters Study	**Parameters**				
	CO_2 Flow rate (g/min)	Co-Solvent (%)	Pressure (bar)	Sample Yield (g)	Percentage Yield (%)
CO_2 Flow rate	15	10	375	0.026	0.52 %
	20	10	375	0.991	19.82 %
	25	10	375	1.561	31.22 %
	30	10	375	0.220	4.4 %
	35	10	375	0.120	2.4 %
Co-Solvent	25	5	375	0.416	8.32 %
	25	15	375	0.428	8.57 %
	25	20	375	0.994	19.89 %
	25	25	375	1.178	23.57 %
	25	30	375	2.046	40.93 %
Pressure Behavior	25	10	1 magnitude	1.467	29.35 %
	25	10	3 magnitudes	1.576	31.52 %
	25	10	5 magnitudes	1.638	32.77 %
	25	10	7 magnitudes	1.757	35.14 %
	25	10	9 magnitudes	1.973	39.47 %

Effect of CO_2 Flow rate on Extraction Yield

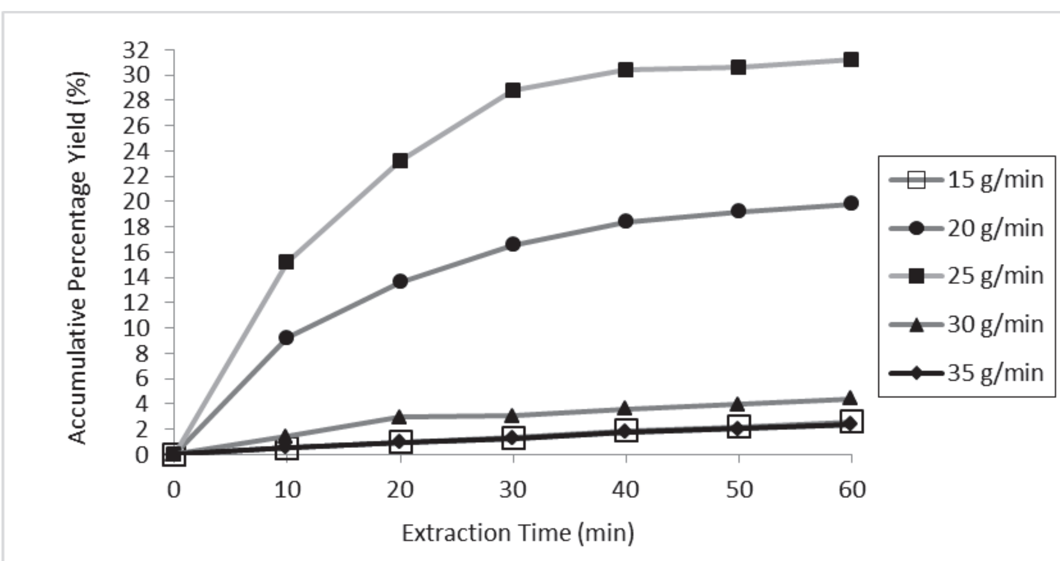

FIGURE 1. Accumulative Extraction Yield for CO_2

Figure 1 shows the accumulative yield extraction percentage from the effect of different CO_2 flow rates. It shows that the CO_2 flow rate reached its highest peak of extraction yield at 25g/min. This finding explains that, there was an effect of different CO_2 flow rate on extraction yield for MCV leaves. Author used this range of parameter from 15g/min to 35g/min based on several authors which had also extracted herbal plant [12–16]. The drastic declination from 25g/min to 30g/min was due to unfavorable CO_2 flow rate to MCV leaves which was supported by a reference which stated that, different sample compound (plant or part) might affect different optimum parameter to extract the selected compound [16]. This drastic declination occurred due high flow rate of CO_2 that was not suitable for MCV leaves extraction sample matrix. There was also study stated that, extraction on certain sample different from MCV leaves needed low CO_2 flow rate in order to allow the CO_2 pass through overall surface of sample matrix and extract the valuable compounds [17]. Furthermore, study of Cannabis extraction stated that, decreasing flow allowed for the material to be in contact with the solvent even longer eventually increasing the potential yield for specific compound [18].

Effect of Co-Solvent Percentage on Extraction Yield

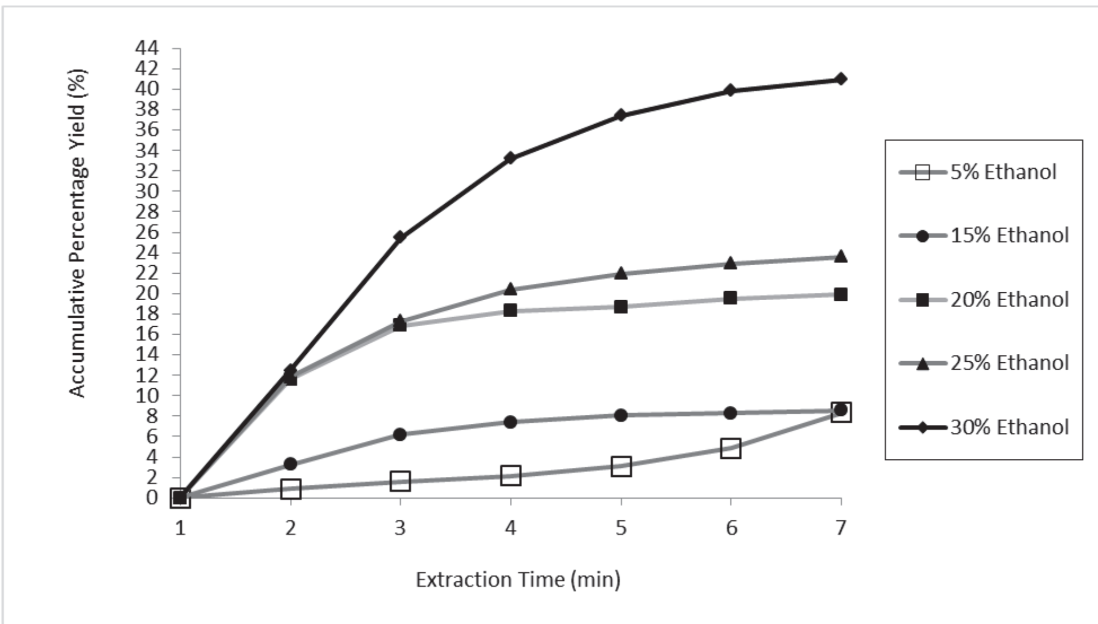

FIGURE 2. Accumulative Extraction Yield for Co-Solvent

For the next parameter which is co-solvent percentage, the result data extraction yield is presented in Figure 2. From the results, the co-solvent percentage really had an effect on the extraction yield. The co-solvent percentage increased eventually increasing the percentage extraction yield of MCV leaves. This result was supported by a reference which stated that, the addition of low levels of modifiers (sometimes called entrainer), such as methanol and ethanol, could also significantly increase solubility, particularly of more polar compounds and have probability to maximize the extraction [19]. This is because CO_2 can remove the lipid of sample matrix while the inner matrix which is phospholipids containing valuable compound or desired oil can be extracted with entrainer or co-solvent [19]. On the other hand, there was other study stated that the high percentage of co-solvent or modifier in SFE might increase the extraction yield but the extract might not contain the valuable desired compound [20]. In addition, the author claimed that in order to perform high quality and quantity yield of specific sample, the use of different modifier might be necessary [20].

Effect of Pressure Magnitude on Extraction Yield

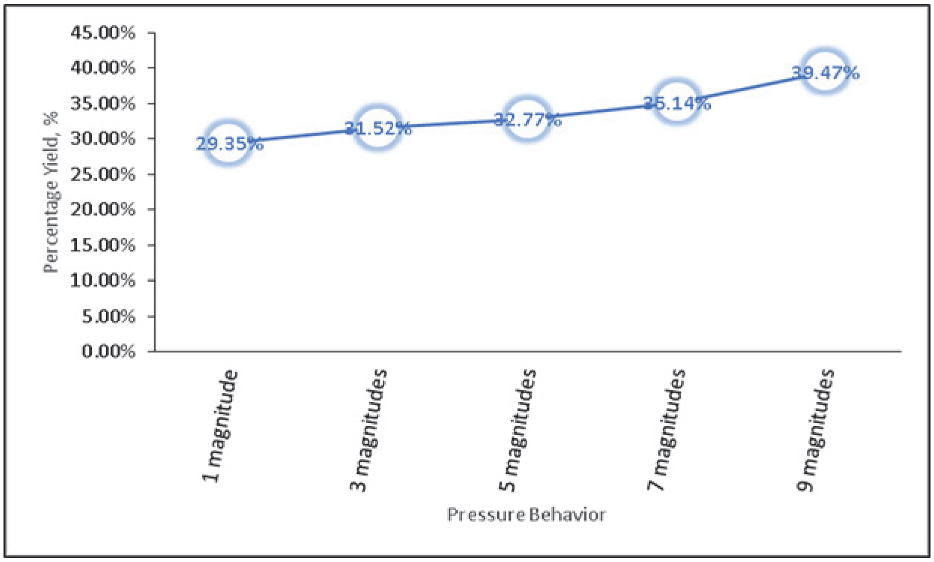

FIGURE 3. Extraction Yield Result versus Pressure Magnitude

Figure 3 results show that the extraction yield was affected by different pressure magnitudes. High pressure of CO_2 might enhance and increase the extraction yield according to single author [21]. Thus, increase pressure would increase the carbon dioxide density and could remove the outside lipid of the sample much easier [21]. Furthermore, from the co-solvent discussion before, the enhancement of modifier increased the extraction yield [19]. The relationship comment between these two authors can be further enlightened that, when the pressure increased at 375bar, the outside matrix of sample would be passed through and when the pressure reduced at 150bar, the modifier did its work to extract the valuable compound from the sample [19,21]. Repeating this system might improve the extraction yield and thus 9 magnitudes pressure gave higher yield in this study. On the other hand, there were others claimed that the higher pressure and higher temperature could cause solute and wax in the sample to be easily removed in order to allow the co-solvent to extract the sample compound [19,22]. Therefore, the high pressure of SFE may result in high penetration matrix to allow high extraction yield and low pressure of SFE with co-solvent present in the system can improve the extraction yield [19,21,22].

CONCLUSIONS

In the nutshell, the study objectives had been achieved. The effects of different CO_2 flow rate, co-solvent percentage and pressure behavior system had been studied. For the CO_2 flow rate, the results show that there were some peaks on different CO_2 flow rate. The optimum CO_2 flow rate in this range of parameters (15g/min – 35g/min) was 25g/min with 31.22% of extraction yield. For co-solvent or modifier effects, the optimum composition of co-solvent in this range of parameters (5% - 30%) was 30% with 40.93% of extraction yield. However, the high extraction yield might not have desired compound [20]. On the other hand, for the new finding, the pressure behavior system had an effect to the extraction yield percentage. Increasing the pressure magnitude might increase the extraction yield due to penetration forth and back to the system. The different pressure magnitude achieved its highest extraction yield of 39.47% at 9 magnitudes of pressure.

ACKNOWLEDGMENTS

The authors would like to recognize all of the Faculty of Chemical Engineering laboratory UiTM Penang Branch staffs for their assistance and wisdom on handling the SCFE equipment. This research has been funded by UiTM under the Internal Research Acculturation Grant Scheme (iRAGS) (600-RMI/DANA 5/3/IRAGS (43/2015)).

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