Supercritical CO_2 extraction of red butterfly wing leaves: process parametric study towards extraction yield

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Abstract. This research illustrates the parametric studies for determining the relationship between the operating parameters used in the supercritical fluid extraction (SFE) and the extraction yield of Red Butterfly Wing (RBW) leaves. In this study, SFE used carbon dioxide (CO_2) as the main solvent and ethanol as the co-solvent. SFE was operated by manipulating few parameters such as temperature, pressure and also particle size in order to determine the extraction yield. The RBW leaves were purchased from the local supplier and then undergone cleaning, drying, grinding as well as sieving processes. 5 grams of grinded leaves was then run through SFE machine. For the temperature, it was observed that the extraction yield increased starting from 40^{o}C to 50^{o}C and dropped after that. The optimum temperature was 50^{o}C with extraction yield of 2.94%. For the pressure, the trend of the extraction yield was directly proportional to the pressure. As the pressure increased, the extraction yield also increased. The optimum extraction yield was 2.45 % at 375 bar. Meanwhile, the particle size was inversely proportional to the extraction yield. The optimum extraction yield was 3.26 % at 63 μm.

1 Introduction

Red Butterfly wing (RBW) plant has gained a huge popularity lately. RBW plant is an ornamental plant Fabaceae, or known scientifically as *Mariposa Christia Vespertilionis* [1], others as red butterfly wing, island peak, mariposa (butterfly in Spanish) or rerama in Malaysia [2]. It is commonly known as “Mariposa” or “Red butterfly wing” because of the similarity of its leaves with the color and shape of a butterfly [3]. In a study by Upadhyay *et al*. [4], RBW plant traditionally can treat snake bites, tuberculosis, heal bone fracture, increase blood circulation bronchitis and cold. Other than that, the crushed leaves of RBW can be applied on body parts as it provides a cure for scabies [4]. Furthermore, an early study had been conducted to highlight the potential of *Mariposa Christia Vespertilionis (MCV)*, focusing on its pharmacology properties. The whole plant was extracted using cyclohexane and was reported to have an anti-plasmodial activity towards Plasmodium falciparum FCB1 with IC50 value of 10.8 μg/ml [5]. The method used by Upadhyay *et al*. [4] had been conducted on MCV as anti-plasmodial agent, in-vitro and in-vivo and bioassay-guided studies were applied to characterize the active compounds. In 2013, Hofer *et al.* [6] conducted a study to find out the effect of MCV on human medullary thyroid carcinoma and human intestinal neuroendocrine tumors. They found that ethyl acetate extracted from MCV plant had high inhibition of cancer cells whilst not affecting normal human fibroblasts. Moreover, the extracts caused a change of gene expression in both the carcinomas and tumors. From the results, it implied a great potential of using MCV against cancer.

SFE uses CO_2 as a medium for the extraction which is faster than the conventional method that resulted in no residual solvent in the final extract because CO_2 is a gas under ambient condition. The physical-chemical properties of supercritical CO_2 possesses a higher diffusivity and lower viscosity than conventional liquid solvents [7]. However, pure CO_2 does not have sufficient solvation power for polar sativoside and needs to add a polar co-solvent. Experimental co-solvents were water, methanol, ethanol, and mixtures of these solvents [8]. Extraction by conventional methods present some problems because the use of flammable or toxic solvents. At certain temperature and pressure condition, liquid and vapor phases of substance become indistinguishable or known as critical condition. The study will use the SFE to extract the RBW leaves. Throughout the study, the effect of manipulating the chosen operating parameters or variables such as temperature, pressure, and particle sample size will be conducted. From the manipulations, different extracted samples will be collected. Supercritical CO_2 offers many advantages over traditional solvents and it can achieve the same results without having the negative side effects of large waste streams. The problem arise when it comes to the yield of extracted sample. The extracted sample could be small in quantity and low in quality. For different methods, the quantity and the quality differed greatly.

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2 Material and methods

In this research, the chemical used was liquid CO_2 as the main solvent and ethanol as a co-solvent in SFE. The RBW leaves were purchased from EES Biotech Herbs Sdn. Bhd.

2.1 Methodology

The RBW leaves were purchased and taken to the Chemical Engineering Laboratory of UiTM Permatang Pauh, Penang for further process. Before drying process, the RBW leaves were cleaned and washed carefully. After washing the leaves, they were undergone drying process. In the oven, the leaves were dried at 35ºC constantly throughout the day. The drying process took 48 hours to ensure that the leaves dried completely. After 48 hours, the leaves and stems were separated. The leaves were grinded and sieved into five different sizes which were 2mm, 1mm, 500µm, 250µm, and 63µm. Following the grinding and sieving procedure, the RBW were run in the SFE machine. 5g of the sample was weighed by using analytical balance model ATY224. After that, the sample was covered in thin cloth and put into the vessel of SFE machine. The extraction process occur in the SFE machine. The extracting parameters such as temperature, pressure and particle size were manipulated at the SFE machine. The temperature used in this study were 40ºC, 45ºC, 50ºC, 55ºC and 60ºC. The pressure used were 275 bar, 300 bar, 325 bar, 350 bar, and 375 bar. The particle size used was the same as the grinding size used. The extraction time was within one hour with 10 minutes time interval. After that, rotary evaporator was used to get the real mass of the extracted sample by separating the ethanol from the extracted. In order to get the extraction yield, the equation below was used:

Yield, y (%) = [(Final weight – Initial weight) / Sample weight] x 100.

3 Results and discussion

3.1 Effect of temperature

The study of temperature effect on the extraction yield had been carried out in the range of 40ºC to 60ºC while the pressure and particle size were kept constant. The pressure and particle applied were 250 bar and 2 mm respectively. The details for the experimental results obtained are presented in Figure 1.

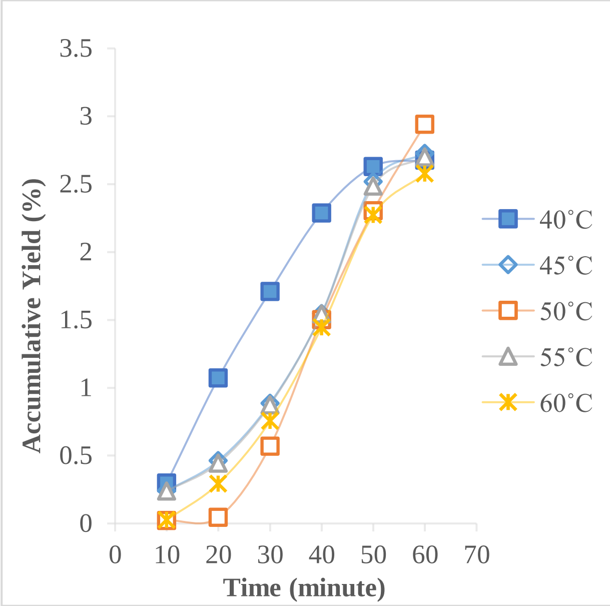

Figure 1. Graph for the extracted RBW by manipulating the temperature

From the results, it shows that when the temperature increases from 40ºC up to 50ºC, it results in the increment of extraction yield. Other studies also had shown similar observations for the extraction yield increment as the temperature increased [9, 10]. After 50ºC, the extraction yield is slightly decreases from 2.94 % at 50ºC to 2.70 % and 2.58 % at 55ºC and 60ºC respectively. The same results also had been made on the supercritical CO_2 extraction of *Hedyotis diffusa* [11]. Meanwhile, the study of temperature effect had also shown that at 50ºC the optimum extraction yield was 2.94 %. The temperature actually has two competing functions which are solute sublimation and supercritical CO_2 solvent density [12]. By increasing the temperature, the solute sublimation will be increased while the supercritical CO_2 density decreased. It will result in extracting more soluble. The enhancement of solute sublimation may be more dominant than the decrement in density which the extraction yield appears to increase from 40ºC to 50ºC. It is also reported that the extraction yield would be decreased when the temperature decreased at low pressure, but the extraction yield would be increased when the pressure increased at high temperature [13].

3.2 Effect of pressure

The effect of pressure on the extraction yield had been carried out within 60 minutes under five different pressure which the range were from 275 bar to 375 bar while keeping the temperature and particle size at constant which were 40ºC and 2 mm respectively. The extraction yields of RBW are presented in Figure 2.

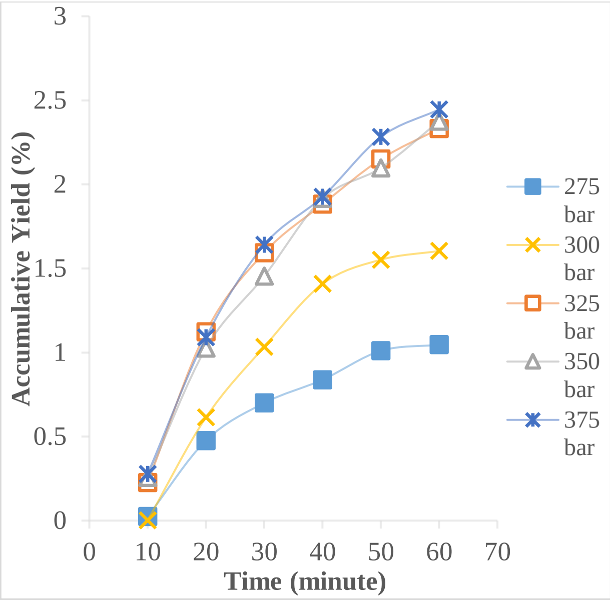

Figure 2. Graph for the extracted RBW by manipulating the pressure

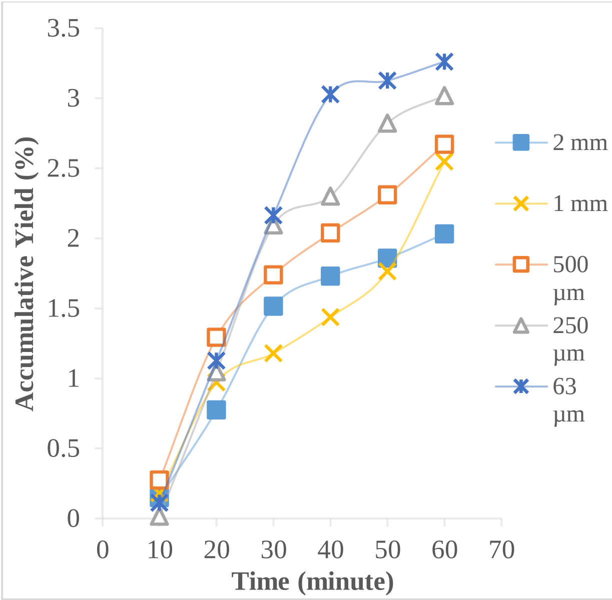

Figure 3. Graph for the extracted RBW by manipulating the particle size

The accumulative yield values obtained under the selected pressures, 275 bar, 300 bar, 325 bar, 350 bar, and 375 bar were 1.05 %, 1.60 %, 2.33 %, 2.38 %, and 2.45 % respectively. The highest total extraction yield was recorded at pressure 375 bar. It is shown from the results that the total extraction yield within 275 bar up to 375 bar had an increasing pattern. It indicates that when the pressure increases, the extraction yield will be increased too. This can be explained by the dependence of supercritical CO_2 density on pressure [14]. When the set pressure is increased, the supercritical CO_2 will bear high density resulting in its solvating power in order to solve the solute in the sample will be higher. The relationship between the density and solvating power will affect the extraction yield. Hence, the higher the solvating power due to high density will resulted in high extraction yield because more extract can be flushed out. Other studies also showed that the extraction yield increased as the pressure increased [14, 15, 16]. For an example, Lisichkov *et al*. [17] had observed the extraction of Cyprinus carpio L. using SFE which the yield increased as the pressure increased. Zhao & Zhang [13, 18] had also stated that the oil yield from both Moringa oleifera seeds and Eucalyptus leaves were significantly increased as the pressure increased. The results actually indicates that the pressure is highly dependent on the dissolving behavior of the extracted sample in supercritical CO_2, which will give more soluble extract to have a higher extraction yield.

3.3 Effect of particle size

To study the effect of particle size, five sizes had been chosen which were 2 mm, 1 mm, 500 µm, 250 µm, and 63µm. Other operating parameters such as temperature and pressure were kept constant at 50°C and 300 bar. The results are presented in Figure 3.

The accumulative extraction yield for samples with size of 2 mm, 1 mm, 500 µm, 250 µm, and 63 µm were 2.03 %, 2.55 %, 2.67 %, 3.02 %, and 3.26 % respectively. From the result obtained, it can be seen that a reduction in particle size had resulted in slightly extraction yield increment. The positive effect of a reduction particle size is that the internal mass transfer resistance in the RBW becomes lower and thus leading to the extraction yield increment [14]. As can be seen from Figure 3, the extraction yield steadily increases with the extraction time, and will slow down and then level off because of extracted component exhaustion. It is similar to the study of temperature and pressure variations. Smaller particle size can produce higher extraction yield compared to larger size because small size of particle can give more solute available at the particle surface [14]. In other words, the particle size actually has a direct impact or effect on the extraction yield because smaller particle can enhance the internal diffusion process.

4 Conclusion

In conclusion, the effect of SFE operating parameters such as temperature, pressure and particle size towards extraction yield had been observed. As the temperature increased with constant pressure and particle size, the total extraction yield of RBW sample would be increased and after 50°C the extraction yield was decreased from 2.94 % to 2.70 % and 2.58 % at 55°C and 60°C respectively. The optimum extraction yield was 2.94 % at 50°C. The effect of pressure showed that when the pressure increased while the temperature and particle size kept constant, the extraction yield would be increased. The optimum yield obtained was 2.45 % at pressure 375 bar. Meanwhile, the effect of particle size showed that when the particle size decreased while other parameters were kept constant, the total extraction yield would be increased. The optimum yield was 3.26 % recorded at particle size of 63μm.

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References

1. G.K. Dash, *An appraisal of Christia vespertilionis (L. F.) bakh. F.: A promising medicinal plant*, Int. J. of Pharmacognosie and Phytochem Research **8(6)**, 1037–1039 (2016)
2. H. Bunawan, S.N. Bunawan, S.N. Baharum, *The Red Butterfly Wing (Christia vespertilionis): A promising cancer cure in Malaysia*, Int. J. Pharm Sci. **7(8)**, (2015)
3. P.A. Whiting, *Commercial production of Christia Subcordata Moench by establishing cultural practices and by applying plant growth regulators* (A Master Thesis, Graduate Faculty of the University Of Georgia, Athens, Georgia, USA), (2007)
4. H.C. Upadhyay, B.S. Sisodia, H.S. Cheema, J. Agrawal, A. Pal, M.P. Darokar, S.K. Srivastava, *Novel anti-plasmodial agents from Christia vespertilionis*. Natural product communications **8**, 1591-1594 (2013)
5. J. Nguyen-Pouplin, H. Tran, T.A. Phan, C. Dolecek, J. Farrar, T.H. Tran, P. Caron, B. Bodo, P. Grellier, *Antimalarial and cytotoxic activities of ethno pharmacologically selected medicinal plants from South Vietnam*, J. Ethnopharmacol **109**, 417-427 (2007)
6. D. Hofer, G. Schwach, N. Ghaffari Tabrizi-Wizsy, A. Sadjak, S. Sturm, H. Stuppner, R. Pfragner, *Christia vespertilionis plant extracts as novel anti-proliferative agent against human neuroendocrine tumor cells*, Oncology reports **29**, 2219-2226 (2013)
7. A. Erkucuk, I. Akgun, O. Yesil-Celiktas, *Supercritical CO_2 extraction of glycosides from Stevia rebaudiana leaves: Identification and optimization*, J. Supercrit. Fluids **51**, 29-35 (2009)
8. J. Pól, E.V. Ostrá, P. Karásek, M. Roth, K. Benešová, P. Kotlaříková, J. Čáslavský, *Comparison of two different solvents employed for pressurized fluid extraction of stevioside from Stevia rebaudiana: methanol versus water*, J. ABC. **388**, 1847-1857 (2007)
9. Z. Huang, M-J. Yang, S-F. Liu, Q. Ma, *Supercritical carbon dioxide extraction of Baizhu: Experiments and modelling*. The J. of Supercritical Fluids **58**, 31-39 (2011)
10. Rai, A, Mohanty, B., and Bhargava, R. (2015). Modelling and response surface analysis of supercritical extraction of watermelon seed oil using carbon dioxide. Separation and Purification Technology, 141, 354-365.
11. M-C. Wei, Y.C. Yang, *Kinetic studies for ultrasound-assisted supercritical carbon dioxide extraction of triterpenic acids from healthy tea ingredient Hedyotis diffusa and Hedyotis corymbosa*, Separation and Purification Technology **142**, 316-325 (2015)
12. L.T. Taylor, *Supercritical fluid extraction vol. 4,* Wiley-Interscience (1996)
13. S. Zhao, D. Zhang, *Supercritical CO2 extraction of Eucalyptus leaves oil and comparison with Soxhlet extraction and hydro-distillation methods*, Separation and Purification Technology **133**, 443-451 (2014)
14. Z. Huang, M-J. Yang, Q. Ma, S-F. Liu, *Supercritical CO_2 extraction of Chinese lantern: Experimental and OEC modelling*. Separation and Purification Technology **159**, 23-34 (2016)
15. G. Wejnerowska, P. Heinrich, J. Gaca, *Separation of squalene and oil from Amaranthus seeds by supercritical carbon dioxide*, Separation and Purification Technology **110**, 39-43 (2013)
16. K. Tomita, S. Machmudah, R. Fukuzato, H. Kanda, A.T. Quitain, M. Sasaki, M. Goto, *Extraction of rice bran oil by supercritical carbon dioxide and solubility consideration*, Separation and Purification Technology **125**, 319-325 (2014)
17. K. Lisichkov, S. Kuvendziev, Z. Zeković, M. Marinkovski, *Influence of operating parameters on the supercritical carbon dioxide extraction of bioactive components from common carp (Cyprinus carpio L.) viscera*. Separation and Purification Technology **138**, 191-197 (2014)
18. S. Zhao, D. Zhang, *A parametric study of supercritical carbon dioxide extraction of oil from Moringa oleifera seeds using a response surface methodology*, Separation and Purification Technology **113**, 9-17 (2013)