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Effects of CO_2 Flow Rate in Supercritical Fluids Extraction of *Polygonum minus* Roots

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ABSTRACT

Polygonum minus (locally known as Kesum) roots has been reported to contain bioactive sesquirtepenes compound, which is β-caryophyllene. In this study, supercritical fluid extraction using carbon dioxide was employed to investigate the effects of CO_2 flow rate (2 ml/min, 3ml/min and 4 ml/min) towards essential oil yield (EO) and β-caryophyllene yields from P.minus roots. The extraction pressure, temperature and time were fixed at 80 bar, 40°C and 240 min, respectively. The results showed the highest amount EO obtained at flow rate 4 ml/min with 33.3% followed by flow rate 3 ml/min and 2 ml/min were 24% and 12.4% respectively. On the other hand, the highest amount β-caryophyllene was obtained at the lowest flow rate of 2 ml/min with 7.69% yield, followed by 3ml/min (1.62%) and 4 ml/min (1.54%). It can be explained that by increasing the CO_2 flow rate, it will increase the initial extraction rate and the overall extraction of EO. However, mass transfer resistance limits the amount of β-caryophyllene transported to the bulk solvents with increasing CO_2 flow rate. Thus, further study is needed to overcome the mass transfer limitation and improve the overall extraction of β-caryophyllene from P.minus roots.

Keywords: Supercritical fluids extraction (SFE); CO_2 Flow rate; Polygonum minus root; β-caryophyllene

INTRODUCTION

Polygonum minus (*P.minus*) is the scientific name of Kesum and it belongs to the *Polygonaceace* family. Traditionally, fresh leaves of *P.minus* have been used as flavouring for certain food due to its unique and strong aroma and taste. For medicinal purposes, it has been widely used to treat headache, digestive disorder and reduce dandruff problem (Zakaria and Mohd 2010). As this plant has high antioxidant activity, P.minus can be used as a therapeutic drug for gastric (Ahmad et al. 2014).

Generally, only the leaves and stem of the *P.minus* are used while the remaining roots would be re-planted or simply discarded as a waste. As reported by Azhari et al. 2020., Ahmad et al. 2014 and Ashraf et al. 2014, the roots of this plant can produce high quality sesquirtepene compound, which is the *β-caryophyllene*. The presence of *β-caryophyllene* which has great anti-fungal properties can mostly be found in various herbs and spices (Fernandes et al. 2007). It is also insecticidal (Sabulal et al. 2006) and has anti-cancer properties (Amiel et al. 2012). In a report by Koyama et al. 2019 based on their experiment on mice, this compound has inflammatory properties which can heal wounds by decreasing inflammatory effect.

Over the decades, several techniques of extraction have been used to extract essential oils and desired compounds from plants. These techniques can be categorized into two types which are the solvent extractions (maceration, soxhlet and sonication) and high-pressure extraction (supercritical fluid extraction (SFE), microwave assisted extraction (MAE), and pressurized solvent extraction (PSE)). When comparing both techniques, it was found that the solvent extraction technique uses a large amount of harmful solvent which could be harmful for long term consumption. Conversely, high-pressure extraction is more reliable in producing safer products for long term consumption (Ariff et al. 2018).

On the other hand, supercritical fluid extraction (SFE) is shown to be more economical and environmentally-friendly compared to other methods (Mohamed@Mahmood et al. 2018). This method can be classified as a clean technology by manipulating the operational condition depending on the specific component that needs to be extracted (Aris et al. 2019). This in turn can improve the quality of the extracted components and increase the extraction yields (Uquiche et al. 2015). Besides, using CO2 as solvent would cause minimal modification of the compound and higher quality of non-polar compounds can be preserved. In this research, SFE has been chosen as the extraction process for the *P.minus* roots, with CO2 as supercritical solvent. The aim of this experiment is to study the effect of CO2 flow rate on essential oil yield and the percentage of *β-caryophyllene* in *P.minus* roots extraction.

MATERIAL AND METHODOLOGY

SAMPLE PREPARATION

Fresh root of *Polygonum minus* were collected from Cameron Highland, Pahang, Malaysia. The fresh samples were cleaned and washed using distilled water. The samples were dried at air-drying for 6 days to removed moisture. The dried samples will cut into pieces before stored in a refrigerator at 4°C.

CHEMICAL AND STANDARD

The reference standard of (β-caryophyllene) was purchased from Fluka at purity 98.5%. Compressed CO_2 (diptube) with purity 99% was purchased from Alpha Gas Solution (Malaysia).

SUPERCRITICAL FLUID EXTRACTION

The extraction was carried by Supercritical fluid extraction in lab scale semi-batch and semi continuous SFE unit. The apparatus setup is schematically shown at figure 1. The CO_2 was chilled to -4°C using chiller to maintain its liquid state before pumped to extractor.

In this study, 0.3 g of dried *P.minus* roots was loaded in extraction vessel with parameter of pressure at 80 bar and temperature at 40°C. Various CO_2 flow rate (2ml/min, 3 ml/min, 4 ml/min) has been investigated for 4 hours of dynamic extraction or dynamic mode which the extractant flows continually through the sample. The extracted samples were collected every 30 minutes with 8 fractions within a time.

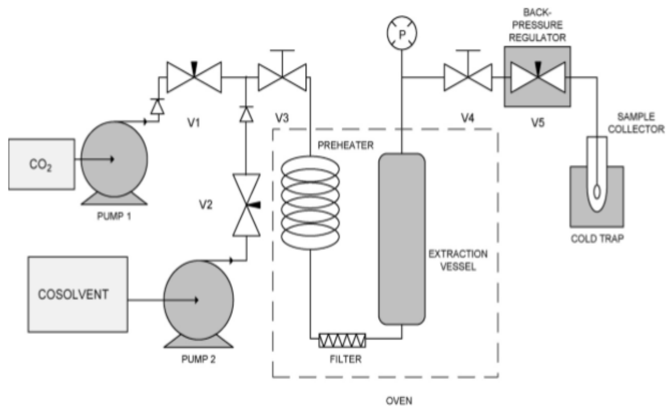


FIGURE 1. Schematic diagram of SFE system

EXTRACT YIELD DETERMINATION

The percentage of extract yield was calculated using Equation 1.

$$Yield\ Percentage\ (\%) = \frac{mass\ extract\ (g)}{mass\ of\ sample\ (g)} \times 100 \quad (1)$$

Determination of Solubility

Solubility of extraction in CO_2 has been determine from the graph of extraction yield (g extract) vs mass of CO_2 (g CO_2) using Equation. 2.

$$Solubility = \frac{g\ extract}{g\ CO2} \quad (2)$$

DETERMINATION OF *Β-CARYOPHYLLENE*

Gas Chromatography (GC) has been used to identify β-caryophyllene. The analysis was conducted using Agilent Technologies Model 5975C gas chromatograph with non-polar column HP-5 (30 m long and 0.25 mm diameter and had a film thickness of 0.25 μm. Helium, with a flow rate of 1.3 mL min-1, was used as the carrier gas. The splitless injection program was set for holding at 50°C for 3 min, increased to 250 C at 6°C per min and was then held at 250°C for 5 min. The GC result will compare with the standard of β-*caryophyllene.*

RESULTS AND DISCUSSION

EFFECT ON OVERALL EXTRACTION CURVE

The overall extractions curves of the essential oil yield with different CO2 flow rate in terms of the extraction time are shown in Figure 2. It can be seen that the extraction yield increased over time for all CO2 flow rates. However, at 210th until 240th minute, at the flow rate of 2 ml/min and 3 ml/min, the extraction curves started to become plateau. Meanwhile, at the flow rate of 4 ml/min, the extraction curve still shows an increasing trend over time. This finding showed that at lower flow rates, the extraction curve reached plateau phase faster than higher flow rates. At the flow rate of 4ml/min, more time was needed to complete the extraction and it this would affect the bioactive compounds in plants (Klein-Júnior et al. 2016).

However, the opposite findings were reported by Aris et al. 2019 who discovered that at higher flow rate, the extraction curve tended to reach plateau phase faster compared to the lower flow rate. Meanwhile, studies from Norodin et al. 2017 revealed that from their extraction experiment on Swietenia mahagoni seeds, different flow rates produced similar duration for the curves to reach the plateau phase to achieve higher extraction yields. Different findings from other studies showed that the type of plants and operating parameter would affect the extraction curve to reach the plateau phase.

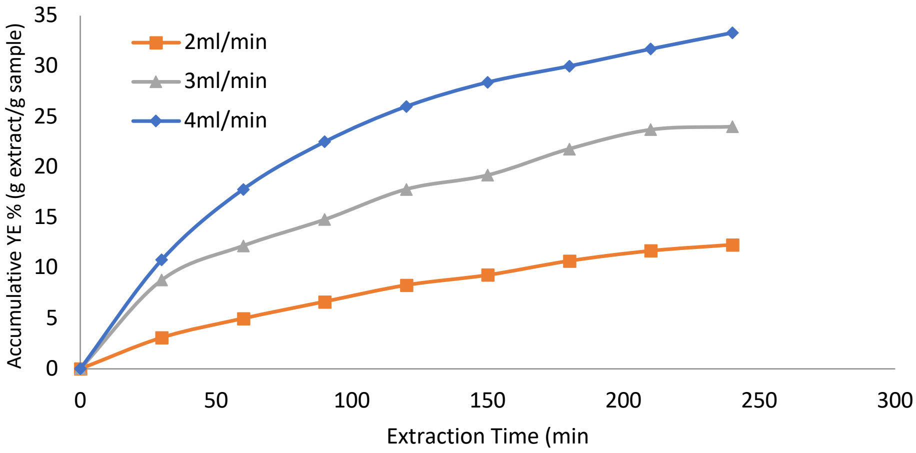


FIGURE 2. Overall extraction curve at different CO_2 flow rate on accumulative yield (%) with parameter: P = 80 bar and T = 40°C

EFFECTS ON ESSENTIAL OIL YIELD

The effects of different CO2 flow rate with essential oil yield obtained from supercritical fluid extraction (SFE) of *P.minus* roots at operating pressure and temperature of 80 bar and 40oC respectively are reported in Table 1. The results showed that the amount of essential oil yield increased with the increase of the CO2 flow rate. The highest essential oil yield was obtained at the flow rate of 4 ml/min with 33.3 % g extract/g sample, followed by 3 ml/min and 2 ml/min which 24% and 12.3% respectively.

One of the explanations for this result is that at low CO2 flow rate, the mass transfer resistance limits the amount of solute to transport as bulk of solvent (Aris et al. 2019). However, the mass transfer resistance decreases as the CO2 flow rate increases (Norodin et al. 2016 and Kumoro and Hassan., 2007). This is because the efficiency of extraction will increase with higher flow rate but the process of extraction would be disturbed. It is due to low loading of SC-CO2 and poor solute trapping.

The findings from these studies are similar with the report made by Icen et al. 2017, which investigated cardamom extraction by supercritical fluid extraction technique. It was found that the extraction yield tended to increase with the increase of the extraction flow rate. In the supercritical carbon dioxide extraction study of *Mariposa Christia Vespertilionis* leaves by Ariff et al. 2018 it has been reported that the increase of the CO2 flow rate increases the initial extraction rate.

TABLE 1. The effect of CO_2 flow rate on essential oil yield (%) at 80 bar and 40°C

CO_2 Flow rate (ml/min)	Extraction Yield (g extract / g sample)	Solubility (g extract/g CO_2)
2	12.3	0.1859
3	24	0.3200
4	33.3	0.3500

EFFECT ON SOLUBILITY STUDIES

The solubility (slope of the extraction curve) effects for different CO_2 flow rate have been shown at Figure 3. The results of solubility at Table 1 showed that with the increase in the CO_2 flow rate, the solubility of extraction yield in the essential oil increased. The highest amount solubility were obtained at flow rate 4 ml/min with 0.35 g extract/g CO_2, followed by 3ml/min and 2 ml/min which 0.32 g extract/g CO_2 and 0.1859 g extract/g CO_2 respectively. The results show that, at the flow rate of 4 ml/min, the increased fluid or solute contact were much stronger than at the flow rate of 2 ml/min. This subsequently increase the amount of extraction yield of the P.minus roots.

Observation from Figure 3 shows that, less amount of CO_2 were consumed to obtain extraction yield more than 10%, which it required 33.348 g for highest flow rate. Meanwhile, 50.022 g and 100.044 g of CO_2 have been consumed at flow rate 3 ml/min and 2 ml/min respectively. Aside from the flow rate, other parameters such as the pressure and temperature needs to be considered in the extraction process of the P.minus roots.

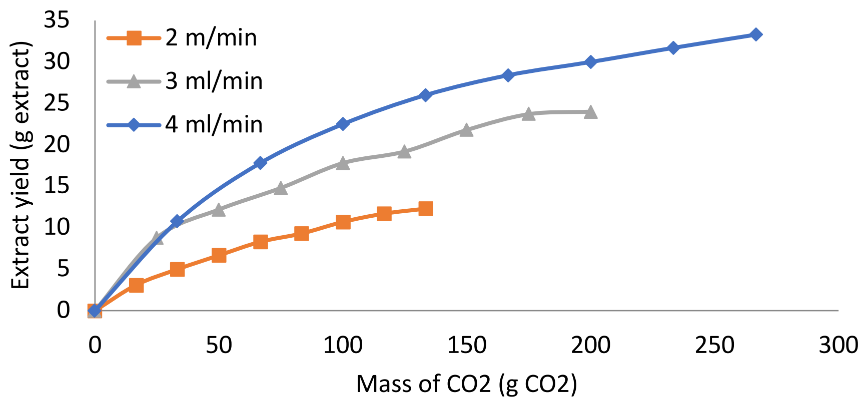
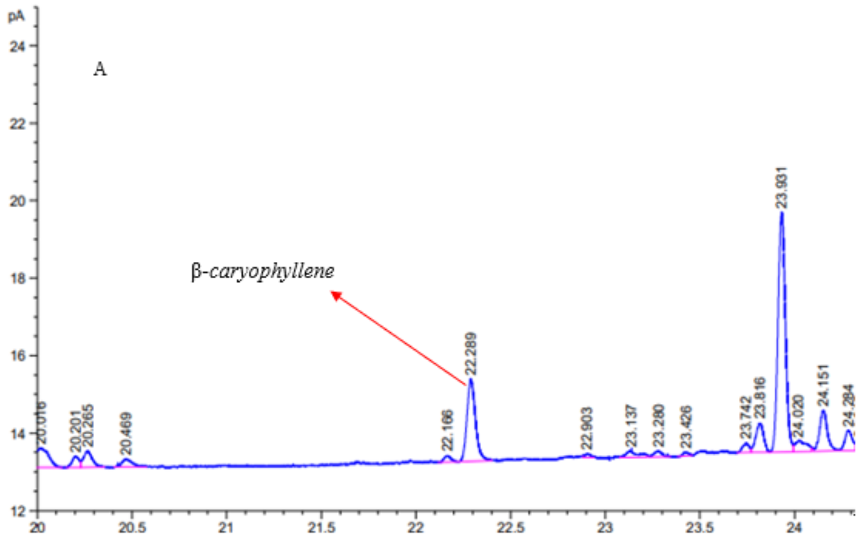


FIGURE 3. Graph of extract yield versus mass of CO_2 with different flow rate (at P= 80 bar and T = 40°C)

EFFECT ON β-caryophyllene IN *P.minus* ROOTS



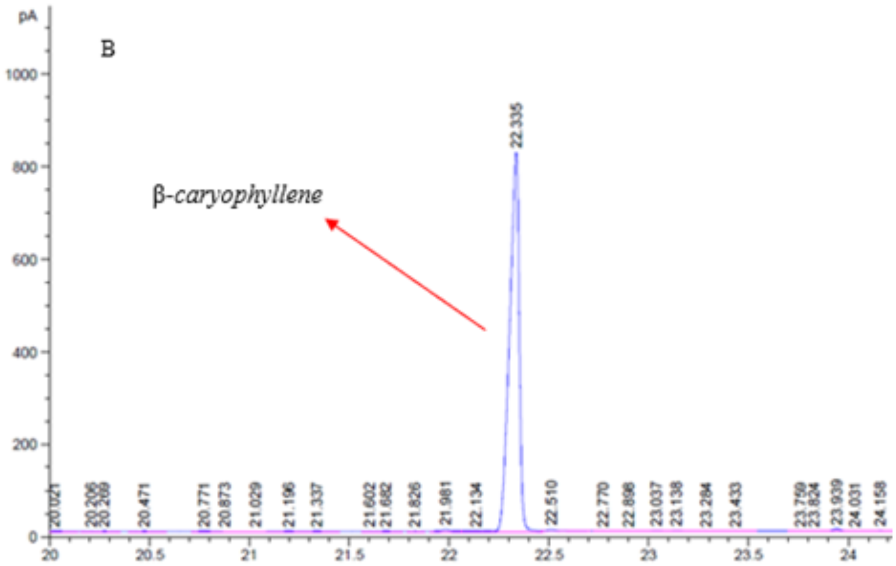


FIGURE 4.GC–MS chromatogram A. Sample at flow rate 2 ml/min, B. β-caryophyllene standard

The percentage of β-caryophyllene from the *P.minus* roots was confirmed by comparing the retention time with the standard as shown in Figure 4. The results of β-caryophyllene with different flow rates are reported in Table 2. The results showed that the most abundance β- caryophyllene can be obtained at the lowest flow rate, which was at 2 ml/min with 7.69% relative peak. This was followed by the flow rate of 3 ml/min and 4 ml/min which were at 1.62% and 1.54% respectively.

Similar finding has been reported by Lu et al. 2005, extraction from Origanum vulgare L. ssp. virens (Hoffm. et Link) letswaart, in their experiment, the result showed that the volatile compounds tended to decrease when the flow rate was increased. The statement was also supported by Chen et al. 2018, in which the extraction of volatile compounds from Finnish wild mushrooms (Craterellus tubaeformis) were the highest at the lowest CO_2 flow rate.

TABLE 2. Relative Peak Area (%) of *β-caryophyllene* at *P.minus* roots with different flow rate. SFE conditions (P= 80 bar and T= 40°C)

CO_2 Flow Rate (ml/min)	Relative Peak Area (%)
2 ml/min	7.69
3 ml/min	1.62
4 ml/min	1.54

CONCLUSION

The effects of CO2 flow rates in the supercritical fluid extraction (SFE) technique on *the P. Minus* roots essential oil extraction and β-caryophyllene percentage have been studied. The findings showed that by increasing the CO2 flow rate, the initial extraction rate and the overall extraction of essential oil increased. However, the percentage of β-caryophyllene from the *P.minus* roots extraction was found to be decreasing. Although the results showed that the flow rate of 2 ml/min produced higher abundance of β-caryophyllene,further studies must also consider other parameters that could affect the yield of *P.Minus* essential oil.

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DECLARATION OF COMPETING INTEREST

None

REFERENCES

Ahmad, R., Baharum, S.N., Bunawaan, H., Lee, M., Noor, N. M., Rohani, E. R., Ilias, N. & Zin, N. M. 2014. Volatile Profiling of Aromatic Traditionaal Medicinal Plant, *Polygonum minus* in Different Tissues and Its Biological Activities. *Molecules*: 19,19220-19242.

Amiel, E., Ofir, R., Dudai, N., Soloway, E., Rabinsky, T. & Rachmilevitch, Sh. 2012. β-Caryophyllene, a compound isolated from the biblical balm of gilead (Commiphora gileadensis), is a selective apoptosis inducer for tumor cell lines. *Evidence-Based Complementary and Alternative Medicine*. doi:10.1155/2012/872394.

Ariff, M.A.M., Yusri.A.M., Razak. N.A.A. & Jaapar. J. 2018. Effect of CO2 flow rate, co-solvent and pressure behavior to yield by supercritical CO2 extraction of Mariposa Christia Vespertilionis leaves. *American Institute of Physic., AIP Conference Proceding*. 2045, 020072 (2018)

Ashraf, M. F., Zain, C. R. C. M., Zainal, Z., Noor, N. M., Anuar, N., Markom, M. & Ismail, I. 2014. Establishment of Persicaria minor hairy roots and analysis of secreted β-caryophyllenein medium broth. *Plant cell Organ Tiss Organ Cult*: DOI 10.1007/s11240-014-0674-2.

Azhari, N. A. M., Markom, M., Ismail, I. & Anuar, N. 2020. Effects of different drying methods on essential oil yield and component profile of *Polygonum minus* root extract. *International Food Research Journal* 27(1): 96 - 102 (February 2020)

Aris, N.A., Idrus, M.N.F., Lee, N.Y., Idham, Z., Ruslan, H.M.S. & Yunus, C.M.A. 2019.The Effect of Fluid Flow Rate and Extraction Time in Supercritical Carbon Dioxide. *Journal of Advanced Research in Applied Sciences and Engineering Technology* 16, Issue 1 (2019) 26-34.

Fernandes, E. S., Passos, G. F. & Medeiros, R. 2007.Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of Cordia verbenacea. *Eur J Pharmacol*: 569(3): 228–236.doi:10.1016/j.ejphar. 2007.04.059.

Icen, H., Tugba, H., Serhat, M. & Guru,M. 2017. Obtaining of β-Sitosterol from *Cardamom* by Supercritical CO2 Extraction. *Preprints* (www.preprints.org) doi:10.20944/preprints201711.0005.v1.

Klein-Junior, Luiz Carlos, Yvan Vander Heyden, & Amelia Teresinha Henriques. 2016."Enlarging the bottleneck in the analysis of alkaloids: A review on sample preparation in herbal matrices." *TrAC Trends in Analytical Chemistry* 80 (2016): 66-82.

Koyama, S., Purk, A., Kaur, M., Soini, H.A., Novotny, M.V, & Davis K. 2019 Beta caryophyllene enhances wound healing through multiple routes. *PLoS ONE* 14(12):e0216104 https://doi.org /10.1371/ journal.pone.0216104

Kumoro, A. C & Hassan. M,. 2007. Supercritical Carbon Dioxide Extraction of Andrographolide from Andrographis paniculata: Effect of the Solvent Flow rate, Pressure, and Temperature. *Chinese Journal of Chemical Engineering*. Volume 15, issues 6, Pages 877-883.

Lu, T., Gaspar, F, & Leeke, G. 2005. Effect of Flow Rate on the Extraction of Volatile Concentrates and Resinoid Compounds from Origanum vulgare L. ssp. virens (Hoffm. et Link) letswaart using Compressed CO2 , *Journal of Essential Oil Research*, 17:1, 89-93, DOI: 10.1080/ 10412905.2005.9698840

Mohamed@Mahmood.M.,Daud.W.W.R., Markom.M & Mansor. C. C.N.A.N. 2018. Cosolvent Selection for Supercritical Fluid Extraction (SFE) of Bioactive Compounds from Orthosiphon stamineus. *Sains Malaysiana* 47(8)(2018): 1741–1747

Norodin, N.S.M., Salleh, M.L., Hartati, & Mustafa.M.N. 2016. Supercritical carbon dioxide (SC-CO2) extraction of essential oil from Swietenia mahagoni seeds. *IOP Conference Series: Materials Science and Engineering*. 162 012030.

Sabulal, B., Dan, M., John, A., Kurup, R., Pradeep, N. S., Valsamma, R. K. & George, V. 2006. Caryophyllene-rich rhizome oil of Zingiber nimmonii from South India: chemical characterization and antimicrobial activity. *Phytochemistry*.67 (22):2469–2473.

Uquiche, E,.Cirano. N. & Millao. S. 2015. Supercritical fluid extraction of essential oil from Leptocarpha rivularis using CO_2. *Industrial Crops and Products*. 77 (2015) 307 – 314.

Zakaria, M. and Mohd. M. A. 2010. Traditional Malay medicinal plants. *Institut Terjemahan Negara (ITNM). Malaysia*. Kuala Lumpur, Malaysia.