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RESEARCH ARTICLE

Comparison of charantin extract from *Momordica charantia* using modified supercritical carbon dioxide and soxhlet extraction method

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Abstract

Charantin extract from *Momordica charantia* fruits for the anti-diabetic compound was proposed via Supercritical Fluid Extraction using carbon dioxide (SC-CO_2) as a solvent. This method is a promising alternative technique to a conventional Soxhlet and shaking water bath extraction method. Experiments were conducted to analyze the charantin i) with and without a modifier (ethanol), ii) Soxhlet extraction with four types of solvent (distilled water, ethanol, dichloromethane, and petroleum ether) and iii) shaking water bath with distilled water as a solvent. A purification step was conducted post extraction to remove intrusive components such as chlorophylls and sugar from the analyte in order to quantify charantin extract using high-performance liquid chromatography (HPLC). The results show that in 2.5 hours SC-CO_2 with ethanol as modifier gives highest charantin yield with 0.7817 mg charantin per gram sample. In contrast, shaking water bath gives the highest charantin yield for a conventional method with 0.712 mg charantin per gram sample during 6 hours of extraction. The result shows that by using SC-CO_2 modified with ethanol can be a promising "green" extraction than a conventional method.

***Keywords*:** Supercritical carbon dioxide extraction, Soxhlet and shaking water bath extraction, charantin, *momordica charantia*, supercritical fluid extraction

INTRODUCTION

For decades, plants have been inevitably powerful sources in providing a vast amount of compounds such as nitrogen, vitamins, phenolic, followed by metabolites that are rich in bioactivities like antioxidant, anti-carcinogenic, anti-bacterial, and anti-inflammatory (Jain and De, 2016). However, this study can be a very thought-provoking subject for the scientist to explore further. Hence, plants have become a major concern among researches in identifying and developing the natural colorants, biodiesel, new drugs and so forth with no or less side effect. According to Horax *et al.* (2010), phenolic extracts from various plants have antioxidant activities. With this intention, the demand and requirement of plant studies are increases among natural product researchers.

Bitter gourd also known as *Momordica charantia* in botanical name belongs to the Cucubitaceae family was selected in this research. Unripe and immature fruits are light green, firm, oblong with white flesh and are the ones harvested for consumption (Raina *et al.*, 2016). Usually, bitter gourd is 4-6 inch long, 100-300 g in weight and the fruit is extremely bitter (Lucas. *et al.*, 2010). Fig. 1 shows unripe bitter gourd used in this experiment. The main constituents of bitter gourd which are responsible for these effects like antidiabetic (Grover and Yadav, 2004; Ojewole *et al.*, 2005), antitumorous (S. Wang *et al.*, 2016), anticancer (Raman and Lau, 1996), anti-flamatory (Lu *et al.*, 2012) are such as alkaloid, proteid, inorganic, lipid, tritepene, steroid and phenolic compound (Budrat and Shotipruk, 2008). As reported by Lucas. *et al.* (2010), bitter gourd contains protein has an ability for fighting against HIV. In addition, a steroid compound like charantin has been proven for its antidiabetic activity (Nerurkar *et al.*, 2008).

Fig. 1 Unripe *Momordica charantia* in January 2017 at the CLEAR laboratory.

The focus material in this research is charantin, which originally formed from a mixture of two compounds,-namely, sitosteryl glucoside

and stigmasteryl glucoside. As mentioned by H. Y. Wang *et al.* (2014), charantin is one of its major bioactive compounds in bitter gourd and is believed to have an insulin-like chemical structure and properties. It consists of a steroidal portion or aglycone, which is highly soluble in a non-polar solvent such as chloroform and dichloromethane. However, the presence of glucoside that attached to its molecule makes it slightly soluble in polar organic solvent such as methanol and ethanol (Pitipanapong *et al.*, 2007).

Recently, a favourable conventional method such as Soxhlet and shaking water bath extraction has been investigated for the extraction of several bioactive compounds from natural materials (Pitipanapong *et al.*, 2007). Nevertheless, these conventional methods required long extraction time, dangerous handling due to exposure of toxic solvent, decreasing demand for the oils extracted with toxic organic solvents and also associated with health problems (Azmir *et al.*, 2013; Belayneh *et al.*, 2017). Due to long extraction time, Soxhlet extraction would require a large volume of solvent as well as loss of flavonoids due to oxidation, hydrolysis and ionisation during extraction.

As an alternative green technology for lipid extraction, SC-CO_2 is considered in this research. Fig. 2 shows the SC-CO_2 instrument at Centre of Lipids Engineering and Applied Research (CLEAR), Universiti Teknologi Malaysia (UTM). SC-CO_2 extraction has many advantages such as less solvent used, shorter time (Shan *et al.*, 2012), better selectivity and efficiency (Arsad *et al.*, 2016; Mohd-Setapar *et al.*, 2014). As carbon dioxide is a non-polar solvent, SC-CO_2 has limitations in the extraction of polar and moderately non-polar compounds. In order to overcome this limitation, polar modifier or co-solvents is introduced in the SC-CO_2 system to aid extraction of polar analytes (Ibrahim and Sarbatly, 2014). In this case, ethanol is used as co-solvent because of its good miscibily with CO_2 and non-toxicity. This co-solvent is necessary in order to adjust the solvent power or the selectivity of CO_2 for a better result in polar lipid components extraction.

Therefore, the objectives of this study are to distinguish the effect of co-solvent on extraction yield of SC-CO_2 and to contrast the extraction method between SC-CO_2 and conventional extraction.

Fig. 2 Supercritical Carbon Dioxide (SC-CO_2) Instrument in September 2017 at CLEAR laboratory.

EXPERIMENTAL

Moisture content determination

In SC-CO_2 of the natural product, fresh plant material are required if the desired bioactive compound is volatile or labile. High moisture content in a fresh sample can cause mechanical difficulties such as restrictor clogging due to ice formation (Lang and Wai, 2001). Therefore, moisture content in a fresh sample of *Momordica charantia* (bitter gourd) needs to be controlled.

Unripe bitter gourd was purchased from a local market. 10 grams of small pieces bitter gourd sample was weighed and placed in the crucible and dried in the oven at 135 ± 2 °C for three hours. The crucible then was closed with its lid and immediately placed in desiccators for a cooling process. The crucible was weighed until constant weight was achieved. The wet basis moisture content is calculated as a percentage by mass of the samples using Eq. (1)

$$\text{Moisture Content (\%)} = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100 \quad (1)$$

Where m_0 = mass of crucible (g)
m_1 = mass of crucible with sample before drying (g)
m_2 = mass of crucible with sample after drying (g)

From equation 1, moisture content in bitter gourd obtained was 93.03%. Due to high moisture content, this sample needs to undergo drying phase before it can be extracted using SC-CO_2. Therefore, the whole sample was oven-dried at temperature 50 °C and 24 hours until it reduces moisture content less than 10%.

Sample preparation and chemicals

The fruits were cut into small pieces after rinsed with distilled water and then oven dried at 50 °C for 24 hours before grinding. The dried bitter gourd was completely crushed using a commercial blender (Philips). The ground sample was sieved (Retshch, Germany) and particle sizes chosen was ± 300 µm. The grounded sample was stored and keep in the freezer (-20°C) (Model Liebherr) until used.

Chemicals used were ethanol, dichloromethane and petroleum ether that purchased from Merck, Malaysia and the charantin standard were purchased from ChromaDex.

Supercritical carbon dioxide (SC-CO_2) extraction

A SC-CO_2 unit was designed as shown in Figure 3, which consists of force ventillation oven fitted with a 50 mL stainless steel extraction vessel (MMM Group, German) and the pressure in the vessel was regulated by means of a back-pressure (Tescom Corp., US) valve installed in the line between the extraction vessel and the separator. Water circulation bath (Daihan Scientific. Co. Ltd, Korea) helps to depressurize using the valve that converted the supercritical state of CO_2 into a gaseous state which then separated CO_2 from the extracted oil. A constant flow rate carbon dioxide gas (Mega Mount Industrial Gases Sdn Bhd, Malaysia) at 4 mL/min and 99.99% purity was liquidized using a refrigerated bath circulator (Daihan Scientific. Co Ltd, Korea) and pumped to an extractor using carbon dioxide liquid pump (Tokyo, Japan).

The supercritical extraction was performed with and without co-solvent, ethanol using 5 g of the ground bitter gourd at 20 MPa and 65 °C for 150 minutes with 25 minutes interval to collect the extract yield. The samples were loaded into the extraction cell and tightly sealed and then placed in the extraction chamber to let the system reach the desired condition. The extraction has begun after the system had reached the desired condition. CO_2 gas was depressurized in order to remove from the separator. The amount of extract oil yield obtained from SC-CO_2 was calculated using Eq. 2 and then it is stored in a freezer (-20 °C) for charantin analysis.

$$\text{Percentage yield (\%)} = \frac{Extract\ yield\,(g)}{Initial\ sample\,(g)} \times 100 \quad (2)$$

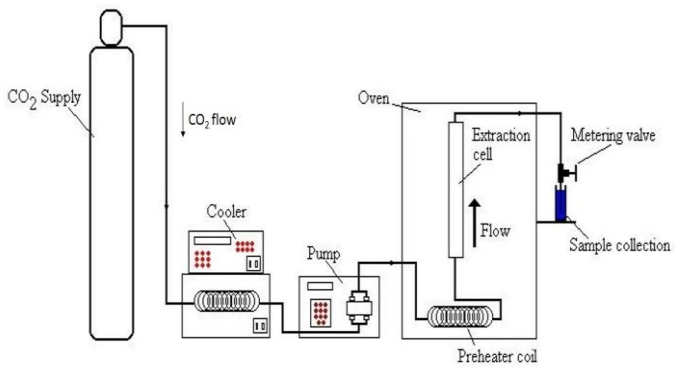

Fig. 3 Schematic diagram for supercritical carbon dioxide extraction.

Water extraction

Water extraction was performed using shaking water bath instrument (NE5-28D Series Clifton, Nickel-Electro Limited, United

Kingdom, UK) at Centre of Lipids Engineering and Applied Research (CLEAR). The extraction was conducted at 80 °C with 6 hours extraction time. Water extract then evaporated using an oven at 60°C and the remaining oil left was kept and stored in a freezer (-20 °C) for charantin analysis.

Soxhlet extraction

Soxhlet extraction was carried out using a Soxhlet apparatus. A 20 g of grounded bitter gourd was added into 4 round bottom flask with 200 mL of different solvent each. The Soxhlet extraction takes about 6 hours to complete. The solvent used was distilled water, ethanol, dichloromethane, and petroleum ether (Merck, Malaysia) with boiling point 100 °C, 78.37 °C, 39.6 °C and 42-62 °C at 1 atm respectively. The next step is, solvents were removed via rotary evaporator (Heidolph, German) at a temperature slightly above the boiling point of the solvent and remaining oil yield then were stored in the freezer (-20 °C) for charantin analysis.

Sample purification

Extract oil was purified according to the previous study with some modification (Pitipanapong *et al.*, 2007). Concisely, 2 mL of methanol-water with ratio 1:1 was added to 0.2 g of extract oil. The mixture was sonicated for 15 minutes and centrifuged at 3500 rpm for another 15 min to separate the supernatant from the precipitate. The precipitate was added with 1 mL of methanol and repeated with sonication and centrifuge steps. The purified solution was filtered through a 0.45 µm nylon membrane filter (Millipore, US) before analyzed using High-Performance Liquid Chromatography (HPLC). HPLC analysis was performed as the previous study as well by Pitipanapong *et al.* (2007) with C-18 Inertsil ODS-3 column (5 µm particle, 4.6 mm x 250 mm ID). The mobile phase was 100:2 (v/v) methanol-water and was delivered at a flow rate of 1 mL/min. The UV detection wavelength was 204 nm and the sample injection volume was 200 µL.

RESULTS AND DISCUSSION

Effect of co-solvent on the SC-CO_2 extraction yield

Figure 4 presents the percentage yield of SC-CO_2 at 20 MPa and 65°C with and without ethanol as co-solvent. It can be seen that the extraction of charantin with co-solvent ethanol gives the highest percentage yield with 1.924% compared to the absence of ethanol, 1.294%. This phenomenon can be explained in terms of diffusivity and target compound extracted. The addition of ethanol as co-solvent was found to be favourable for the extraction in SC-CO_2 at 20 MPa and 65 °C. According to Machmudah *et al.* (2006), the ethanol addition will enhance the solvent power in SC-CO_2 and caused swelling of the matrix, thus increasing the internal volume and the surface area for the contact with SC-CO_2. The addition of ethanol in SC-CO_2 can penetrate into the porous solid materials more efficiently. Therefore, it may render much faster mass transfer resulting in a more rapid and efficient extraction (M. A. C. Yunus *et al.*, 2007).

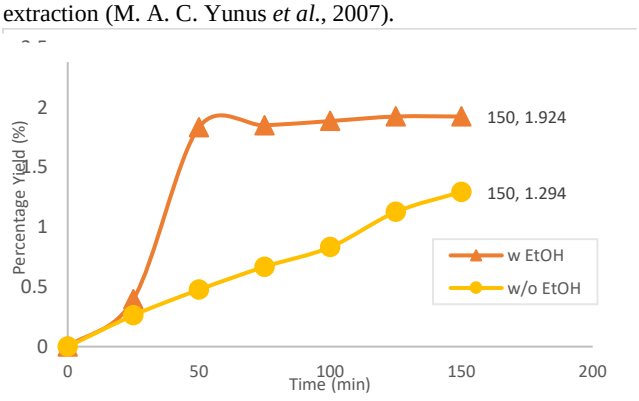

Fig. 4 Percentage oil yield at 20 MPa & 65 °C with ethanol and without ethanol.

Additionally, the polarity of the target compound may affect the extraction yield as it depends on the polarity of the solvent. Extraction using pure SC-CO_2 (without ethanol) gives the lowest percentage yield compared to extraction with ethanol as co-solvent. As pure carbon dioxide is non-polar solvent and the presence of ethanol increase the polarity of the solvent, this obvious gap explains that most of the target compound in bitter gourd were polar compound. Since the polar lipid dissolves and soluble in the polar solvent, it can be said that extract yield of bitter gourd is a polar lipid. Same results trend were reported by Machmudah *et al.* (2006) where the presence of 1.67% ethanol using SC-CO_2 extraction was found to enhanced the amount of astaxanthin extract from 10 to 20.5% at higher pressure (40MPa) while at low pressure (20MPa) the extract was from 0.6 to 6.6%.

Comparison of extraction yield between SC-CO_2 and conventional method

The extraction of bitter gourd oil in this research was done by using three extraction methods namely SC-CO_2, shaking water bath and Soxhlet extraction. The results obtained for the extract oil yield were compared between these three methods.

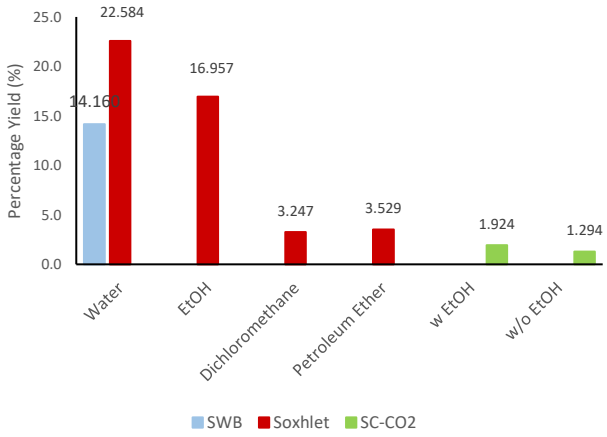

Fig. 5 Percentage yield of *Momordica charantia* in shaking water bath extraction, Soxhlet extraction with a different solvent (ethanol, dichloromethane, petroleum ether) and SC-CO_2 extraction (with and without ethanol).

Figure 5 shows percentage yield of bitter gourd in shaking water bath extraction; water as solvent, Soxhlet extraction with solvent water (b.p 100 °C), ethanol (b.p 78.3 °C), dichloromethane (b.p 39.6 °C) and petroleum ether (b.p 60-80 °C) and SC-CO_2 extraction with pure carbon dioxide and addition of co-solvent ethanol.

Based on Figure 5, it can obviously be seen that using water as a solvent in shaking water bath and Soxhlet extraction gives the highest percentage yield among others solvent and SC-CO_2 method with 14.16% and 22.58% respectively. It clearly shows that most compounds in bitter gourd were a slightly polar molecule. According to Arsad *et al.* (2016), Soxhlet extraction traditionally used in determining the total oil and also capable to extract a group of resin and waxes, so the oil yield of Soxhlet extraction may be more than other methods. This trend also applies to shake water bath extraction. On the other hands, Soxhlet with ethanol, dichloromethane and petroleum ether solvents give 16.96%, 3.25% and 3.53% respectively meanwhile SC-CO_2 with and without the addition of ethanol gives 3.70% and 1.29% respectively. From the result obtained between Soxhlet and SC-CO_2 extraction, it shows a comparison of percentage yield between the polar solvent (water and ethanol in Soxhlet; SC-CO_2 with ethanol) and non-polar solvent (dichloromethane and petroleum ether in Soxhlet; pure SC-CO_2) where both polar and non-polar solvent in the Soxhlet method resulted in higher percentage of extract yield compared to SC-CO_2 extraction. The same result's trend was recorded in the previous research by M. Yunus (2007) where SC-CO_2 gives lower extracted oil yield than Soxhlet in *Pitchecellobium Jiringan* (Jack) prain seeds extraction. In addition, Soxhlet extraction was capable to extract the groups of oleoresin and waxy materials in the outer part of the ground raw materials (Arsad *et al.*, 2016; Lee *et al.*, 2013).

On the other hand, Soxhlet extraction with water as a solvent also comparable to shaking water bath extraction as shaking water bath extraction involves most of the Soxhlet disadvantages but none of its advantages. Although both of these methods used a high volume of solvent, Soxhlet extraction gives high extraction efficiency compared to shaking water bath as it does not require filteration after the extraction step. Even though the extraction using a shaking water bath with some modification to improve the overall efficiency, it hardly surpasses that Soxhlet extraction (De Castro and Garcıa-Ayuso, 1998).

Comparison of Charantin Yield between SC-CO_2 and Conventional Method

Figure 6 shows the comparison of charantin contains in bitter gourd extract from SC-CO_2 and conventional (shaking water bath and Soxhlet) method. It is clearly shown that charantin obtained from SC-CO_2 with ethanol is the highest compared to the other two conventional methods. In addition, dichloromethane gives higher charantin contain in Soxhlet extraction compared to the other solvent.

Based on this comparison, it shows that the SC-CO_2 extraction is better than the conventional method in order to obtain the higher value of charantin contain in extract yield. Even though SC-CO_2 gives lower extract yield compared to the conventional one, it is proven that SC-CO_2 is high selectivity as the extract yield contain rich charantin. According to M. C. Yunus *et al.* (2013), SC-CO_2, have the ability to modify their selectivity by modifying the fluid density (varying the temperature and pressure).

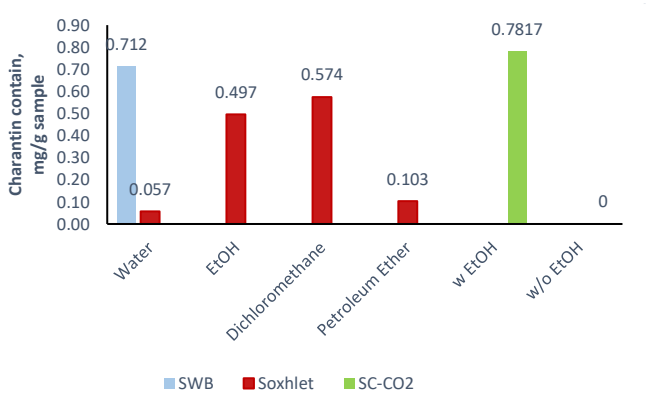

Fig. 6 Amount of charantin contain in *Momordica charantia* extract in shaking water bath, Soxhlet extraction with a different solvent (ethanol, dichloromethane, petroleum ether) and SC-CO_2 extraction (with and without ethanol).

Although charantin contain in shaking water bath extraction gives slightly lower than extraction using SC-CO_2 with ethanol, it is not practical as the extraction using shaking water bath consumes a longer extraction time which is in this case 6 hours compared to SC-CO_2, 150 minutes. In addition, consuming long extraction time required a lot of energy during the extraction process, thus the process is not green to the environment.

CONCLUSION

Generally, there have many methods to extract charantin from *Momordica charantia* (bitter gourd). In this research, the SC-CO_2 which is known as green technology extraction was used to extract charantin from bitter gourd. Moreover, a conventional method such as shaking water bath and Soxhlet extraction were also used in this experiment. Comparison of extraction and charantin yield were investigated in this research. The highest extraction yield (22.584%) is obtained by using Soxhlet extraction with water as solvent meanwhile lowest yield (1.294%) is obtained by using pure SC-CO_2. Despite the extraction yield obtained from SC-CO_2 was lower than Soxhlet and shaking water bath extraction, with the presence of modifier ethanol, the SC-CO_2 extraction method was more selective compared to conventional extraction as the charantin contain using SC-CO_2 method with modifier ethanol (0.7817 mg charantin/g sample) was higher than that obtained by conventional extraction method. Therefore, SC-CO_2 modified with ethanol can be a promising "green" extraction method than the conventional extraction method.

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