Supercritical fluid extraction of bioactive flavonoid from *Strobilanthes crispus* (pecah kaca) and its comparison with solvent extraction

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Abstract: Supercritical carbon dioxide extraction (SC-CO_2) of bioactive flavonoid from Strobilanthes crispus (Pecah Kaca) was performed to study the effects of various parameters such as pressure, temperature and dynamic extraction time on the yield and composition of bioactive flavonoid. The results were also compared with those obtained by conventional Soxhlet extraction in lab conditions. The results from SFE showed that the effect of extraction variables on extraction yields decreased in the following order: pressure, temperature and dynamic extraction time. The extraction pressure played a dominant role in the yield of the sample while the effect of time could be ignored. This study also revealed that both Soxhlet extraction and SC-CO_2 extraction can be used to obtain flavonoid compound. Under the optimum conditions, the highest bioactive flavonoid compound content was obtained at 3.98% and eight flavonoid compounds were identified by HPLC.

Keywords: Component, supercritical fluid extraction (SFE), bioactive flavonoid, solvent extraction

Introduction

Flavonoids are a group of polyphenolic compounds (Figure 1) which found in various sources of fruits vegetables and plants. Flavonoids are ubiquitous in vascular plants, and apparently more than 4000 of these compounds have been identified (Harbone *et al*., 1974). The benefit of flavonoid can be seen in their capability to act as an antioxidant. Pecah Kaca (*Strobilanthes crispus*) has been used traditionally as antidiabetic, diuretic, antilytic, and laxative (Sunarto, 1977). It is commonly consumed in the form of herbal tea. Recent investigations on ethno pharmacological studies demonstrated that *S. crispus* leaves extract was an effective antioxidant in with an ability as antihyperglycemic and antilipidemic agent. The extract has the effect on minimizing the glucose level in blood and also reduces the risk of blood vessels and heart muscle/ cardiovascular ailments (Abu *et al*., 2006).

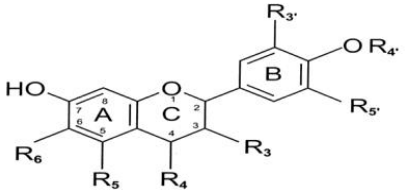

Figure 1. Basic structural feature of flavonoid

In the past three decades, SFE technique has been extensively studied for the extraction and isolation of valuable compounds from natural products (Beatriz *et al*., 2006). The SFE technique helps to minimize sample handling expedites sample preparation and reduces the disposal of environmentally aggressive solvents. Additionally, SFE also extracts an active compound from herbs and plants that are even better than conventional solvent extraction. In the SFE process, the extraction operates at low temperature and with absence of the light and oxygen due to prevent any oxidation reaction, thermal degradation and decomposition of labile compounds of the plants (Del Valle *et al*., 1999). Furthermore, there are less information on comparison study of SFE and solvent Soxhlet extraction on flavonoid compound from plant. Therefore, the objectives of this work are; (i) to investigate the influences of parameters such as temperature, pressure and dynamic extraction time on the SFE of *S. crispus*; (ii) to identify the bioactive flavonoid compound obtained from *S.crispus*; (iii) to compare the results obtained from conventional Soxhlet extraction method and SC-CO_2 in which the optimum parameters of SC-CO_2 will be selected as the comparison to Soxhlet extraction method.

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Materials and Methods

Materials

The leaves of *S. crispus* were harvested from the herbal garden of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia. The leaves were separated from the stalks, thoroughly washed with tap water and rinsed with distilled water. The leaves were dried in ventilated drying oven (1350FX, USA) at 40°C for 24 h. Immediately prior to the extraction process, the dried leaves was ground in a dry mill blender (MX-335, Panasonic, Malaysia) to form a powder in order to increase the surface area of the sample. The dried leaves were stored in a dark place at room temperature for 20 days.

Chemicals

Commercial grade liquid carbon dioxide (purity 99.99%), supplied in cylinder with dip tube, was purchased from Malaysian Oxygen (MOX), Malaysia. Ethanol (EtOH, 99.5%, analytical grade) was obtained from Scharlau Chemical, European Union and methanol (MeOH, HPLC grade) was purchased from Fisher Scientific Chemical, USA. Triflouroacetic acid (TFA≥98%) was obtained from Sigma, Aldrich, Germany. The flavonoid standards including (+)−catechin, (−)- epicatechin, apigenin, rutin, luteolin, kaempferol, myricetin and naringenin were purchased from Sigma, Aldrich, Germany.

Supercritical fluid extraction (SFE)

Exactly thirty grams (±0.1 mg) of dry powdered plant materials was mixed with ninety grams of 2.0 mm diameter glass beads, placed into the extractor vessel. The SC-CO_2 extraction system was operated with different independent variables pressure (100, 150 and 200 bar), temperature (40, 50 and 60°C) and dynamic extraction time (40, 60 and 80 min). A schematic design of the SFE unit used in this work is shown in Figure 2. Liquid carbon dioxide and co-solvent (ethanol) were pumped into the extraction vessel after desired temperature was achieved.

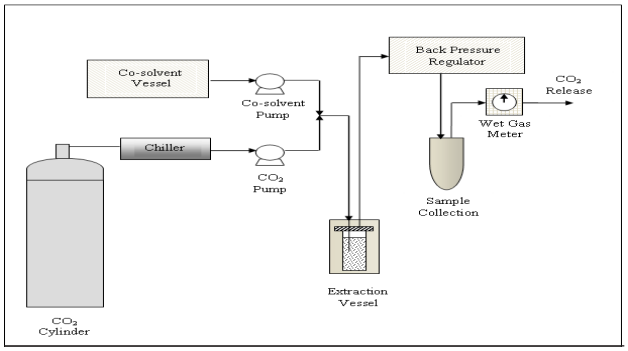

Figure 2. A schematic design of the supercritical fluid extraction (SFE) unit

The flow rate of CO_2 and co-solvent were maintained at 10 and 1 g/min, respectively. Static extraction was performed for 30 min after the desired pressure and temperature were reached. The extract samples were taken at every 10 min and experiments were terminated after extraction time as setting was achieved and the total amount of solute collected was weighed. The extract collected was gravimetrically determined using a balance (Mettler Toledo, model AG 204) with an accuracy of ± 0.0001 g. For each trial, at least two experiments were carried out where the total amount of CO_2 that passed through the cell slightly varied, hence varying the total amount of solute collected.

Soxhlet extraction (SE)

About three gram of dried ground *S. crispus* leaves were weighed and quantitatively transferred into a filter paper extraction thimble and insert into 500 ml reflux flask. The apparatus of SE was fitted with 500 ml round bottom flask containing 150 ml of extraction solvent. The extraction was performed for 6 hour and the temperature extraction was kept at boiling point temperature depend on the solvent used. In this experiment four solvents were used: Pure ethanol, methanol, petroleum ether and 70% methanol.

Determination of extraction yield

After extraction, the extraction mixture was cooled and the residue of the co-solvent from the extract was removed by evaporating using rotary vacuum evaporator at 50°C (Eyela, A-1000S, Japan). The dry extract then cooled for 30 min in desiccators, and weighed. All extractions were performed in duplicate. The extract was then placed in the oven at 40°C for 30 min before transferring into the desiccators for final constant weight and all of the steps were performed with the exclusion of light. The results of the experiments were based on extraction yields and expressed as the equation below:

$$Y_{extract}(\%) = \left[\frac{m_{extract}}{m_{feed}}\right] x100 \quad (1)$$

Where; $Y_{extract}$ is percentage of extraction yield, $m_{extract}$ is the crude extract mass (g) and m_{feed} is the feed mass (g).

Determination of bioactive flavonoids compounds by HPLC analysis

The flavonoid components of the *S. crispus* extracts were analyzed by high performance liquid chromatography (HPLC) method (Wang *et al*., 2001). The HPLC analyses were performed with a

Water 600 pump Controller, 9486 tunable absorbance UV detector and equipped with an Eclipes XDR-C18 reversed-phase column (25 cm×4.6 mm×5 µm, Supelco, USA). Classic Millenium 2010 software was used for manipulation of data processing. The temperature was set to room temperature with flow rate set at 1.0 ml/min and the wavelength was set for detected flavonoid at 280 nm.

Results and Discussion

Effect of pressure on extraction yield

Figure 3 presents the effect of pressure on extraction yield of *S. crispus* in SC-CO_2 at three levels namely 100, 150 and 200 bar at constant temperature. According to the results, as pressure increases from 100 to 200 bar, the extraction yield increased. At a constant temperature, increasing the pressure will increase the density of the SC-CO_2. The solvent strength of SC-CO_2 increases with the density of CO_2. As the density increased, the distance between the molecules decreased therefore the interaction between the analytes and CO_2 increased, leading to greater solubility of the analytes in CO_2 (Castro De *et al*., 1994). Therefore the increase in pressure will also accelerate mass transfer analytes and solvent in supercritical extractor vessel system and improve the extraction yield. This suggests that the solubility of flavonoids in SC-CO_2 is proportional to the density of SC-CO_2. This result was clearly shown for higher temperature at 50 and 60°C.

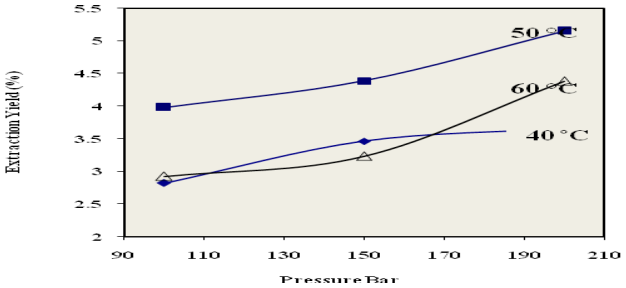

Figure 3. The effect of pressure on extraction yield (%) at constant temperature (°C)

Effect of temperature on extraction yield

Figure 4 presents the effect of temperature on extraction yield of *S. crispus* in SC-CO_2 at three levels namely 40, 50 and 60°C at constant pressure. The influence of temperature on the yield extraction was studied. Density of CO_2 at constant pressure decreases with increasing temperature and hence reduces the solvent power for SC-CO_2. On the other hand the increase of temperature can increase the vapor pressure of analytes. Therefore the tendency of compounds to be extracted passing through the supercritical fluid will increase (Reverchon *et al*., 2006). A moderate increase in temperature can lead to a large decrease in fluid density, with a consequent reduction in solute solubility (Roop *et al*., 1989). In this study the dual effect was clearly shown at the three constant pressures. Results showed that the extraction yield increased as temperature was increased from 40 to 50°C. This can be explained in a way that increasing temperature affected the enhancement of vapor pressure of analytes which is greater than the reduction of density of CO_2. However, a temperature increase from 50 to 60°C caused a decrease in the extraction yield which probably is due to reduction in the density of CO_2.

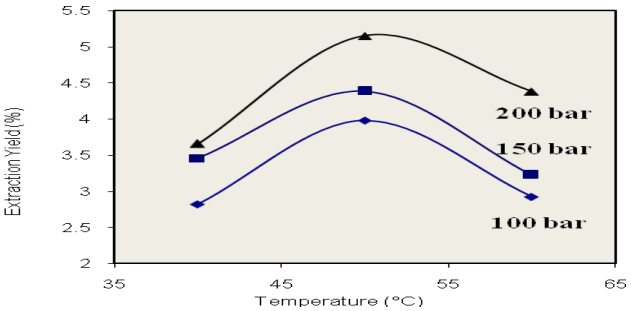

Figure 4. The effect of temperature on extraction yield (%) at constant pressure (bar)

Effect of dynamic time on extraction yield

Figure 5 shows the effect of mean value of dynamic extraction time on extraction yield of *S. crispus* in SC-CO_2. Extraction was performed with SC-CO_2 at the static extraction time of 30 min, followed by three levels of dynamic extraction times set at 40, 60 and 80 min. According to the result obtained, by increasing the dynamic extraction time, the extraction yield was enhanced. However, since the difference between percentages of extraction yield obtained for 60 and 80 min was not significantly different, so 60 min is a reasonable time to be used for the extraction which contributes to less utilization of CO_2 gas. Based on the probability value (*P*-value), time has no significant effect on the extraction yield with $P > 0.05$.

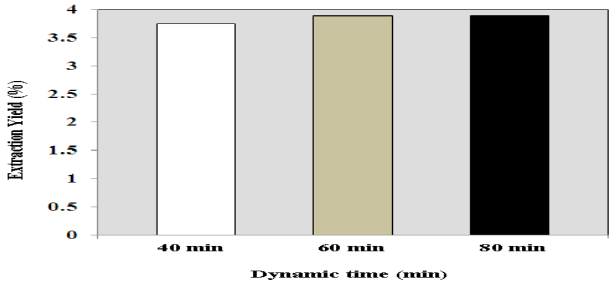

Figure 5. The effect of dynamic time (min) on the extraction yield

Identification and quantification of the extracted compound from S. crispus

The best conditions obtained for the extraction of flavonoids from *S. crispus* leaves extracts were pressure at 200 bar, temperature at 50°C and dynamic extraction time of 60 min. The extract at optimum

Table 1. Identification and quantification of the flavanoid compounds extract from *S. crispus* by SC-CO_2 extraction under different conditions

Pecah Kaca *(S.crispus)* extraction mode	Extraction Yield (%)	Flavonoid Content [d] (%)	Contents of flavonoid (mg/g)							
			Catechin (mg/g)	Epicatechin (mg/g)	Rutin (mg/g)	Myricetin (mg/g)	Luteolin (mg/g)	Apigenin (mg/g)	Naringenin (mg/g)	Kampferol (mg/g)
Minimum [a]	2.37	3.24	9.52	-	13.55	-	-	-	-	-
Optimum [b]	5.17	3.98	4.83	4.55	8.47	4.10	12.52	3.75	3.63	19.45
Maximum [c]	4.38	3.53	4.64	5.64	14.14	3.01	12.32	-	2.44	4.19

[a] Minimum level of each studied parameter (100 bar, 40°C and 40 min)
[b] Optimum level of each studied parameter (200 bar, 50°C and 60 min)
[c] Maximum level of each studied parameter (200 bar, 60°C and 80 min)
[d] Flavonoid content (%) = [the amount of total flavonoids (mg)/the amount of crude extracts (mg)] x 100 %

Table 2. Comparison of result Soxhlet solvent extraction and supercritical carbon dioxide (SC-CO_2) extraction

	Soxhlet Solvent extraction (70% Etoh)	(SC-CO_2)
Extraction Yield (%)	3.22	5.17
Flavonoid Content (%) (g/crude extract g) extract)	2.41	3.98
Catechin (mg/g)	2.39	4.83
Epicatechin (mg/g)	4.11	4.55
Rutin (mg/g)	5.55	8.47
Myricetin (mg/g)	3.24	4.10
Luteolin (mg/g)	6.32	12.52
Apigenin (mg/g)	2.16	3.75
Naringenin (mg/g)	2.59	3.63
Kampferol (mg/g)	12.21	19.45
Condition: Solvent Temperature Time Other	 Ethanol (70%) Boiling point 6 hr	 CO_2 (66.66%) and Ethanol (33.33%) 50°C 60 min CO_2 flow rate: 10 g/min Co-solvent (Ethanol): 5 g/min

conditions was analyzed by HPLC in order to determine the contents of main flavonoid compounds. For the comparison of bioactive flavonoid identification with other extraction condition, the extracts at two other SC-CO_2 conditions for minimum (100 bar, 40°C, 40 min) and maximum (200 bar, 60°C, 80 min) levels were carried out for HPLC analysis. In this study, a problem existed in applying the standard method for hydrolyzing the flavonoid glycosides in *S. crispus* extracts. Even when using abrasive hydrolysis conditions (refluxing for 2 hr with 6M HCL, pure methanol and water) it was not possible to perform complete hydrolysis to produce all free aglycone for quantification. All flavonoid compounds from the extraction yield were identified by matching the retention time and their spectral characteristics against those of standards as comparison. Detailed identification and quantification of the compounds extracted by SFE under different conditions are presented in Table 1.

Comparison of extraction yield and bioactive flavonoid compound of S.crispus by Soxhlet solvent extraction and supercritical carbon dioxide (SC-CO_2) extraction

In this study different solvents were used to determine, which solvent gives the highest recoveries of extraction yield of bioactive flavonoid compound. Based on the results obtained, the best solvent which produces the higher extraction recovery and flavonoid content is 100% methanol. However for the safety effect, 70% ethanol was preferable in this Soxhlet extraction. Therefore, as comparison to SC-CO_2 extraction, result based on 70% ethanol extraction of *S.crispus* leaves extract was selected. As discussed by other researcher, the different method and nature of solvent extraction will consequently affect the extraction yield and efficiency of extraction process (Grigonis *et al*., 2005). Most of the extraction process was set in order to determine preferable process condition, which enables to obtain the highest yield of bioactive compounds. Better extraction yields mean lower economic cost, which often a primary task in production of natural products. The composition of the SC-CO_2 extraction and 70% ethanol was shown as in Table II. The yield extraction result for SC-CO_2 was selected based on the optimization condition under the effect of co-solvent flow rate. Based on the table shown, it shows that flavonoid content in SC-CO_2 extraction is higher compare to the Soxhlet extraction with difference about 1.57%. In addition, solvent extraction with 70% ethanol, have the same capability as SC-CO_2 extraction method of separating bioactive flavonoid compounds. Furthermore, the result revealed that there was a significant different between this two extraction method.

Conclusion

According to our results, the optimum conditions of SC-CO_2 for *S. crispus* bioactive flavonoid compounds were pressure at 200 bar, temperature at 50°C and dynamic time at 60 min. Based on mean value, it can be shown that the effect of extraction variables on extraction yields decreased in the following order: pressure, temperature and dynamic

extraction time. The extraction pressure played a dominant role in the yield of the sample while the effect of time could be ignored. Under the optimum conditions, highest bioactive flavonoid compound content was at 3.98% and eight flavonoid compounds were identified. From identification of bioactive flavonoid compounds by HPLC in this study, it clearly revealed that temperature at 50°C is more convenient to be selected for SC-CO_2 extraction, in order to avoid thermal degradation of the sample. Despite of good result obtained from soxhlet extraction method, SC-CO_2 extraction has been already proven for faster, less solvent consumed and more convenient for food and pharmaceutical industrial purpose.

Acknowledgement

We thank Universiti Putra Malaysia for providing the leaves of *S. crispus* and for the financial support for our research purposes.

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