Optimization of supercritical carbon dioxide extraction of Piper Betel Linn leaves oil and total phenolic content

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Abstract. Supercritical Carbon Dioxide (SC-CO₂) Extraction was applied to extract piper betel linn leaves. The piper betel leaves oil was used antioxidant, anti-diabetic, anticancer and anti-stroke. The aim of this study was to optimize the conditions of pressure, temperature and flowrate for oil yield and total phenolic content. The operational conditions of SC-CO₂ studied were pressure (10, 20, 30 MPa), temperature (40, 60, 80 °C) and flowrate carbon dioxide (4, 6, 8 mL/min). The constant parameters were average particle size and extraction regime, 355µm and 3.5 hours respectively. First order polynomial expression was used to express the extracted oil while second order polynomial expression was used to express the total phenolic content and the both results were satisfactory. The best conditions to maximize the total extraction oil yields and total phenolic content were 30 MPa, 80 °C and 4.42 mL/min leading to 7.32% of oil and 29.72 MPa, 67.53 °C and 7.98 mL/min leading to 845.085 mg GAE/g sample. In terms of optimum condition with high extraction yield and high total phenolic content in the extracts, the best operating conditions were 30 MPa, 78 °C and 8 mL/min with 7.05% yield and 791.709 mg gallic acid equivalent (GAE)/g sample. The most dominant condition for extraction of oil yield and phenolic content were pressure and CO₂ flowrate. The results show a good fit to the proposed model and the optimal conditions obtained were within the experimental range with the value of R² was 96.13% for percentage yield and 98.52% for total phenolic content.

1. Introduction

Piper betel Linn belongs to family of Piperaceae which is originated from South East Asia like Malaysia, Indonesia, India, Thailand and China. In Malaysia, this plant known as ‘DaunSirih’ which is a perennial dioecious creeper and grows about one metre height. This plant has very unique love shaped leave that usually chewed together with areca nut and lime by old folks [1,2]. The Piper betel leaves are used by local to treat cold, headache, asthma, pimples, cut, improve digestion and many more. The leaves juice was used in eyedrops and as an inedible ink for marking and labelling garments [3] and to enhance the intoxicating effect in alcoholic beverages [4].

The leaves contain vitamins, mineral, protein, fibre, carbohydrate, fat and essential oil that medicinally important for body [5]. Previous researchers have proved that piper betle leave contain of phenolic compound which is medicinal importance or biological properties that can possess as antioxidant [6], antimicrobial [7], antidiabetic [8], antifertility [9], antihemolytic [10], antibacterial [11], [12], antihepatotoxic [13], antidepressant [14], anti-inflammatory [15] and many more. Phenolic compounds of piper betel linn has been reported by some researchers which are chavicol by [16], chavibetol and chavibetol acetate by [17] and eugenol by [18]. Hydroxychavicol and Eugenol are the main bioactive compound in the extract of piper betel leaves [19]. Folin-Ciocalteu method was used to access the total phenolic content in the piper betel leaves.

Extraction and product recovery from various plant parts were considered as the most crucial steps in the evaluation of target molecules [20]. Conventional extraction processes are time consuming, laborious and involve long operational techniques, bulk amount of solvents required and can cause...
thermal degradation of the target molecules at continuously high temperature. In addition, traces of toxic solvents are rarely removed from the extracts hence low quality of the yield of product and large quantity of solvent waste. Therefore, supercritical fluid extraction is an alternative extraction technique with better selectivity and efficiency. The most common solvent used in supercritical fluid extraction is carbon dioxide (SC-CO$_2$) because CO$_2$ is inexpensive, non-toxic, non-flammable and considered as a GRAS (Generally Recognized as Safe) solvent. The low polarity is the only major drawback of CO$_2$ hence co-solvent can be used to increase the polarity of CO$_2$.

The aim of this study to optimize SC-CO$_2$ variables such as temperature, pressure and flowrate of the solvent for the maximum extract yield and total phenolic content from piper betel leaves by using response surface methodology (RSM) with Box-Behnken design via Design Expert software version 6.0. RSM was used to develop a model on the percentage yield and total phenolic content (TPC) where the model could be used to optimize the extraction parameters.

2. Materials and methods

2.1. Materials
The green piper betel linn leaves were used and obtained from a market place. The leaves were rinsed using distilled water 2 to 3 times to remove any dust and precipitate stick on the leaves. The clean betel leaves were cut into small pieces and placed in the aluminium tray. Then, the tray was placed into the oven-dryer at 40$^\circ$C for few hours. Next, the dry leaves were ground using grinder and sieve by using sieving tray for particle size of 355µm, 500µm and 710µm. Lastly, the ground betel leaves were put inside the sealed plastic bag and kept in the freezer at -20$^\circ$C to maintain the freshness of the sample.

2.2. Supercritical Carbon Dioxide (SC-CO$_2$)
10g of ground Piper betel leaves were weighed and placed into the extraction cell. The extraction cell was fitted and placed in an oven to maintain the desired temperature. CO$_2$ supplied from a cylinder was chilled and pump using HPLC pump (Lab Alliance, Series II Pump). The pressure in the extractor cell was controlled by adjusting back pressure regulator to achieve desired pressure. The extracts were collected in the collection vial attached at the outlet stream. The extraction takes places for 5hours extraction regime with 30minutes fractionation to determine the optimum time of extraction. Experiment regime was reduced to 3.5hours with 30minutes fraction after obtaining the optimum extraction time. The average particle size of the ground Piper betel leaves was constant at 355µm since during preliminary study of the average particle size of 500µm and 710µm produced the lowest yield compared to 355µm. Three parameters were tested in this study which are temperature (40, 60, 80$^\circ$C), pressure (10, 20, 30MPa) and flowrate of CO$_2$ (4, 6, 8mL/min) in order to obtain high yield of the extracts and phenolic content simultaneously.

2.3. Total Phenolic Content
Folin-Ciocalteu method (FC method) was used to determine the total phenolic content of piper betel linn leaves. The piper betel extract was diluted in methanol and 1mg/ml of the solution was added into 6ml of distilled water, 0.5ml Folin-Ciocalteu reagent and 1.5ml sodium carbonate. Next, the solution was diluted in 7.9 ml distilled water and incubate for 90 minutes. The absorbance of the mixture was measured at 725nm using a UV-VIS spectrophotometer. The total phenolic content is expressed as gallic acid equivalents (GAE) in mg/g dry weight of the plant.

2.4. Experimental Design
In this study, an experimental design was performed in order to optimize three important parameter of SC-CO$_2$ extraction which are pressure ($X_1$), temperature ($X_2$) and flowrate of CO$_2$ ($X_3$) at constant average particle size and extraction regime in order to achieve high yields of Piper betel extracts and phenolic content simultaneously. Box Behnken was applied for experimental design of piper betel leaves SC-CO$_2$ extraction. The parameters were assigned at three coded levels of -1, 0 and +1 for
temperature of 40, 60 and 80˚C, pressure of 10, 20 and 30Mpa, and flowrate of CO$_2$ of 4, 6 and 8mL/min. The result obtained were analysed using Design Expert Software version 6. Table 1 shows the experimental design for the SC-CO$_2$ of piper betel leaves.

Table 1. Design of the experiment

| Pressure, $X_1$ | Temperature, $X_2$ | Flowrate of CO$_2$, $X_3$ | Percentage Yield [%] | Total Phenolic Content [mg GAE/g] |
|-----------------|-------------------|-----------------------------|----------------------|----------------------------------|
| -1              | -1                | 0                           | 0.671                | 181.951                          |
| 1               | -1                | 0                           | 4.930                | 120.815                          |
| -1              | 1                 | 0                           | 2.451                | 276.565                          |
| 1               | 1                 | 0                           | 8.012                | 475.983                          |
| -1              | 0                 | -1                          | 1.777                | 208.327                          |
| 1               | 0                 | -1                          | 6.723                | 374.090                          |
| -1              | 0                 | 1                           | 2.073                | 596.798                          |
| 1               | 0                 | 1                           | 6.155                | 625.910                          |
| 0               | -1                | -1                          | 3.779                | 413.943                          |
| 0               | 1                 | -1                          | 4.294                | 406.114                          |
| 0               | 1                 | 1                           | 3.307                | 470.160                          |
| 0               | 0                 | 0                           | 3.925                | 480.350                          |

Response surface methodology (RSM) was applied to optimize the operating parameters of SC-CO$_2$ for the extraction of piper betel leaves. RSM model or regression equation which include linear and quadratic variables as well as interaction terms were used to fit the first and second-order polynomial equation based on the experimental data as follows:

$$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum_{i} \sum_{j} B_{ij} X_i X_j$$  

(1)

Y is the predicted response, $B_0$ is a constant, $B_i$, $B_{ii}$, $B_{ij}$ are the coefficient for linearity, $X_i$ and $X_j$ are independent variables. The statistical testing of the model was assessed through F-test to obtain the mathematical relationship between input and output parameters. F value calculated must be higher than F value tabulated so that the relationship was significant. While, the significance of the model was performed by analysis of variance (ANOVA) through calculation of the correlation factor $R^2$.

3. Result and Discussion

3.1. Percentage Yield

Table 1 shows that the response of percentage yield of the piper betel leaves extraction by using SC-CO$_2$. The highest percentage yield of piper betel extracts is 8.012% at high temperature and pressure with medium flow of carbon dioxide which are 80˚C, 30Mpa and 6mL/min. More than 8.00% of the yield was consider good enough according to [21] if using SC-CO$_2$. The regression model from Eq.1 for percentage yield of extract was first order polynomial as shown in Eq. 2 below;

$$Y = -4.672 + 0.087X_1 + 0.118X_2 - 9.75 \times 10^{-3}X_3$$

(2)

From the Eq. 2, the 3D response surface graph as a function of pressure and temperature on percentage yield of piper betel extract at the constant flowrate (6 ml/min) was obtain as shown in Figure 1. At constant pressure, the increase in temperature increases the vapour pressure of the extract contributein enhanced the extraction yield. Therefore, SC-CO$_2$ density increases with increasing pressure hence improved solubility of the extract and increased the extraction yield. As can be seen in Eq. 2, the temperature shows the most dominant parameter which contributes positive effect with the coefficient
of 0.188 compared pressure with the coefficient of 0.087. The slope of the increases temperature shows steeper compared to the increases pressure proves that temperature was the most dominant factor on the yield recovery of piper betel.

Figure 1. 3D Response Surface for Percentage Yield

ANOVA was performed as in Table 2. F value calculated was 73.12 while F value tabulated at 95% confidence level was 3.86 (Fc>Ft). Hence, there is significant relationship between the parameters. The calculated coefficient of correlation (R^2) and adjusted coefficient of correlation (R^2a) were 96.13% and 94.74% respectively. These values indicate that the model adequately represented the experimental data and 94.74% of the variations could be covered by the fitted model Eq. 2. The best operating conditions where the percentage yield at maximum were 80˚C, 30MPa and 4.42mL/min with the percentage yield was 7.32%.

| Source      | df | Sum of Square | Mean Square | Fc value | Ft value | R^2  | R^2a |
|-------------|----|---------------|-------------|----------|----------|------|------|
| Regression  | 3  | 50.45         | 16.82       | 73.12    | 3.86     | 0.9613 | 0.9474 |
| Residual    | 9  | 2.03          | 2.3E-01     |          |          |       |      |
| Total       | 12 | 52.48         |             |          |          |      |      |

3.2. Total Phenolic Content

The amount of total phenolic content of the extracts of *piper betel* leaves using the FC method was shown in Table 1. The highest TPC from the *piper betel* extracts was 804.854 mg GAE/g at experimental conditions of 60˚C, 30MPa and 8mL/min. The RSM model for TPC was second order polynomial as in Eq. 3:

$$Y = -202 + 44.04X_1 - 6.72X_2 - 312.24X_3 + 0.33X_1X_2 - 0.85X_1X_3 + 4.28X_2X_3 - 0.36X_1^2 - 0.72X_2^2 + 28.86X_3^2$$

(3)

From Eq. 3, the 3D response surface graph was generated by the software and showed in Figure 2a-c as a function of pressure, temperature and flowrate parameters. From the Eq. 3, flowrate of carbon dioxide was the most dominant factor towards total phenolic content response. From Figure 2a, as the temperature increase from 40˚C to 60˚C, it leads to increases the phenolic compound vapour pressure resulting in enhanced recovery of phenolic content. On the other hand, increasing the temperature from 60˚C to 80˚C leads to lower SC-CO₂ density and reduces the solubility of phenolic compound.
thus causes lower recovery of phenolic compound. As well as, there is possible when operate at high temperature, the phenolic content may degrade. Figure 2b and 2c indicate that increases of the flowrate at constant pressure and temperature lead to high value of phenolic content.

Figure 2. 3D Response Surface for Total Phenolic Content
Table 3 represents ANOVA table for TPC. F value calculated was 22.2 while F value tabulated at 95% confidence level was 8.81 ($F_{c}>F_{t}$). Hence, there is significant relationship between parameters. The calculated coefficient of correlation ($R^2$) and adjusted coefficient of correlation ($R^{2\text{a}}$) were 98.52% and 94.08% respectively. These values indicate that the model is good agreement to represent the experimental data and 94.74% of the variations could be covered by the fitted model Eq. 3. The optimum operating conditions where the extracts rich with phenolic compound were 67.53°C, 29.72MPa and 7.98mL/min with the value of TPC was 845.085mg GAE/g.

| Source         | df | Sum of Square | Mean Square | $F_c$ value | $F_t$ value | $R^2$   | $R^{2\text{a}}$ |
|----------------|----|---------------|-------------|-------------|-------------|---------|----------------|
| Regression     | 9  | 4.38E05       | 48702       | 22.2        | 8.81        | 0.9852  | 0.9408         |
| Residual       | 3  | 6582.45       | 2194.2      |             |             |         |                |
| Total          | 12 | 4.45E05       |             |             |             |         |                |

### 4. Conclusion

In this work the optimization of the supercritical fluid extraction of piper betel leaves was carried out for three process conditions, namely temperature (40-80°C), pressure (10-30MPa) and flow rate of CO$_2$ (4-8mL/min), using Box–Behnken design of experiments and upon application of the response surface methodology. The main objective of this research was to determine the optimum operating conditions of SC-CO$_2$ for the extraction of piper betel leaves. The best conditions to maximize percentage of the extraction yield are 80°C/30MPa/4.42mL/min, which lead to 7.32%. In terms of total phenolic content in the extracts, the optimized operating conditions are 67.53°C/29.72MPa/7.98mL/min, providing 845.085mg GAE/g. The optimum condition for two responses simultaneously where maximum percentage yield as well as high value of TPC are 78°C/30MPa/8mL/min, which lead to 7.05% yield and 791.709mg GAE/g.

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