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RESEARCH ARTICLE

Optimization and effect of supercritical carbon dioxide extraction conditions on global oil yield and eugenol from *piper betle* leaves

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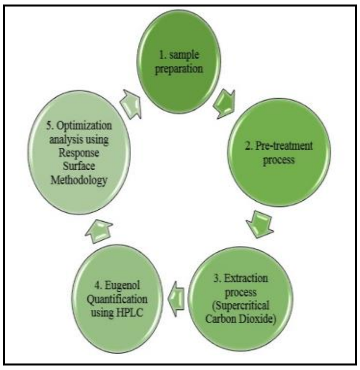
Graphical abstract



Abstract

Todays, medicinal plants have been of great importance to the health of people and societies in Malaysia, and the entire world. *Piper betle* leaves, a member of family Piperaceae is an edible plant. The leaves of *Piper betle* have been traditionally utilized in India for inhibition of oral diseases. Scientific research shows that the leaves possess many biological activities with a good medicinal and commercial value. Nowadays, advance technologies have been used to develop high quality products. This study concentrates on supercritical fluid extraction technology which carbon dioxide, CO_2 play as a solvent. The purpose of this study was to optimize and look into the effects of supercritical CO_2 (SC-CO2) extraction process variables, namely pressure (10–30 MPa), temperature (40–80 °C) and CO_2 flowrate (2-8 mL/min) on global oil yield and percentage of Eugenol in *Piper betle* Leaves. The result shows that as the pressure, temperature and flow rate of CO_2 increased the oil yield of *Piper betle* leaves increased. However, further increased, resulting in decreasing the amount of global oil yield. Meanwhile, the percentage of Eugenol increased as the CO_2 flow rate increased. However, as the pressure and temperature increased, the percentage of Eugenol decreased. Second order polynomial model was used to express the extracted oil and percentage of Eugenol with the both results was satisfactory. The best conditions to maximize the global oil yields and percentage of Eugenol extracted were 19.0 MPa, 40.0 °C and 7.0 mL/min leading to 0.228g of oil and 8.21 % of Eugenol. The most dominant factor for both responses was CO_2 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 R^2 was 69.06% for global oil yield and 82.79% for amount of Eugenol.

Keywords: Supercritical carbon dioxide extraction, *Piper betle* Leaves, Piperaceae, Optimization, Eugenol

INTRODUCTION

Currently the studies on natural products are one of the most coveted research in this universe. As we cognize, plant based products have been expended throughout the world to treat many diseases. It is therefore important to establish their quality. In this study we selected widely available plant namely *Piper betle* leaves. This plant is locally known as 'Sirih' and belongs to the genus Piper of *Piperaceae* family [1]. The betel plant is a stunning and aromatic creeper. It has an alternate, heart shaped, smooth, shining, and long stalked leaves with pointed apex. This plant arose from the central and eastern part of Peninsular Malaysia [2]. Today, this plant is extensively cultivated by thousands of betel growers in various parts of India where about 40 varieties are found in India and more so 30 in West Bengal [3-5].

Several constituents have been identified in leaf oils of *Piper betle* depending on the predominance of compounds such as alkaloid/amide group [6], propenylphenol group [7], prenylated hydroxybenzoic acid group [8], terpene/sesquiterpene group and steroid group [9]. Eugenol ($C_{10}H_{12}O_2$) has been regarded as the major volatile of essential oil compounds belonging to the propenylphenol group. Eugenol ($C_{10}H_{12}O_2$) that present in *Piper betle* leaves was bacteriostatic and fungistatic [10-12]. The chemical structures of Eugenol are shown in Fig. 1. To date, Eugenol ($C_{10}H_{12}O_2$) is utilized as an analgesic in dental preparations, as an insect repellent and as a flavoring agent in foods [13]. The FDA permitted its use in foods and status of GRAS (generally regarded as safe) was appointed by the Flavor and Extract Manufacturers Association. Eugenol ($C_{10}H_{12}O_2$) also has been reported shown to possess anti-inflammatory effects in various animal models of studies with various inflamogens [14]. It has a long antiquity of use as a major and active ingredient in traditional medicines. [15].

Supercritical fluid extraction has developed as an attractive separation system for the food and pharmaceutical industries due to a growing necessitate for "natural" processes that do not bring in any residual organic chemicals. Supercritical carbon dioxide (SC-CO_2) extraction is extensively used as an alternative technique in nutraceuticals industry because of the comparatively low critical temperature (Tc = 31°C), inertness, and non-toxicity of carbon dioxide (CO_2) [16-17]. SC-CO_2 extraction processes are generally carried out at near-environmental temperatures and in the nonexistence of air, which lessens heat requirements, and avoids thermal and oxidative impairment of labile compounds [18-19] Furthermore, one of the main benefits of this technology is the capability of the process in varying the pressure and temperature in order to modify the fluid density.

Hence, supercritical fluids are frequently used to extract selectively or separate specific compounds from a mixture [20].

In industrial perspective, enhancing the extraction process and recovery of target compound is important. Therefore, in supercritical fluid extraction (SFE) process, optimization of the process condition is one of the main aspects that should be studied. Response surface methodology (RSM) is a set of techniques used in industrial process control and engineering to find the best levels of input variables of a response [21]. This experimental methodology combines mathematics with statistics for producing a mathematical model to depict the process, studying the effects of the independent variables and optimizing the processing operations [22].

Comparing the RSM method and other method such as Taguchi method, it shows that RSM is more capable due to its giving very low average error towards modelling and experimental validation as well as the significance of interactions and square terms of parameters are more undoubtedly predicted in RSM [23]. RSM has a major advantage over the one-factor-a time approach in that it lets the assessment of the effect of multiple variables and their interactions on the output variables with lessened number of tests [24]. RSM has been successfully applied to model and optimize supercritical CO_2 extraction of oils from Swietenia Mahagoni Seed [25], *Quercus infectoria* [26] and *squalene* from palm oil [27].

Hence, using RSM in SC–CO_2 process would be an outstanding method to optimize the extraction and, by manipulating the combined arrangement of pressure, temperature and flowrate. Thus, the objective of this study was to optimize the extracted yield and concentration of Eugenol from *Piper betle* leaves using SC-CO_2.

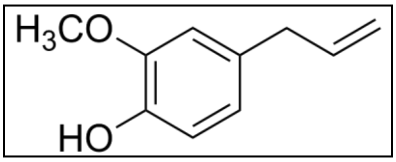


Fig. 1 Structure of Eugenol

METHODOLOGY

Sample Preparation

The fresh leaves of *Piper betle* were collected in bulk from Banting, Selangor, Malaysia. The leaves were separated from the stalks and washed thoroughly with distilled water to remove any dirt and dust present. The clean leaves were cut into small pieces and were dried at 40 °C for 12 hours. The dried leaves were ground just before extraction in a grinder (mill machine, Pulverisett 14, Ider- Oberstein, Germany) to produce a powder with an approximate particle size of 355 µm and stored at 0−5 °C prior to use.

Supercritical Carbon Dioxide System

In this study, SFX TM 220 extraction system (ISCO, Lincoln, NE, US) comprising of high pressure syringe pump (Model: OM51K40GN-CW2), 10 mL stainless steel extraction cell, extractor chamber, extractor controller (Model: TESCOM 10000), a chiller (Model: Wisezcircu) and CO_2 with 99.99 % purity (MOX Gases Sdn. Bhd. Selangor) as a solvent were used throughout the process. The equipment set up of the extraction process SFX TM 220 is shown in Fig. 2.

5.0 g of ground dried *Piper betle* leaves that have reached room temperature were loaded into the extraction cell and tightly sealed. Next, the extraction cell was set in the extraction chamber and let the system to reach the desired condition. The extraction started after reaching the desired condition. Volume of CO_2 used and extracted oil was recorded every 30 minutes of the extraction process. The extraction duration was 3 hours and 30 minutes. Extracts are finally separated from the CO_2 phase and collected in collector at ambient temperature and atmospheric pressure. The CO_2 gas was depressurized to remove from the separator.

Experimental Design

An experimental design was performed to optimize three important parameters of SC-CO_2 extraction which are flowrate of CO_2 (X_1), temperature (X_2) and pressure (X_3) at constant average particle size and extraction time to achieve high percentage of global oil and high percentage of Eugenol ($C_{10}H_{12}O_2$) from *Piper betel* extracts.

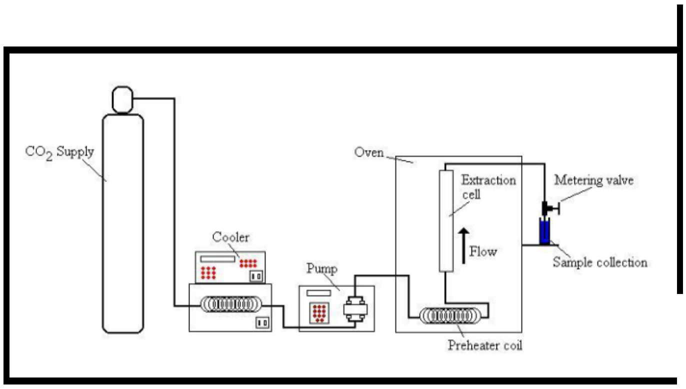


Fig. 2 Schematic diagrams of Supercritical fluid extraction apparatus

Response surface methodology (RSM) was applied to analyzing the effect of the three factors on the two responses and identify the combination that will optimize the extraction. Historical data Design was used to perform this experiment. 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 30 Mpa, and flowrate of CO_2 of 4, 6 and 8 mL/min. The result obtained were analysed using Design Expert version 6.0.4.

The coded and uncoded independent variables employed in RSM design are shown in Table 1. The experimental points used according to this design are shown in Table 2 where Y_1 represent oil yield and Y_2 represent percentage of Eugenol ($C_{10}H_{12}O_2$) of *Piper betle* leaves extracted. In the research, the experimental data were fitted with the second order polynomial model which indicated a requirement of 27 experiments to cover all possible combinations of factors levels. The general equation of the second-degree polynomial is stated as follows:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i=1}^{k}\beta_{ii} x_i^2 + \sum\sum_{i<j}^{k}\beta_{ij} x_i x_j + \in \quad (1)$$

Where the β_i are the regression coefficients for the first-degree terms, the β_{ii} are coefficients for the pure quadratic terms, the β_{ij} are the coefficients for the cross-product terms, and $\in$ is the random error term

Table 1 Experimental levels of the factors

Symbol	Independent Variables	Coded Level -1	0	+1
Factor Level				
X_1	Flowrate (mL/min)	4	6	8
X_2	Temperature (°C)	40	60	80
X_3	Pressure (MPa)	10	20	30

Table 2 Experimental and estimated values for different levels of experimental design

X_1	X_2	X_3	Y_1	Y_2
-1	0	-1	0.1927	6.3273
-1	0	0	0.2385	6.4179
-1	0	1	0.126	5.3768
-1	1	-1	0.2351	5.9418
-1	1	0	0.2473	5.6038
-1	1	1	0.0879	6.2379
-1	-1	-1	0.1672	8.1849
-1	-1	0	0.2228	6.5266
-1	-1	1	0.0927	6.2179
0	0	-1	0.2071	7.5693
0	0	0	0.2577	6.0849
0	0	1	0.2983	5.1617
0	1	-1	0.1804	8.7075
0	1	0	0.1952	7.2734
0	1	1	0.2384	6.3968
0	-1	-1	0.2065	7.7344
0	-1	0	0.2427	6.5930
0	-1	1	0.276	6.2521
1	0	-1	0.1402	10.231
1	0	0	0.1776	9.1760
1	0	1	0.1917	8.5523
1	1	-1	0.1725	9.2323
1	1	0	0.1908	8.2770
1	1	1	0.2502	6.0574
1	-1	-1	0.1636	8.7878
1	-1	0.	0.2439	6.6748
1	-1	1	0.361	3.5580

Eugenol Determination

Identification and Quantification of Eugenol was performed using Agilent Eclipse Plus C18 (150 x 4.6 mm I.D., particle size 5 µm) (Agilent Technologies, California, USA). A reverse-phase HPLC assay was carried out using an isocratic system with a flow rate of 1.0 mL/min, a column temperature of 25^{o}C and a mobile phase of acetonitrile: methanol (85:15, v/v). The peaks were detected at 280 nm and identified using reference standards of Eugenol. A quantitative determination of Eugenol was completed by comparison of the peak area of the sample injection to the corresponding peak area of a standard injection. Identification of Eugenol from SC-CO_2 extraction of Piper betle leaves were made by comparing the chromatographic appearance of the peak and retention time of standards reference as presented in Fig. 3.

RESULTS AND DISCUSSION

As referred earlier, a RSM was applied to measure, both, the main and the relations effects of the operating parameters. In this research, the RSM was employed to create and discover an estimated functional connection among variables such as temperature, pressure and flow rate of CO_2 utilized with yield of global oil and chemical compound extracted. The pressure studied was in the range of 10 to 30 MPa and the temperature studied was in the range of 40 and 80 ^{o}C while the flow rate of CO_2 studied was between 4 to 8 mL/min (Table 1).

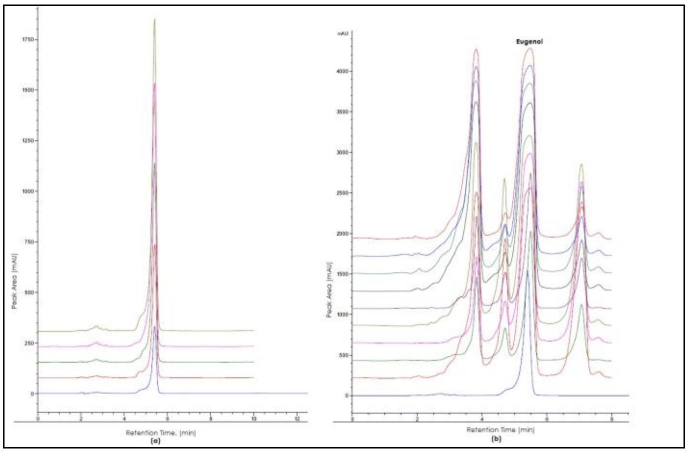


Fig. 3 HPLC chromatograms of Eugenol: (a) standard Mixture and (b) SC-CO_2 extract of Piper betle leaves

The oil yield and percentage of Eugenol obtained from all the experiments is listed in Table 2 according to RSM design. From the data obtained, the highest global oil yield of *piper betel* extracts was 0.361 g at high conditions of SC-CO_2 process where temperature at 80 ˚C, pressure at 30 MPa and flow rate of CO_2 at 8mL/min.

Fig. 4 shows three-dimensional plot of the response surface for global oil yield for which the polynomial function is

$$\text{Oil yield (g)} = 0.413 + 0.032f - 0.00976f^2 - 0.0082T + 0.000029T^2 - 0.00696P - 0.00025P^2 + 0.0000695fT + 0.00256fP + 0.002fP^2 - 0.000049TP \quad (2)$$

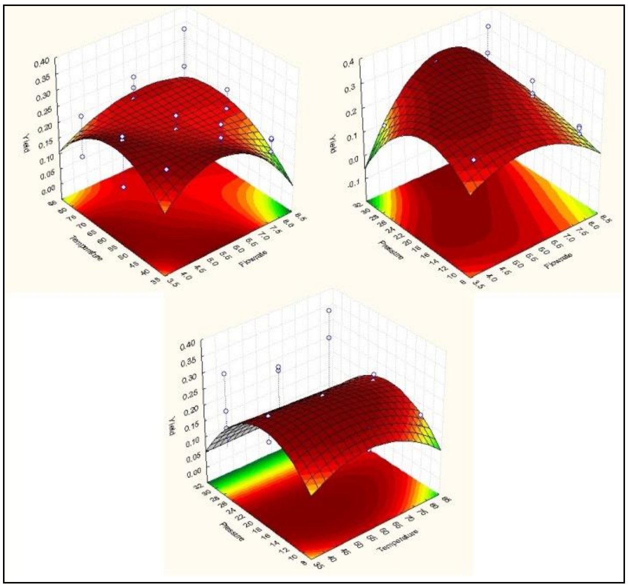


Fig. 4 Three-dimensional plot of the response surface for global oil yields of *Piper Betle* leaves

As can be seen in Eq. 2, the flow rate of CO_2 shows the most dominant parameter which contributes positive effect with the coefficient of 0.032 compared with pressure and temperature where the coefficient obtained is 0.00696 and 0.0082. From the graph shown in Fig. 4, mostly all the relations between the parameters were in quadratic pattern. As the pressure, temperature and flow rate of CO_2 increased the global oil yield of *Piper betle* leaves increased. However, further increased, resulting in decreasing the amount of global oil yield.

This phenomenon occurs most likely due to the increasing the CO_2 solvent density as the pressure increased that resulting in an improvement in solvent power [28]. However, the declining of oil yield with further increasing the pressure probably a reflection of the increased repulsive solute–solvent interactions resulted from the highly compressed carbon dioxide at high-pressure levels.

Next, the progressive effect of temperature can be described in reference to its power to modify the physical properties of the sample matrix, causing it accessible for the CO_2 to perforate that resulting in increasing the mass transfer speed. However, declining of oil yield with further increase of temperature most probably because the density of the supercritical fluid decreased at a higher temperature.

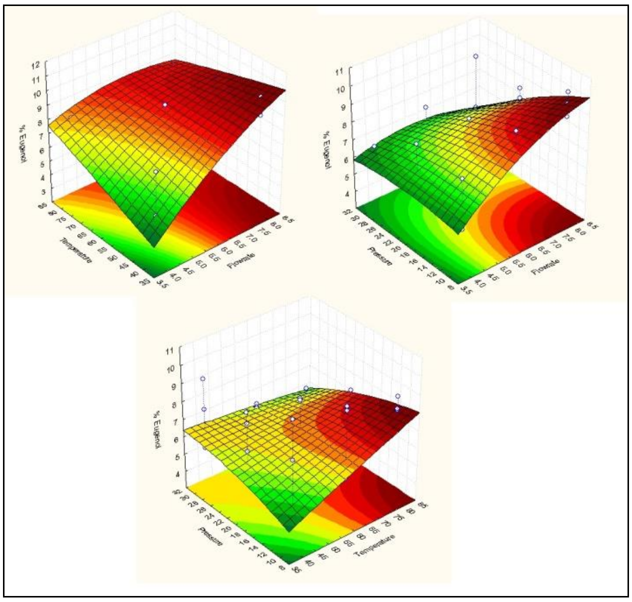


Fig. 5 Three-dimensional plot of the response surface for percentage of Eugenol of *Piper Betle* leaves

From the experimental data, the highest percentage of Eugenol obtained was 10.275 % at low temperature and pressure with high flow of CO_2 which were 40 ˚C, 10 MPa and 8 mL/min. Fig. 5 shows three dimensional plot of the response surface for percentage of Eugenol in *Piper Betle* leaves extract for which the polynomial function is

$$\% \text{ Eugenol} = -68.127 + 24.709f + 1.6505T + 1.7258P - 0.2447fT - 0.3109fP - 0.0152TP \quad (3)$$

As can be seen in Eq. 3, the flow rate of CO_2 shows the most dominant parameter which contributes positive effect with the coefficient of 24.709 compared with pressure and temperature where the coefficient obtained is 1.7258 and 1.6505. From the graph shown in Fig. 5, the effect of increasing flow rate of CO_2 on the yield of Eugenol ($C_{10}H_{12}O_2$) was more obvious than that of temperature and pressure. The slope of the increases flow rate shows steeper compared to the increases pressure and temperature proves that flow rate was the most dominant factor on the Eugenol recovery of *piper betel.*

In supercritical fluid extraction process, flow rate of solvent is another factor that affecting the extraction process to produce a high yield of extracts [29]. From Fig. 5, it shows that by increasing the CO_2 flow rate, the percentage of Eugenol also increased. This phenomenon occurs could be due to the reducing of mass transfer resistance of solute-solvent. Furthermore, by increasing the CO_2 flowrate, the intermolecular interaction between solvent and the solute increase due to the increase of CO_2 molecules per volume in the extraction vessel [29]. In this condition, the mass transfer increases as the flowrate increases.

However, as the pressure and temperature increased, the percentage of Eugenol decreased. The decreasing could be due to the degradation of the interest compound. As shown in Fig. 5, as the temperature constant at low condition which is 40ºC, the percentage of Eugenol increased as the pressure increase. This process occurs due to the improvement of oil solubility resulted from the increased carbon dioxide density with the rise of pressure.

Table 3 Analysis of Variance for oil yield and the percentage of Eugenol

Source	Sum of Square	df	Mean Square	F_c	F_t	R_2	$R^2{}_a$
Oil yield							
Regression	0.065	9	0.0072	4.22	2.98	0.6906	0.54
Residual	0.029	17	0.0017				
Total	0.094	26					
% Eugenol							
Regression	4860.46	6	810.08	16.03	3.87	0.8279	0.78
Residual	1010.53	20	50.53				
Total	5870.99	26					

The results of the experimental parameters were measured by statistical analysis of the experimental data shown in Table 2. The enter method was used to calculate the estimated coefficients of the polynomial functions of response surfaces for both global oil yield and the amount of Eugenol extracted. The analysis of variance (ANOVA) is presented in Table 3 for global oil yield and the amount of Eugenol extracted in term of % w/w.

Analysis of variance was performed as in Table 3. The result of oil yield for F value calculated was 4.22 while F value tabulated at 95 % confidence level was 2.98 ($F_c > F_t$). Hence, there is significant relationship between the parameters. The calculated coefficient of correlation (R^2) and adjusted coefficient of correlation (R^2a) were 69.06 % and 54.0 % respectively. These values show that the model satisfactorily represented the experimental data and 54.0 % of the variations could be covered by the fitted model Eq. 2.

Meanwhile, for the percentage of Eugenol, the result of percentage of Eugenol for F value calculated was 16.03 while F value tabulated at 95 % confidence level was 3.87 ($F_c > F_t$). Hence, there is significant relationship between the parameters. The calculated coefficient of correlation (R^2) and adjusted coefficient of correlation (R^2a) were 82.79 % and 78.0 % respectively. These values show that the model satisfactorily represented the experimental data and 78.0 % of the variations could be covered by the fitted model Eq. 3. The analysis indicated that both models were statistically significant at 95 % confidence.

CONCLUSION

In this study, an experimental design was performed in order to optimize three important parameters of SC-CO_2 extraction which are temperature (40-80 ˚C), pressure (10-30 MPa) and flowrate CO_2 (4-8 mL/min), using RSM. The main objective of this research was to determine the optimum operating conditions of SC-CO_2 for the extraction of global oil and Eugenol from *piper betel* leaves. The best conditions to maximize both responses which were the global oil yield and amount of Eugenol were 40.0 ˚C, 19 MPa and 7.00 mL/min. which lead to 0.228 g of global oil yield and 8.21 % .in terms of amount of Eugenol in the extracts. The results show a good fit to the proposed model and the optimal conditions obtained were within the experimental range with the value of R^2 was 69.06 % for global oil yield and 82.79 % for amount of Eugenol.

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