THE EFFECT OF SUPERCritical CO$_2$ ON THE
ANTIOXIDANT ACTIVITY OF THE SWIETENIA
MAHAGONI SEED EXTRACT BY BOX-BEHNKEN
DESIGN

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Abstract. In this study, the influence of supercritical carbon dioxide parameter on the antioxidant activity on Swietenia mahagoni seed extract was investigated. Response surface methodology was employed, using Box-Behnken statistical design to evaluate the effects of three independent variables. Pressure (20-30 MPa), temperature (40-60°C) and particle size (0.25-0.75 mm) were the three variables with total time and CO$_2$ flow rate being held constant at 180 minutes and 2 ml/min respectively, for each extraction. The extracts were screened for possible antioxidant activity by free radical scavenging activity 2,2-diphenyl-1-picryl hydrazyl (DPPH) assays. The study reveal that the highest antioxidant activity which was 94.84% was obtained at temperature of 40°C, pressure of 25 MPa and particle size of 0.25 mm. They stated that the dominant effect on the antioxidant activity of the Swietenia mahagoni seed extracts was the pressure compared to temperature and particle size.

Keywords: Swietenia mahagoni; Supercritical carbon dioxide; Antioxidant; Box-Behnken design

INTRODUCTION

Swietenia mahagoni (Linn.) Jacq. seeds have been applied as traditional medicine for treatment of hypertension, diabetes, and malaria, while the decoction of its bark has been used as a febrifuge (Chen, et al. 2007). The therapeutic effects associated with the seeds are mainly caused by the biologically active ingredients, fatty acids and tetrnortriterpenoids (Bascal, et al 1997). There are reports of S. mahagoni seeds having anti-inflammatory, antimutagenicity, and antitumour activities (Guevara et al., 1996). The plant extracts have been accounted to possess antibacterial and antifungal activities (Alraade, et al.2010).

Supercritical carbon dioxide (SC-CO$_2$) was successfully used in the extraction of edible oils from a wide range of seeds, including amaranth (Westerman, D, et al., 2006), hiprose (Reverchon, et al., 2000), cuphea (Eller, et al., 2011), flax (Ozkul, S.G. 2009), sunflower and rape (Boutin, and Badens. 2009).

Response surface methodology (RSM), originally described by Box and Wilson (Box, and Wilson, 1951), is a collection of mathematical and statistical techniques useful for modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery, D.C.. 1991). Recently, RSM has been successfully applied to optimize SC-CO$_2$ extraction of oils from Salvia mirzayanii (Wei, et al. 2009), silkworm pupae (Liu, et al., 2009), Passiflora seed (Ku, and Mun. 2008), wheat germ (Shao, et al., 2008), cottonseed (Bhattacharjee, et al., 2007), Curcuma longa


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(Chang, et al., 2006), rosehip seed (Machmudah, et al., 2007), Cyperus rotundus (Wang, et al., 2012) and amaranth seed (Kraujalis, F.and Venskutonis. 2013).

In the present study, SC-CO\textsubscript{2} was used to extract the oil from S.mahagoni seed. The aim was to investigated the influence of supercritical carbon dioxide parameter on the antioxidant activity on Swietenia mahagoni seed extract.

METHOD

1. Plant material preparation

S.mahagoni seeds were rinsed with tap water to remove any foreign particles and dirt prior to drying. Then, the cleaned seeds were cut into small pieces and dried in an oven at the temperature of 50°C for one week to remove the moisture. The seeds were then ground by a blender (Panasonic).

2. Supercritical CO\textsubscript{2} extraction

The ground sample of 5 g was placed in an extractor vessel. Two independent variables studies were extraction pressure (MPa) and temperature (°C). These independent variables and their levels were selected based on the preliminary experiments in our laboratory (data not shown). The extraction was run with 5 g of sample placed in the extraction vessel for extraction. The oil was extracted at temperature of 40, 50 and 60°C with operating pressure 20, 25, and 30 MPa using carbon dioxide flow rate 2 ml/min and extraction time of 180 min. After each extraction, the obtained extract was placed into glass vials. The oil yield was calculated by the weight increment at the end of the extraction and keep at -20°C ready for analysis.

3. Box-Behnken design (BBD)

Box-Behnken design (BBD) was applied to determine the optimum extraction pressure, temperature and particle size for supercritical CO\textsubscript{2} extraction of S. mahagoni seed. The pressure (A), temperature (B) and particle size (C) were independent variables studied to optimize the oil yield (Y) from S.mahagoni seed. The CO\textsubscript{2} flow rate was constant. Box-Behnken design requires an experiment number (N) according to the following equation:

\[ N = 2k(k_1) + cp \] (1)

Where k is the factor number and c\textsubscript{p} is the replicate number of the central point. There are three levels of design (-1, 0, +1) with equally spaced intervals between these levels. The investigated factors and tested levels are reported in Table 1.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Symbol</th>
<th>Low (-1)</th>
<th>Middle (0)</th>
<th>High (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (MPa)</td>
<td>A</td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>B</td>
<td>40</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Particle size (mm)</td>
<td>C</td>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
</tr>
</tbody>
</table>

The experimental data were fitted with the second order response surface model of the following form:

\[ y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j \] (2)

Where y is the response (extraction yield in %); \( \beta_0, \beta_i, \beta_{ij} \) are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively;and \( X_i \) and \( X_j \) are coded independent variables (pressure, temperature or particle size). Analysis was performed using commercial software Design-Expert\textsuperscript{®} v.6.0.4 . The analysis of variance (ANOVA) was also used to evaluate the quality of the fitted model. The test of statistical difference was based on the total error criteria with a confidence level of 95%.
4. Antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The free radical scavenging activity was measured by using DPPH assay. The quantitative estimation of radical scavenging activity was determined according to the methods described by Millauskas et al. (1994) with a slight modification. Extract solution was prepared by dissolving 0.025 g of dry extract in 10 ml of methanol to give final concentration at 2.5 mg/ml. Then, 77 μL of the extract solution was mixed with 3 ml of 6 x 10⁻⁵ M methanolic solution of DPPH. The mixtures were vortex-mixed and kept under darkroom condition for 30 min. The optical density (OD) was measured at 517 nm. Radical scavenging activity of the sample was calculated: (Ao – A1) x 100% / Ao, where Ao is the absorbance of the control reaction and A1 is the absorbance in the presence of the sample of the tested extracts.

RESULT AND DISCUSSION

1 DPPH Free Radical Scavenging

The available methods to measure free radicals and other reactive (ROS) species contributing to the development of several diseases by oxidative damage have been revised (Willcox et al., 2004; Halliwell et al., 2004). In order to evaluate the antioxidant activity of plants, DPPH free radicals scavenging activity was used. DPPH is a common abbreviation for an organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl. DPPH is a potent tool to determine antioxidant capacity of the extracted compound. Hence, Table 1 depicts the DPPH free radical scavenging by the S. mahagoni seed extract using different parameter condition of SC-CO₂.

<table>
<thead>
<tr>
<th>Run</th>
<th>Pressure (MPa)</th>
<th>Temperature (°C)</th>
<th>Particle Size (mm)</th>
<th>DPPH free radicals scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>40</td>
<td>0.5</td>
<td>86.95±0.75⁷</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>40</td>
<td>0.5</td>
<td>93.47±1.89⁶</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>60</td>
<td>0.5</td>
<td>89.42±0.66⁶</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>60</td>
<td>0.5</td>
<td>92.53±0.82⁶</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>50</td>
<td>0.25</td>
<td>91.59±0.90⁶</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>50</td>
<td>0.25</td>
<td>95.13±0.43⁷</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>50</td>
<td>0.75</td>
<td>88.55±0.25⁶</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>50</td>
<td>0.75</td>
<td>92.31±2.89⁶</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>40</td>
<td>0.25</td>
<td>94.84±0.25⁬</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>60</td>
<td>0.25</td>
<td>89.71±0.25⁶</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>40</td>
<td>0.75</td>
<td>89.71±0.25⁶</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>60</td>
<td>0.75</td>
<td>88.84±1.25⁥</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>50</td>
<td>0.5</td>
<td>89.27±1.64⁥</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>50</td>
<td>0.5</td>
<td>88.55±0.90⁥</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>50</td>
<td>0.5</td>
<td>88.84±2.18⁥</td>
</tr>
<tr>
<td>16</td>
<td>25</td>
<td>50</td>
<td>0.5</td>
<td>90.63±0.51⁥</td>
</tr>
<tr>
<td>17</td>
<td>25</td>
<td>50</td>
<td>0.5</td>
<td>87.82±1.15⁥</td>
</tr>
</tbody>
</table>

SFE has become a popular method of extraction for use with botanical materials as it can greatly reduce the time needed to complete the extraction and can also give an extract equally as good better than that obtained using classical extraction methods (Wang et al., 2008). The critical point of carbon dioxide is reached above a temperature of 31.1°C and pressure of 73.8 bar. Above this point, carbon dioxide becomes a supercritical fluid (Ramsey et al., 1998) and possesses the properties of both a liquid and a gas (Herrero et
The main aim when using SFE is to find the most suitable combination of various operating parameters in order to obtain an extract which contains the desired components. Pressure is perhaps the most important operating parameter in SFE. It is the principle parameter that can influence the efficiency of the extraction. An increase of pressure, at a certain temperature, increases the fluid density, which increases the solubility of the compounds contained in the matrix (Pourmortazavi et al., 2007). It is recommended to keep the temperature between 35-60°C when using SFE with natural materials. Going above this range may cause damage to thermolabile compounds present in the material. Increasing the extraction temperature, at a certain pressure, reduces the fluid density, which reduces the solvent power of the fluid but increases the vapour pressure of the compounds present in the matrix (Reverchon et al., 2006). Every type of botanical material will differ when using SFE to extract certain desirable compounds. Because of the competition between the pressure and temperature parameters it must be determined through experimentation which parameter has the most influence on the extraction being carried out.

Increasing pressure increased the percentage yield (Table 1) and percentage DPPH free radical scavenging activity (Table 1) from S. mahagoni seed. This indicate that as the pressure increases, at a certain temperature, the fluid density increases, helping the solvent to penetrate into the matrix, allowing for better extraction (Zarena et al., 2012). For the percentage yield of S. mahagoni seed and also the percentage DPPH free radical scavenging activity, a high pressure had a positive effect on the responses, with the temperature having little to no effect. This shows that a high fluid density, as opposed to high vapour pressure, is most beneficial for the outcome of the extraction.

Free radicals scavenging activity (Table 1) showed that S. mahagoni seed extract of sample 1 (86.95±0.75) was significantly different when comparing with the sample 2, 4, 5, 6, 8 and 9 (p<0.05) with the respective value of 93.47±1.89, 92.53±0.82, 91.59±0.90, 95.13±0.43, 92.31±2.89 and 94.84±0.25, but its not significantly different to sample 3, 7, 10, 11, 12, 13, 14, 15, 16 and 17 with the respective value of 89.42±0.66, 88.55±0.25, 89.71±0.25, 89.71±0.25, 88.84±1.25, 89.27±1.64, 88.55±0.90, 88.84±2.18, 90.63±0.51 and 87.82±1.15. Table 1 also showed that at each constant pressure (30 MPa), the antioxidant activity increase with the increase of temperature from 40°C to 50°C and started to decrease when further increase from 50°C to 60°C. The study shows that the effect of SC-CO2 parameter on the antioxidant activity did not have the same traits as the effect of the SC-CO2 parameter on the extracted oil. The decrease of the antioxidant activity occurred due to the degradation of the antioxidant compounds. It can be assumed that above the temperature of 60°C, certain antioxidant compounds were degraded predominately through oxidation. According to Mandana et al. (2011), the reason of the change in the antioxidant activity when the temperature changed from 40°C to 60°C due to the critical pressure range, the effect of temperature on solute solubility was different. The CO2 density was very sensitive to temperature nearing the critical pressure. Therefore they also stated that, due to the thermo-sensitivity the decrease in antioxidant activity occurred.

Other researchers who studied on the antioxidant activity on the extracted oil using supercritical carbon dioxide were Chun et al. (2009), Qiuhi et al. (2007), Wei et al. (2009), Mandana et al. (2011). The effect of SC-CO2 condition on the antioxidant activity on the extraction of Chlorella pyrenoidosa was studied by Qiuhi et al. (2007). The parameters used in their study were temperature (32°C, 40°C, 47°C and 55°C, pressure (25 MPa, 30 MPa, 35 MPa and 40 MPa), modifier (0, 0.5, 1.0 and 1.5 mL ethanol/g), CO2 flow rate (15, 20, 25 and 30 L/h) and time of extraction (1.5 h, 2 h, 2.5 h and 3 h). The results showed that the highest antioxidant activity (54.16%) was obtained at 40°C, 40 MPa and 1.0 mL ethanol/g with 20 L/h of CO2 flow rate for 2.5 hours of extraction time. Meanwhile, the highest oil yield (7.78%) was obtained at 32°C, 40 MPa and 1.5 mL ethanol/g with 20 L/h of CO2 flow rate for 3 hours of extraction time. The findings show that the condition used in the process was different in order to achieve maximum value of oil yield and antioxidant activity.

Mandana et al. (2011) studied on the effect of the SC-CO2 extraction on the antioxidant activity of the Mentha spicata leaves extracts. The condition of SC-CO2 extraction process used were temperature ranging from 40°C to 60°C, pressure ranging from 10 MPa to 30 MPa and co-solvent flow rates ranging from 3 g/min to 9 g/min. They reported that the highest antioxidant activity which was 71.00% was obtained at temperature of 60°C, pressure of 20 MPa and co-solvent flow rate of 6 g/min. They stated that the dominant effect on the antioxidant activity of the Mentha spicata extracts was the pressure campared to temperature and co-solvent flow rate.

**CONCLUSION**

The study reveal that the highest antioxidant activity which was 94.84% was obtained at temperature of 40°C, pressure of 25 MPa and particle size of 0.25 mm. They stated that the dominan effect on the
antioxidant activity of the *S. mahagoni* seed extracts was the pressure compared to temperature and particle size.

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**REFERENCES**