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 ^oC) 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 activitiy 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^{o}C, pressure of 25 MPa and particle size of 0.25 mm. They stated that the dominan effect on the antioxidant activity of the *Swietenia mahagoni* seed extracts was the pressure campared 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 tetranortriterpenoids (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 (Alrdahe, *et al.*2010).

Supercritical carbon dioxide extraction (SC-CO_2) is an attractive alternative to conventional liquid extraction due to its mild environment in the process and no residue of harmful solvents. Furthermore, employing carbon dioxide as a supercritical solvent is a promising technology. It does not affect thermally sensitive materials with it low critical temperature (31.1^{o}C) and pressure (7.28 MPa), that makes it an ideal solvent for extracting. Also being nontoxic, inflammable, widely available and cheap (Nik Norulani *et al*, 2004). 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 (Ozkal, 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*

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

In the present study, SC-CO_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_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_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_2 flow rate was constant. Box-Behnken design requires an experiment number (N) according to the following equation:

$$N = 2k(k_1) + cp \qquad (1)$$

Where k is the factor number and c_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 1. The coded and uncoded levels of independent variables used in RSM design

Independent variable	Symbol	Level		
		Low (-1)	Middle (0)	High (+1)
Pressure (MPa)	A	20	25	30
Temperature (°C)	B	40	50	60
Particle size (mm)	C	0.25	0.50	0.75

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

$$y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i \leq i}^{k} \beta_{ij} X_i X_j \qquad (2)$$

Where y is the response (extraction yield in %); β_0, β_j, β_{jj}, β_{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® 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-CO_2.

TABLE 1. DPPH free radicals scavenging activity of *S. mahagoni* seed extracts at different parameter SC-CO_2

Values are expressed as mean ± SD of 3 replicate measurement in which different letters for each column (a-f) indicate significantly different from each other according to the Tukey's test ($p<0.05$).

Run	Pressure (MPa)	Temperature (°C)	Particle Size (mm)	DPPH free radicals scavenging activity (%)
1	20	40	0.5	86.95±0.75[a]
2	30	40	0.5	93.47±1.89[def]
3	20	60	0.5	89.42±0.66[abcd]
4	30	60	0.5	92.53±0.82[cdef]
5	20	50	0.25	91.59±0.90[bcdef]
6	30	50	0.25	95.13±0.43[f]
7	20	50	0.75	88.55±0.25[abc]
8	30	50	0.75	92.31±2.89[cdef]
9	25	40	0.25	94.84±0.25[f]
10	25	60	0.25	89.71±0.25[abcd]
11	25	40	0.75	89.71±0.25[abcd]
12	25	60	0.75	88.84±1.25[abc]
13	25	50	0.5	89.27±1.64[abcd]
14	25	50	0.5	88.55±0.90[abc]
15	25	50	0.5	88.84±2.18[abc]
16	25	50	0.5	90.63±0.51[abcd]
17	25	50	0.5	87.82±1.15[ab]

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* *al*., 2006). 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^oC 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 apposed 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^oC to 50^oC and started to decrease when further increase from 50^oC to 60^oC. The study shows that the effect of SC-CO_2 parameter on the antioxidant activity did not have the same traits as the effect of the SC-CO_2 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^oC, 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^oC to 60^oC due to the critical pressure range, the effect of temperature on solute solubility was different. The CO_2 density was very sensitive to temperature nearing the critical pressure. 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), Qiuhui *et al*. (2007), Wei *et al*. (2009), Mandana *et al*. (2011). The effect of SC-CO_2 condition on the antioxidant activity on the extraction of *Chlorella pyrenoidosa* was studied by Qiuhui *et al*. (2007). The parameters used in their study were temperature (32^oC, 40^oC, 47^oC and 55^oC, pressure (25 MPa, 30 MPa, 35 MPa and 40 MPa), modifier (0, 0.5, 1.0 and 1.5 mL ethanol/g), CO_2 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^oC, 40 MPa and 1.0 mL ethanol/g with 20 L/h of CO_2 flow rate for 2.5 hours of extraction time. Meanwhile, the highest oil yield (7.78%) was obtained at 32^oC, 40 MPa and 1.5 mL ethanol/g with 20 L/h of CO_2 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-CO_2 extraction on the antioxidant activity of the *Mentha spacita* leaves extracts. The condition of SC-CO_2 extraction process used were temperature ranging from 40^oC to 60^oC, 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 50^oC, pressure of 20 MPa and co-solvent flow rate of 6 g/min. They stated that the dominan 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^oC, 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 campared to temperature and particle size.

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