OPTIMIZATION OF *SWIETENIA MAHAGONI* SEED IN SUPERCRITICAL CARBON DIOXIDE EXTRACTION

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To my beloved family

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ABSTRACT

Supercritical carbon dioxide extraction has been used for extraction of essential oil from *Swietenia mahagoni* seed. The effect of different particle sizes on diffusivity coefficients, D (m^2/s) were studied by applying second Fick's law of diffusion proposed by Crank. Particle size of 710 μm showed the highest D of 3.1 x 10^{-12} m^2/s. Gas compression at pressure of 30 MPa and temperature of 60 ^oC on seeds caused swelling thus allowed faster diffusion of carbon dioxide through porous structure of *Swietenia mahagoni* seed. For optimization of *Swietenia mahagoni* seed oil extraction, a three-level factorial design in response surface methodology was used to analysis the effect of pressure (20 – 30 MPa) and temperature (40 - 60 ^oC) on extraction oil yield. The highest extraction oil yield of 29.70% was obtained at pressure of 30 MPa and temperature of 40 ^oC. This explained that increased pressure and low temperature increased the carbon dioxide density. Thus, higher solute bonding in solvent phase and consequently higher the extraction yield was obtained. Qualitative phytochemical analysis showed the presense of alkaloid, saponin, triterpenoid, phenolic hydroquinone and tannin but absense of flavonoid. Toxicity test of *Swietenia mahagoni* seed extract using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on human skin fibroblast cell (HSF 1184) showed cell viability above 80%. The result showed that *Swietenia mahagoni* seed extracts at studied concentrations (10, 1, 0.1, 0.01, 0.001, 0.0001 mg/ml) are non toxic.

ABSTRAK

Aplikasi pengekstrakan menggunakan bendalir lampau genting karbon dioksida telah digunakan untuk mengekstrak minyak dari biji *Swietenia mahagoni*. Kesan perbezaan saiz zarah kepada pekali keresapan, D (m^2/s) telah dikaji menggunakan hukum kedua resapan Fick's yang disarankan oleh Crank. Saiz zarah 710 μm menunjukkan D yang tertinggi sebanyak 3.1×10^{-12} m^2/s. Pemampatan gas pada tekanan 30 MPa dan suhu 60 ^oC ke atas biji *Swietenia mahagoni* menyebabkannya bengkak dan seterusnya membenarkan resapan karbon dioksida menembusi struktur ruangnya dengan lebih cepat. Untuk tujuan pengoptimuman pengekstrakan minyak biji *Swietenia mahagoni*, reka bentuk faktorial tahap-tiga dalam kaedah gerak balas permukaan telah digunakan untuk menganalisa kesan tekanan (20 – 30 MPa) dan suhu (40 – 60 ^oC) ke atas hasil pengekstrakan minyak. Hasil tertinggi pengekstrakan minyak sebanyak 29.70% telah diperoleh pada tekanan 30 MPa dan suhu 40 ^oC. Ini menjelaskan bahawa pada peningkatan tekanan dan suhu rendah, ketumpatan karbon dioksida meningkat. Oleh itu, ikatan bahan larut yang lebih tinggi dalam fasa pelarut dan seterusnya lebih banyak hasil pengekstrakan telah diperoleh. Analisis fitokimia kualitatif menunjukkan kewujudan alkaloid, saponin, triterpenoid, fenolik hidrokuinon dan tanin tetapi tiada flavonoid. Ujian ketoksikan biji *Swietenia mahagoni* yang diekstrak menggunakan assai 3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolium bromida ke atas sel fibroblas kulit manusia (HSF 1184) menunjukkan kebolehhidupan sel melebihi 80%. Ini menunjukkan ekstrak biji *Swietenia mahagoni* pada kepekatan yang berbeza (10, 1, 0.1, 0.01, 0.001, 0.0001 mg / ml) adalah tidak toksik.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
C	-	Concentration
CO_2	-	Carbon Dioxide
HSF	-	Human Skin Fibroblast
LM	-	Logistic Model
MAE	-	Microwave-Assisted Extraction
MEM	-	Modified Eagle Medium
MOX	-	Malaysian Oxygen
OEC	-	Overall Extraction Curve
PAF	-	Plattelet-Activating Factor
PBS	-	Phosphate Buffer Saline
RSM	-	Response Surface Methodology
SC-CO_2	-	Supercritical Carbon Dioxide
SFE	-	Supercritical Fluid Extraction
SSP	-	Simple Single Plate
TPC	-	Total Phenolic Content
UAE	-	Ultrasonic-Assisted Extraction
USA	-	United State America

LIST OF SYMBOLS

a, b, c	-	Constants in the Density Based Model
°C	-	Degree celcius
D	-	Diffusivity coefficient
k	-	Associated Number
K	-	Kelvin
M_A	-	Molecular weight of the solute
M_B	-	Molecular weight of the gas solvent
MPa	-	Mega Pascal
ρ	-	Density
P	-	Pressure
R	-	Gas constant
T	-	Temperature
Y	-	Oil yield

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Traditional plants have contributed to development of novel pharmaceutical, neutraceutical and cosmetic applications. Plant poses a vast array of natural products, bioactive primary and secondary metabolites. Seed is part of plant that important as primary stage of plant life cycle. Therefore, it has strong defense mechanisms. It is dues to attributions of biologically active phytoconstituents in them.

Swietenia mahagoni comes from Meliaceae family is a large, deciduous, and economically important timber tree native to the West Indies which is commonly known as mahogani. *Swietenia mahagoni* is a medium-sized semi-evergreen tree growing to 30–35 meters tall. The leaves are pinnate, 12 to 25 centimeters long, with four to eight leaflets. The flowers are small, produced in panicles. The fruit is a woody capsule 5 to 10 centimeters long and 3 to 6 centimeters broad, containing numerous winged seeds (Khare *et al*., 2012).

Swietenia mahagoni seed has potentials as antimicrobial activity, antioxidant activity (Mayur *et al*., 2011), cytotoxic activity, antiulcer activity, antifungal activity, anti-HIV activity, anti-inflamatory and antipyretic activity (Majid *et al*., 2004), and hypoglycemic activity (Sahgal *et al*., 2009; Debasis *et al*., 2011). *Swietenia mahagoni* seed was traditionally used as antidiabetic in several countries including

Malaysia, Indonesia, China and India. Furthermore, studies on antidiabetic potential of *Swietenia mahagoni* seed extracts on diabetic induced rats have been done and it shows a positive result (Debasis *et al.*, 2011). From the previous study, *Swietenia mahagoni* afforded with two limonoids which are swietenolide and 2-hydroxy-3-O-tigloylswietenolide (Tan, 2009).

Due to fast development, people have better understanding on natural products. Thus, many researched have been conducted in studying the active natural products as medicines, food additives, cosmetic application and natural pesticides. Extraction is a separation process of oil from plant. There are two categories of extraction, conventional and non-conventional methods. Compared with conventional method of extraction, supercritical fluid extraction has gained wide acceptance in many analytical and industrial processes. Moreover, supercritical fluid extraction has been used for decades on extraction of essential oil from plants. Previous literature reported that the applications of supercritical fluid extraction have focused more on edible oil extraction as examples, palm kernel oil (Hassan *et al.*, 2000), black pepper (Dang *et al*., 2014), sunflower seed (Salgin *et al*., 2006), olive oil (Fornari *et al*., 2008) and Leptocarpha rivularis (Edgar *et al*., 2015).

Wai (2003) reported that supercritical carbon dioxide extraction has many beneficial as it has low toxicity, in expensive and not harm to nature. This is because, physical properties of carbon dioxide at supercritical state has low critical temperature and critical pressure made supercritical carbon dioxide environmentally friendly and green solvent.

1.2 Problem Statement

Swietenia mahagoni has been used traditionally as treatments such as diabetes, asthma, eczema, premenstrual syndrome and migraine. Especially seed, it has potential of antioxidant activity, antimicrobial activity, antifungal activity and

hyperglycemic activity. Selection of extraction method is crucial to ensure the extract in high purity, rich with components and non toxic.

Supercritical fluid extraction method has been used for separation and extraction of essential oil from plants commercially. But, the high capital, high operating investment and higher pressure needed in the process contributes to problem for commercial. Temperature and pressure are two important parameters that will control whole extraction process. Size of particles also plays important roles for extraction yield. Carbon dioxide is selected as solvent for extraction process. Therefore, optimization of extraction process is crucial. Moreover, different plants have different optimization process especially on pressure, temperature, sample particle size and polarity of compounds.

Carbon dioxide as supercritical fluid as extraction solvent has low viscosity and higher diffusivity compared to other liquids. Moreover, supercritical fluid has beneficial as it has great transport properties which can diffuse through solids more easily compared to liquid. For optimizing the operating conditions and extraction process design a vase area of knowledge on the mechanism and kinetics of extraction processes is required. Mathematical modelling of extraction processes from different herbaceous materials has been of great importance for design purposes because it allows generalization of the experimental results and successful prediction of the extraction kinetics. A mathematical model based on the second Fick's Law, which was introduced by Crank, has been widely used to describe the process of unsteady diffusion in the solid phase for different extraction processes and particle geometries. From constant temperature and pressure parameter, the best diffusivity coefficients of sample with different particle size will be chosen for optimization process.

1.3 Objectives of Research

The researched is conducted based on following objectives;

1. To investigate the effect of diffusivity coefficients on particle size of *Swietenia mahagoni* seed using diffusivity model.

2. To study the effect of pressure and temperature on extraction yield and biological analysis of *Swietenia mahagoni* seed.

1.4 Scopes of Research

The extraction of *Swietenia mahagoni* seeds was done at selected range of conditions using supercritical carbon dioxide extraction. In order to achieve the objectives stated in section 1.3, the scopes of study are as follows:

1. Extraction of *Switenia mahagoni* seeds using supercritical carbon dioxide extraction process

2. Determination of diffusivity coefficients of carbon dioxide at different particle size of Swietenia mahagoni seeds by application of mathematical model second Fick's law proposed by Crank.

3. Optimization of pressure and temperature on extraction process using Response surface methodology (RSM)

4. Statistical analysis using ANOVA

5. Biological analysis on quality of phytochemicals and toxicity of *Swietenia mahagoni* seed oil.

1.5 Thesis Summary

This thesis is divided into five main chapters. Chapter 1 is the introduction of the research that included background of the research, problem statement, objectives

and scopes of the research. Chapter 2 included the overview of *Swietenia mahagoni*, extraction process involved, mathematical modeling namely Crank model and process optimization. Chapter 3 discussed the overall methods used in the research including the procedures of chemicals and raw material preparation, extraction process involved (soxhlet and supercritical carbon dioxide extraction), diffusivity measurement and modeling, and process optimization also biological study methods on qualitative phytochemical and toxicity of *Swietenia mahagoni* seed oil. Chapter 4 discussed the results and discussion for overall experiments while the conclusion and some recommendations were discussed in Chapter 5.

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