STUDIES ON THE OIL YIELD, ANTIOXIDANT ACTIVITIES AND SOLUBILITY FROM THE EXTRACTION OF *Pithecellobium Jiringan Jack* SEEDS USING SUPERCRITICAL CARBON DIOXIDE

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To my beloved family and friends

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ABSTRACT

Studies on the effects of oil yield, antioxidant activities and solubility from the extraction of *Pithecellobium Jiringan* (Jack) *Prain* (*P.Jiringan)* seeds by using supercritical carbon dioxide (SC-CO_2) were carried out at temperatures and pressure ranging from 40 ^oC to 70 ^oC, and 27.6 MPa to 44.8 MPa, respectively. Two grams of ground *P.Jiringan* seeds with the particle size of 215 µm and moisture content of 3.5% were used in this study. The extraction process was carried out for 60 minutes. The antioxidant activities of *P.Jiringan* oil in SC-CO_2 were analyzed by using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) method. The study showed that the maximum oil yield was 89.9 mg, at a pressure and temperature of 44.8 MPa and 70 ^oC, respectively. Moreover, the highest antioxidant activity was 55.4% for scavenging activity at 34.5 MPa and 60 ^oC. In addition, the dominant factor for oil yield was pressure, while that for antioxidant activity was temperature. In this study, the solubility data of *P.Jiringan* oil in supercritical CO_2 was measured by plotting experimental data of mass of oil extracted against the mass of CO_2 used. At constant temperature, the solubility of oil increased as the density of CO_2 increased. Meanwhile, at constant pressure, the solubility of oil increased as the temperature increased even though the density of CO_2 decreases. The experimental solubility data was correlated with Chrastil model and del Valle-Aguilera model. The solubility was correlated with Chrastil model at average absolute percent deviation (AAPD%) of 0.213% as compared to the del Valle Aguilera model with AAPD% of 0.570%.

ABSTRAK

Kajian kesan tekanan dan suhu ke atas kesan hasil minyak, aktiviti antioksida dan keterlarutan dari pengekstrakkan *Pithecellobium Jiringan* (Jack) *Prain* (biji jering) melalui kaedah penyarian bendalir lampau genting dengan menggunakan karbon dioksida (CO_2) sebagai pelarut telah dijalankan pada suhu dan tekanan masing-masingnya di antara 40 ^oC sehingga 70 ^oC dan 27.6 MPa sehingga 44.8 MPa. Dua gram biji jering dikisar dengan garispusat 215 μm dengan kadungan lembapan 3.5% telah digunakan. Proses pengekstrakkan telah dijalankan selama 60 minit. Kajian berkaitan aktiviti antioksida minyak jering telah dijalankan dengan menggunakan kaedah 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH). Kajian menunjukkan bahawa hasil maksimum minyak jering yang dapat dihasilkan ialah 89.9 mg pada tekanan dan suhu masing-masingnya 44.8 MPa dan 70 ^oC. Tambahan pula, pada tekanan 34.5 MPa dan suhu 60 ^oC, aktiviti antioksida yang tertinggi dihasilkan ialah 55.4%. Di samping itu, faktor dominan bagi hasil minyak ialah tekanan manakala bagi aktiviti antioksida pula ialah suhu. Dalam kajian ini, data keterlarutan minyak jering di dalam CO_2 lampau genting telah dikira dengan membina graf data experimen jisim minyak yang diekstrak terhadap jisim CO_2 yang digunakan. Pada suhu malar keterlarutan minyak meningkat dengan ketumpatan CO_2 meningkat. Sementara itu, pada tekanan malar, keterlarutan minyak meningkat walaupun ketumpatan CO_2 menurun. Data keterlarutan minyak yang diperolehi telah dikolerasi dengan menggunakan model Chastil dan model del Valle-Aguilera. Data keterlarutan minyak telah berjaya dipadankan dengan model Chrastil dengan purata peratus sisihan mutlak (PPSM%) 0.213% berbanding model del Valle-Aguilera dengan nilai PPSM% bersamaan 0.570%.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
SC-CO_2	Supercriticl carbon dioxide
SFE	Supercritical fluid extraction
ASE	Accelerated Solvent Extraction
DPPH	Accelerated Solvent Extraction
	2,2-Diphenyl-1-Picrylhydrazyl
P.Jiringan	*Pithecellobium Jiringan* (Jack) *Prain*
AAPD	Average absolute percent deviation
O_2	Oxygen
CO_2	Carbon dioxide
HAT	hydrogen atom transfer
SET	single electron transfer
ORAC	Oxygen Radical Absorbance
TRAP	Total Peroxyl Radical-Trapping Antioxidant Parameter
CUPRAC	Copper Reduction Assay
FRAP	Ferric Reducing Antioxidant Power
TEAC	Trolox Equivalent Antioxidant Capacity
HD	Hydro distillation
HPLC	High-performance liquid chromatography
CER	Constant extraction rate
FER	falling extraction rate
DOE	Design of experiment
VCO	Virgin coconut oil

BHA	Butylated hydroxyanisole
LDL	Low density lipoprotein
AH	Antioxidant
R•	Radical species
MCTs	Medium-chain triglycerides
RVE	Rotary vacuum evaporator
PORIM	Palm oil research institute of Malaysia
RPM	Revolution per minute
MAE	Microwave
PAHs	Polycyclic aromatic hydrocarbons
ABS	Absolute
CCD	Central composite design
ANOVA	Analysis of variance
UV-Vis	Ultra-violet visible
DE	Diatomic Earth
R134a	1,1,1,2-Tetrafluoroethane
CCC	tricaprylin
MMM	trimyristin
PPP	tripalmitin
OOO	triolein
SSS	tristearin
LLL	trilaurin

LIST OF SYMBOLS

%	-	Percentage
Y%	-	Percentage of overall oil yield
M_c%	-	Percentage of moisture content
Y_s	-	Solubility
m_0	-	Mass in grams of dish
m_1	-	Mass in grams of dish and sample before drying
m_2	-	Mass in grams of dish and sample after drying
W_{oil}	-	Weight of extracted oil, g
W_i	-	Weight of sample before extraction, g
W_f	-	Weight of sample after extraction, g
Mm	-	Micro meter
G	-	gram
mL	-	mililiter
MPa	-	Mega pascal
K	-	kelvin
^oC	-	Degree celcius
P_c	-	Critical pressure
T_c	-	Critical temperature
IC_{50}	-	half maximal inhibitory concentration
X_i	-	Variables
Y_i	-	Responses
d_1	-	degree of freedom of regression
d_2	-	degree of freedom of residue
h/hr	-	hour

ft	-	feet
C	-	Critical point
p	-	probability
wt%	-	Weight percentage
Kg/h	-	Kilogram per hour
α	-	Alpha
β	-	Beta
γ	-	Gamma
δ	-	Delta
mm	-	Millimeter
nm	-	nanometer
N_2O	-	Nitrogen oxide
NH_3	-	Ammonium
N_2	-	Nitrogen
ppm	-	Part per million
psi	-	Pound per square inch
ρ	-	density
ΔH_{vap}	-	Heat of vaporization
ΔH_{solv}	-	Heat of salvation
ΔT	-	Increment of temperature
ΔP	-	Increment of pressure
Δt	-	Increment of time
>	-	More than

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Malaysia has about 12,000 species of plants of which about 1,300 are said to be medicinal (Burkill, 1935), and only a small number of these plants have been fully investigated for their potential. One of them is *Pithecellobium Jiringan (Jack) Prain.*

Pithecellobium Jiringan (*P.Jiringan*) is not normally considered to be a medicinal plant as it contains undesired compounds such as volatile oil and also toxic acid namely djenkolic acid (Mohd, 2007). Even though the *P.Jiringan* seeds contain undesired compounds, it is also rich in essential compounds including protein, carbohydrates, fiber, calcium, phosphorus, vitamins and sterols which make it possible to be developed into medicinal drugs especially for anti-diabetics and anti-hypertension (Vimala *et al.,* 2003).

Nowadays the studies on natural products are one of the most sought after research in this world. As we know, plant based products have been used throughout the world to treat many diseases. According to Roslina (2010), studies have revealed crucial findings such as anti-oxidant, anti-cancer and anti-diabetic substances, mosquito pheromones and ways to reduce fuel usages.

Many years ago, conventional separation methods such as steam distillation, water extraction, evaporation and spray drying have been used to extract bioactive compounds from medicinal plants. These methods could not guarantee the production of a safe product. The use of these conventional methods was not sufficient to achieve the exact separation between the desired and the undesired compounds (Mohd, 2007).

Nowadays, advance technologies such as membrane separation, ultrasonic-assist extraction, molecular distillation, supercritical fluid extraction (SFE), polymeric adsorbent technology and accelerated solvent extraction (ASE) have been used to improve high quality products and to reduce the amount of solvent required. This study focuses on supercritical fluid extraction technology which carbon dioxide, CO_2 act as a solvent. In addition, ASE and Soxhlet extraction were used as a comparison method.

Supercritical CO_2 (SC-CO_2) extraction is the most commonly use for SFE application because of its moderate critical properties that include: 1) critical temperature at 31.1 °C and 2) critical pressure at 7.38 Mpa. With critical temperature less than body temperature (37 ^{o}C), little thermal degradation of sensitive compounds occurs especially for natural antioxidants. Besides that, SC-CO_2 extraction is nontoxic, chemically inert and available in high purity at low cost. These properties make SC-CO_2 extraction as an 'environmental friendly' technology to extract natural products. In many countries, more strict regulations regarding the use of organic solvents (eg: hexane) to address safety, health and environmental issues is forcing the industry to search for alternative processes. Therefore, as expected the 21st century is a tertiary peak with the mounting interest in the application of supercritical fluid extraction technology.

In the SFE system it contains of a pump for the CO_2, a pressure cell to containing the sample, a means of maintaining pressure in the system and a collecting vessel. CO_2 is pumped to a heating zone, where it is heated to supercritical conditions. It then passes into the extraction vessel, where it quickly diffuses into the solid matrix and dissolves the material to be extracted. The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out. The CO_2 can then be cooled, re-compressed and recycled, or discharged to atmosphere. Usually, SFE can be completed in 10 to 60 minutes. This is due to the solvent used in supercritical condition. The diffusivities are much faster in supercritical fluids than in liquids and therefore extraction can occur faster. Besides, in supercritical condition, CO_2 has no surface tension and viscosities are much lower than in liquids, so the solvent can penetrate into small pores within the matrix inaccessible to liquids. Both the higher diffusivity and lower viscosity significantly increase the speed of the extraction (Skoog, 2007).

Meanwhile, ASE is one of the advanced technologies in separation process that required small volume of solvent and give the faster extraction process. The ability in increasing the sample extraction kinetics by performing solid–liquid extractions at temperatures above the solvent's boiling points, ASE significantly reduces extraction times (Dionex, 2004). ASE utilizes high pressure to maintain the solvent liquid at higher temperatures and also to increase the matrix insight by the solvent. The combination of high temperature and pressure changes the ASE extractor to perform fast extractions with minimum solvent usage while ensuring excellent analyte recoveries. The efficiency of ASE in extracting oil depends upon the ASE operating parameters such as extraction temperature, extraction pressure and static extraction time.

Furthermore, soxhlet extraction process is one of the conventional methods of separation processes. This process was studied to compare between advanced technology and conventional technology. During soxhlet extraction, the vapors of the solvent were generated from the round-bottomed flask, pass through the thimble and get condensed in the condenser. The extraction occurs when condensed fresh solvent comes in contact with the *P.Jiringan* seeds in the thimble. When the liquid reaches the overflow level in the thimble, the liquid moves through the siphon back into the reservoir, carrying extracted solutes into the bulk liquid.

1.2 Problem Statement

P. Jiringan seeds are good sources of natural antioxidants. Previous researcher claimed the presence of vitamin E as one of the major component in the extracted oil from *P. Jiringan* seeds using SC-CO_2 extraction. However, the level of the antioxidant activity has not been studied. To-date, no studies have been done on the mathematical modeling for the extraction of oil from *P.Jiringan* seeds using SC-CO_2 extraction. In this research, the main goals were to determine the best condition of SC-CO_2 extraction that gives the highest level of antioxidant activity and to represent the solubility behavior with an appropriate model.

1.3 Objectives

The objectives of the research are:

a) To investigate the effects of pressure and temperature of SC-CO_2 extraction on oil yield and antioxidant activity of extracted oil from *P. Jiringan* seeds.
b) To determine the best combination of SC-CO_2 condition (pressure and temperature) extracting high oil yield and the antioxidant activity level of extracted oil from *P. Jiringan* seeds.
c) To apply the solvent density-based model (Chrastil Model and del Valle and Aguilera Model) on the solubility of *P.Jiringan* oil in SC-CO_2.

1.4 Scope of Research

The scopes of research are:

a) To study the pre-treatment process involving in sample preparation.

b) To determine the best extraction time and flow rate in SC-CO_2 extraction of oil from *P.Jiringan* seeds.

c) To extract oil from *P.Jiringan* Seeds using SC-CO_2 extraction, Accelerated Solvent Extraction (ASE) and Soxhlet extraction.

d) To evaluate the antioxidant activities using 2, 2-Diphenyl-1-Picrylhydrazyl, DPPH method.

e) To determine the correlation of the solubility of *P.Jiringan* oil behavior with a Chrastil's model and del Valle and Aguilera model.

1.5 Thesis Summary

This thesis is divided into five chapters. Chapter 1 is the introduction of the overall project. It contains the research objectives and scope of study after the research problems are clearly identified. Chapter 2 gives the review of the raw material used, antioxidant components and characteristic, process involved and mathematical modeling of the extraction curve of oil seeds. Chapter 3 describes the research methodology of the project. This chapter discusses details experimental procedures of field sampling, sample preparation, particle size, moisture content determination, and process involved which are soxhlet extraction, accelerated solvent extraction and supercritical CO_2 extraction and antioxidant activity analysis using the DPPH method. The results and discussion fully discuss in chapter 4. Lastly, chapter 5 gives the conclusion of the overall study and also some recommendation.

1.6 Research Contribution

The contributions of this research are:

a) The knowledge and study on the SC-CO_2 condition to obtain the high oil yield and high level of antioxidant activity was very useful to be applied in the pharmaceutical and nutraceutical industry.

b) The coefficient value (*a*, *b*, *c* & *k*) of Chrastil and del Valle & Aguilera model were established through this study and important in industrial design and scale up purpose

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