

**EXTRACTION OF OIL FROM *CALOPHYLLUM INOPHYLLUM* SEEDS:  
OPTIMIZATION AND KINETICS STUDIES OF ULTRASONIC-ASSISTED  
EXTRACTION**

**by**

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## LIST OF ABBREVIATIONS

BBD	Box-Behnken design
CV	Coefficient of Variance
DOE	Design of experiment
GAE	Gallic acid equivalents
GC-MS	Gas chromatography-mass spectrometry
HIV	Human immunodeficiency virus
L/S	Liquid to solid
MAE	Microwave-assisted extraction
MRPD	Mean relative percentage deviation
NIST	National Institute of Standards and Technology
OFAT	One-factor-at-a-time
PSE	Pressure-assisted solvent extraction
Rpm	Revolution per minute
RSM	Response surface methodology
SE	Soxhlet extraction
SFE	Supercritical fluid extraction
UAE	Ultrasonic-assisted extraction

## LIST OF SYMBOLS

$b$	washing coefficient in Unsteady diffusion model
$B$	constant incorporating the characteristics of the carrier–active agent system in Power Law model
$\beta_0$	intercept of the BBD model
$\beta_i, \beta_{ii}, \beta_{ij}$	regression coefficients of BBD model
$C_0$	initial yield of oil at $t = 0$
$C_1$	oil yield at equilibrium for the washing step
$C_2$	oil yield at equilibrium for the diffusion step
$C_t$	oil yield at any time, $t$
$\text{CO}_2$	carbon dioxide
$i$	values from 1 to the total number of variables
$j$	values from 1 to the total number of variables
$k$	slow diffusion coefficient
$K_1$	Peleg's rate constant
$K_2$	Peleg's capacity constant
$k_1$	mass transfer coefficient for the washing step
$k_2$	mass transfer coefficient for the diffusion step

$m_i$	initial mass of seeds sample
$m_d$	final mass of seeds sample
$n$	diffusional exponent in Power Law model
$N$	number of observations
$q_o$	total oil content in the seeds
$q$	oil content in the seeds during the extraction
$R^2$	determination coefficient
$X_i$	linear terms of BBD model
$X_i^2$	quadratic terms for a single variable of BBD model
$X_i, X_j$	interaction terms for variables of BBD model
$y_o, y_1$	parameters in Parabolic Diffusion model
$t$	time
$Y, y$	response variable



**PENGEKSTRAKAN MINYAK DARIPADA BIJI *CALOPHYLLUM*  
*INOPHYLLUM*: KAJIAN PENGOPTIMUMAN DAN KINETIK BAGI  
PENGEKSTRAKAN ULTRASONIK**

**ABSTRAK**

Minyak daripada biji *Calophyllum inophyllum* (*C. inophyllum*) telah diekstrak dengan menggunakan dua teknik yang berbeza iaitu, teknik pengekstrakan ultrasonik (UAE) dan pengekstrakan Soxhlet (SE). Hasil ekstrakan minyak tertinggi yang telah direkodkan daripada teknik pengekstrakan ultrasonik adalah  $55.44 \pm 0.53$  % pada masa pengekstrakan 20 minit, suhu pengekstrakan 40 °C, kuasa ultrasonik 210 W, nisbah cecair kepada pepejal (L/S) 20 mL/g dan *n*-heksana sebagai pelarut. Manakala,  $70.21 \pm 0.36$  % hasil ekstrakan telah diperolehi bagi pengekstrakan Soxhlet pada masa pengekstrakan 240 minit, suhu pengekstrakan 85 °C, nisbah cecair kepada pepejal 35 mL/g dan *n*-heksana sebagai pelarut. Minyak biji *C. inophyllum* yang diekstrak daripada kedua-dua teknik mengandungi jenis asid lemak yang sama, iaitu asid oleik, asid palmitik, asid linoleik, asid stearik dan asid behenik. Pengoptimuman bagi proses pengekstrakan ultrasonik telah dikaji dengan menggunakan kaedah permukaan sambutan (RSM) melalui reka bentuk 'Box-Behnken' (BBD). Ramalan hasil minyak maksimum sebanyak 56.20 % telah diperolehi pada 21 minit masa pengekstrakan, 42 °C suhu pengekstrakan, 210 W kuasa ultrasonik, 21 mL/g nisbah cecair kepada pepejal dan *n*-heksana sebagai pelarut. Untuk kajian kinetik bagi proses pengekstrakan ultrasonik, model Patricelli didapati sangat berpadanan dengan data pengekstrakan dengan  $R^2 > 0.974$  dan nilai MRPD  $< 10$  %. Oleh itu, ia sesuai digunakan untuk menggambarkan proses pengekstrakan minyak daripada biji *C. inophyllum*.

**EXTRACTION OF OIL FROM *CALOPHYLLUM INOPHYLLUM* SEEDS:  
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**ABSTRACT**

*Calophyllum inophyllum* seeds oil was extracted using two different extraction techniques, namely, ultrasonic-assisted extraction (UAE) and Soxhlet extraction (SE). The highest oil yield attained from UAE was  $55.44 \pm 0.53$  % at an extraction time of 20 min, extraction temperature 40 °C, ultrasonic power 210 W, liquid to solid (L/S) ratio 20 mL/g and *n*-hexane as the solvent. Meanwhile,  $70.21 \pm 0.36$  % of oil yield was obtained from SE when the extraction time was 240 min, extraction temperature 85 °C, L/S ratio 35 mL/g and *n*-hexane as the solvent. *C. inophyllum* seeds oil extracted from these two techniques contained similar types of fatty acids such as oleic acid, palmitic acid, linoleic acid, stearic acid and behenic acid. The UAE process of *C. inophyllum* seeds oil was further optimized using Response Surface Methodology (RSM) via Box-Behnken design (BBD). The maximum predicted oil yield of 56.20 % was obtained at an extraction time of 21 min, extraction temperature 42 °C, ultrasonic power 210 W, L/S ratio 21 mL/g with *n*-hexane as the solvent. For the kinetics evaluation of UAE process of *C. inophyllum* seeds oil, it was found that the Patricelli's model fitted well to the extraction data with  $R^2 > 0.974$  and MRPD value  $< 10$  %. Thus, it is adequate to describe the extraction process of oil from *C. inophyllum* seeds.

# CHAPTER 1

## INTRODUCTION

### 1.1 Research Background

Since ancient times, plant materials such as juices, gums, fatty oils and essential oils are considered as a rich source of natural, effective and safe medicines (Tiwari, 2008). Fatty oils, usually obtained from vegetable sources are essential in human diets because of their nutritional and functional properties against numerous diseases and microorganisms (Li et al. 2014). They are composed of triglycerides of different fatty acids, accompanied by monoglycerides, diglycerides and free fatty acids. In small amounts, they may contain phospholipids, free sterols, tocopherols (tocopherols and tocotrienols), triterpene alcohols, hydrocarbons and fat soluble vitamins (Badea et al., 2015). Conventionally, vegetable oil extraction is carried out by mechanical pressing or solvent extraction method (Uquiche et al., 2008). Vegetable oils are an important and widely exploited source of lipids, vitamins, waxes, pigments, lecithins and hydrocarbons (Cerutti et al., 2012). They are normally isolated from the seeds of oleaginous plants and cereals (Avelar et al., 2013). In addition, it is also possible to isolate them from fruits, such as palm and olive (Trindade et al., 2015).

Vegetable oils offer tremendous benefits in broad range of applications. In fact, they have gained great popularity as consumers have developed a particular growing awareness towards the use of natural ingredients in their daily used products (Turek and Stintzing, 2013). Numerous seed oils from non-conventional sources, recovered from a wide variation of plants, have been locally used for food, pharmaceutical and cosmetic purposes (Nonviho et al., 2015). Other than that, they are used as an

alternative to liquid fossil fuels, and the products of transesterification of such oils with alcohols are now widely used as biodiesel (Hellier et al., 2015). In the polymer industry, the applications of these oils as lubricant, plasticizer or as other types of additives for polymer materials have also been practiced worldwide (Gobin et al., 2015).

Nut oil, seed oil and oil of fruits and vegetables are receiving significant attention due to their high concentration of bioactive lipid components, such as polyunsaturated fatty acids and phytosterols, which have shown various health benefits (Straccia et al., 2012). Therefore, these oils have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing (Saravanan et al., 2011). They have been considered as an alternative to treat infectious diseases and some of them have documented to possess pharmacologically important activities including antimicrobial, antifungal and antitumor properties (Nair et al., 2005; Cordova-Albores et al., 2014). Seed oils are beneficial in the prevention and treatment of several diseases including arthritis, diabetes and hypertension (Niu et al., 2014). In addition, they are also being used widely in skin care products, such as lotions, moisturizers and soothing creams (Palla et al., 2014).

Today, due to the interdisciplinary approaches through research and technological innovations in oleo-chemistry, biosciences, biotechnology and engineering, it is possible to design eco-friendly specialty chemicals from nature's abundant renewable resources (Alam et al., 2014). Vegetable oils are considered as a promising alternative to conventional fuel because of their similar properties (Melo-Espinosa et al., 2015). Such oils include soybean oil, palm oil, sunflower oil, safflower oil, rapeseed oil, coconut oil and peanut oil (Bhuiya et al., 2014). In the production of

polymers, vegetable oils have been identified as possible substitute for the petrochemical derivatives (Nicolau et al., 2009). They find innumerable industrial applications such as plasticizers, lubricants, adhesives, biodegradable packaging materials, printing inks, paints and coatings (Alam et al., 2014).

The use of plant oils have become increasingly important for scientific research and industrial applications in recent years (Chou et al., 2012). Plant oils are one of the important products of agriculture based industry such as biodiesel, food, pharmaceutical and cosmetic industries (Raut and Karuppayil, 2014; Rabiah Nizah et al., 2014). The World Health Organization (WHO) projected that nearly 80 % of the population in developing countries rely predominantly on medicinal plants for primary health care needs and sources for clinically useful drugs (Boukhatem et al., 2013; Salem et al., 2013). Additionally, modern pharmacopoeia also contains at least 25 % drugs derived from plants (Saravanan et al., 2011). Similarly, the demand for plant oils in the food industry are growing because of their important application as food preservatives (Asbahani et al., 2015). In particular, a great demand for vegetable oils is expected as they are highly valuable in the preparation of many value-added products (Maran and Priya, 2015). According to Ixtaina et al. (2011), despite the vast range of vegetable oils sources available, the world consumption was dominated by palm, soybean, rapeseed, and sunflower oils with 38.1, 35.7, 17.8, and 18.2 million tons consumed per year, respectively.

Nowadays, the natural product industries (food, cosmetics, personal care, beauty and pharmaceutical) have become a multibillion dollar international market (Martins et al., 2014). As a consequence, the global and national markets for natural oils are growing rapidly and significant economic gains are being realized (Kyarimpa et al., 2014). For instance, the total world production of important oilseeds is forecasted to

reach up to 395 million tonnes for the 2005 and 2006 crop year (Dumont and Narine, 2007). In addition, the production rate of essential oils of 40, 000 to 60, 000 tonnes per annum with estimated market value of 700 million US dollar indicates that the production and consumption of essential oils is also increasing (Raut and Karuppayil, 2014).

## 1.2 Problem Statement

The availability of sufficient amount and continuous supply of raw materials for vegetable oil production is important in order to meet the demand in various fields. Recently, numerous oil-bearing seeds including safflower (Han et al., 2009), pumpkin (Mitra et al., 2009), grape (Passos et al., 2010), pomegranate (Liu et al., 2012), hemp (Kostić et al., 2014), jatropha and moringa seeds (Kibazohi and Sangwan, 2011) were exploited industrially as an alternative to the conventional palm, soybean and rapeseeds as the main source of vegetable oils. According to Avram et al. (2014), the worldwide demand for oil seed production is expected to increase in the next thirty years due to the growing interests in using natural-based products in pharmaceutical, cosmetics, biodiesel and polymer industries. The response to the increasing demand for vegetable oil lead to the needs of exploring more alternative sources which are reliable and promising.

Therefore, the present study focus on the utilization of *Calophyllum inophyllum* (*C. inophyllum*) seeds as a potential alternative source of vegetable oil. This non-edible plant is well known for its high seeds oil content and it can be found abundantly in Malaysia. However, the seeds are currently underutilized and less exploited. *C. inophyllum* seeds make a great source of vegetable oil as the oil can be used in wide range of applications such as in cosmetic products, soap making, biodiesel production and in the pharmaceutical industry. In fact, the use of non-edible plants as a source of

vegetable oil have the advantage of being used for afforestation to reclaim wastelands and it does not compete with food crops for limited lands (Yang et al., 2014). Furthermore, they can be grown easily in barren soil with less maintenance and low moisture (Haldar et al., 2009).

Traditionally, the extraction of seed oils is performed using mechanical pressing and Soxhlet extraction (SE) techniques (Evon et al., 2013). Mechanical extraction process consumes high energy, requires high level equipment and produces lower extraction yield (Liu et al., 2009; Qu et al., 2013). Moreover, it is a time and labour intensive process (Jahirul et al., 2013). Meanwhile, SE technique is time consuming and requires a huge amount of organic solvent (Lopresto et al., 2014; Shao et al., 2014). Additionally, this technique may results in the degradation of target compounds due to its high operating temperature (Amirah et al., 2012). The problems associated with extraction performance in isolating oil in the aforementioned techniques lead to the investigation of an indirect ultrasonic-assisted extraction (UAE) technique which is more convenient and straightforward.

In recent years, UAE is considered as an inexpensive and simple extraction technique as it effectively extracts targeted components from plant materials and increased the extraction yield along with shorter time, lower operating temperature and less solvent consumption (Fakhari et al., 2010; Kamazani et al., 2014). The enhancement of extraction efficiency in UAE is attributed to a phenomenon of acoustic cavitation in which bubbles are produced in the solvent as a respond to an ultrasonic wave. During the application of ultrasound, the cavitation bubbles grow and finally collapse when they reach a critical size. The bubbles collapse produces a micro jet which travels through the solvent system and onto the solid surface, thus resulting in the cell wall disruption which allows better penetration of the solvent into solid

particles and promotes the release of intracellular compounds into the solvent (Wardhani et al., 2013).

The efficiency of oil extraction process from plant materials is strongly affected by several experimental factors including extraction time, extraction temperature, liquid to solid (L/S) ratio as well as type of solvent. However, detailed study on the influence of these critical parameters on *C. inophyllum* seeds oil extraction process in an UAE system is not being reported in the literature. From an engineering point of view, the knowledge gained in kinetics study of a process is highly beneficial for scaling up and industrial processing purposes. Nonetheless, an in-depth evaluation of the kinetics of UAE of *C. inophyllum* seeds oil is still scarce in the literature. Therefore, the main aim of this study is to evaluate the potential of UAE technique in extracting oil from *C. inophyllum* seeds and further optimize the extraction process parameters for better oil yield.

### **1.3 Research Objectives**

The measurable objectives in this study are:

- 1) To optimize the extraction parameters of UAE and SE process of *C. inophyllum* seeds oil using one-factor-at-a-time (OFAT) method.
- 2) To optimize the UAE process parameters of *C. inophyllum* seeds oil using RSM via Box-Behnken Design (BBD).
- 3) To evaluate the kinetics of UAE of *C. inophyllum* seeds oil using a mathematical model.

### **1.4 Scope of Study**

This study focused on the extraction of oil from *C. inophyllum* seeds by using UAE and SE techniques. Effects of different process parameters such as type of