OPTIMIZATION OF SOLVENT EXTRACTION AND MATRIX SOLID-PHASE DISPERSION METHODS FOR AGARWOOD OLEORESIN AND ESSENTIAL OIL PRODUCTION

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ABSTRACT

Agarwood essential oil is the most expensive resinous wood fragrance, indicating that it is extremely valuable. Generally, Malaysian producers use traditional method of hydro-distillation for extracting agarwood essential oil. However, this method has several limitations such as low product yield, essential oil quality inconsistency, time and energy consuming which resulting in a high operating cost of agarwood essential oil production. A low-grade agarwood (grade D) from the species of *Aquilaria malaccensis* was utilized in this study to improve the yield and quality of oleoresin and its essential oil using two consecutive solvent extractions, that were ethanol and n-hexane, respectively. The oleoresin was firstly extracted using reflux extraction, and then the essential oil was extracted using matrix solid-phase dispersion (MSPD). Based on their polarity, the combination of these solvents is the most ideal for producing high quality agarwood essential oil. Central composite design was used to investigate the effect of process parameters on extraction yield and the presence of chemical compound in the extract using regression analysis. Oleoresin yield of 7.08 ± 0.38 % (w/w) with total resin content of 5.75 ± 0.81 % (w/w) were obtained under the following optimal conditions: particle size of 0.5 - 1.0 mm, raw material to ethanol ratio of 1:29.9 g/ml and extraction time of 4.97 hours. Agarwood essential oil of 0.46 ± 0.10 % was achieved through optimization of essential oil extraction using the MSPD method at optimal condition: oleoresin to sorbent ratio of 1:1.46, oleoresin to n-hexane ratio of 1:39.19, extraction duration of two hours and applied silica gel as the solid support material. There were seven sesquiterpenes compounds detected through gas chromatography – mass spectrometry (GCMS) profiles for qualitative assessment, with the highest relative peak areas of 14.11 % and 16.80 % in oleoresin and essential oil extracts, respectively. This study revealed that both optimal extraction conditions produced the desired extracts in lesser extraction time and lower agarwood to solvent ratio. The best quality of agarwood essential oil obtained in improved extractions was proven by comparing with GCMS chromatogram pattern. There was a 3.67-fold increase in peak area % of the discovered seven compounds when compared to Soxhlet extraction, and the amount of 4-phenyl-2-butanone compound was found to be the highest (0.53 mg/ml) when compared to other analyzed extraction methods. Overall, the MSPD extraction approach was able to produce the highest yield of agarwood essential oil, with a 21.05 % increase of essential oil yield compared to the Soxhlet extraction. The findings of this study provide a new insight about mass transfer theory by observing the surface morphology and particle size distribution on oleoresin-silica gel blended powders prior to extraction. On top of that, the disrupted mixture sample could achieve a smaller particle size and increase solute diffusion in the dispersion process, resulting in a high essential oil yield and retain most of the agarwood components. In conclusion, the findings strongly suggested that extraction using MSPD is a potential strategy for improving the overall extract yield and quality in the production of agarwood essential oil.

ABSTRAK

Minyak pati gaharu adalah wangian kayu damar paling mahal yang menunjukkan bahawa ia sangat berharga. Umumnya, pengeluar- pengeluar di Malaysia menggunakan kaedah tradisional penyulingan hidro untuk mengekstrak minyak pati gaharu.Walaupun begitu, kaedah ini mempunyai beberapa batasan seperti hasil produk yang rendah, kualiti minyak pati gaharu yang tidak konsisten, serta penggunaan masa yang lama dan banyak tenaga yang mengakibatkan kos operasi yang tinggi dalam penghasilan minyak pati gaharu. Kajian ini menggunakan gaharu gred rendah (gred D) dari spesis *Aquilaria malaccensis* dengan pengekstrakan pelarut berturutan, iaitu etanol dan n-heksana untuk meningkatkan hasil serta kualiti oleoresin and minyak patinya. Oleoresin diekstrak terlebih dahulu menggunakan kaedah pengekstrakan refluks, dan kemudian minyak patinya diekstrak melalui kaedah penyebaran matriks fasa pepejal (MSPD). Berdasarkan aspek kekutuban pelarut, kombinasi pelarut-pelarut ini adalah yang paling sesuai dalam menghasilkan minyak pati gaharu yang berkualiti tinggi. Reka bentuk composit berpusat telah digunakan untuk menentukan kesan parameter proses pada hasil pengekstrakan dan kehadiran sebatian kimia dalam ekstrak melalui analisis regresi. Hasil oleoresin sebanyak 7.08 ± 0.38 % (w/w) dengan kandungan keseluruhan resin sebanyak 5.75 ± 0.81 % (w/w) telah diperoleh pada keadaan optimum: saiz partikel 0.5-1.0 mm, nisbah bahan mentah kepada etanol 1:29.9 g/ml dan tempoh pengekstrakan selama 4.97 jam. Sebanyak 0.46 ± 0.10 % minyak pati gaharu telah berjaya diperoleh melalui pengoptimuman pengekstrakan minyak pati menggunakan kaedah MSPD pada keadaan optimum: nisbah oleoresin kepada sorben 1:1.46, nisbah oleoresin kepada n-heksana 1:39.19, tempoh pengekstrakan selama dua jam dan menggunakan gel silika sebagai bahan sokongan pepejal. Terdapat tujuh sebatian *sesquiterpene* yang dikenalpasti untuk penilaian kualitatif melalui profil kromatografi gas – spektrometri jisim (GCMS) dengan nilai puncak relatif tertinggi 14.11 % dan 16.80 %, iaitu masing-masing bagi ekstrak oleoresin dan minyak pati. Penyelidikan ini juga mendapati bahawa kedua-dua prosedur pengekstrakan yang ideal menghasilkan ekstrak yang diingini dalam masa yang lebih singkat dan nisbah gaharu kepada pelarut yang lebih rendah. Kualiti minyak pati gaharu terbaik yang diperoleh dalam pengekstrakan optimum telah dibuktikan melalui kajian perbandingan menggunakan corak kromatogram GCMS. Terdapat kenaikan sebanyak 3.67 kali ganda dalam keluasan puncak bagi tujuh sebatian tersebut berbanding dengan kaedah pengekstrakan Soxhlet, dan sebatian 4-fenil -2-butanon diperoleh pada nilai yang tertinggi (0.53 mg/ml) berbanding dengan kaedah pengekstrakan lain yang dikaji. Keseluruhannya, pengekstrakan dengan kaedah MSPD mampu menghasilkan minyak pati gaharu yang tertinggi, dan peningkatan sebanyak 21.05 % hasil minyak berbanding dengan kaedah pengekstrakan Soxhlet. Hasil kajian ini menyediakan pandangan baru mengenai teori pemindahan jisim melalui pemerhatian terhadap morfologi permukaan dan taburan saiz partikel pada serbuk campuran oleoresin-gel silika sebelum pengekstrakan. Di samping itu, sampel yang terubah suai mampu memperoleh ukuran partikel yang lebih kecil dan memudahkan penyebaran zat terlarut dalam proses penyebaran, yang mana akhirnya dapat menghasilkan minyak pati yang banyak dan mengekalkan sebahagian besar komponen gaharu. Kesimpulannya, hasil penemuan ini menunjukkan bahawa pengekstrakan menggunakan MSPD adalah strategi yang berpotensi untuk meningkatkan hasil dan kualiti keseluruhan ekstrak dalam pengeluaran minyak pati gaharu.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
AO	-	Aluminum Oxide
ASE	-	Accelerated Solvent Extraction
B.C.	-	Before Christ
CAS-No	-	Chemical Abstracts Service Registry Number
CITES	-	Convention on International Trade in Endangered Species of Wild Flora and Fauna
DE		Diatomaceous Earth
DOE	-	Design of Experiment
et al	-	*et alia* (and others)
g.b.h.	-	Girth at Breast Height (girth at 1.3 m above ground)
GAE	-	Gallic Acid Equivalent
GC	-	Gas Chromatography
GC-FID	-	Gas chromatography - Fame Ionization Detector
GC-MS	-	Gas Chromatography – Mass Spectrometry
i.e.	-	In Other Words
LLE	-	Liquid - Liquid Extraction
MSPD	-	Matrix Solid-Phase Dispersion
NWFPs	-	Non-Wood Forest Products
OFSG	-	Octadecyl - Functionalized Silica Gel
PERHILITAN	-	Department of Wildlife and National Parks
PLE	-	Pressured Liquid Extraction
PNGFA	-	Papua New Guinea Forestry Authority
Reag. Ph Eur	-	Reagents section of the European Pharmacopoeia
RSM	-	Response Surface Methodology
SFE	-	Supercritical Fluid Extraction
SG	-	Silica Gel
US EPA	-	United States Environmental Protection Agency
USAE	-	Ultrasonic Assisted Extraction
UTM	-	Universiti Teknologi Malaysia
w/v	-	Weight per Volume

w/w	-	Weight per Weight
WM	-	Without Sorbent Material

LIST OF SYMBOLS

$	-	Singapore Dollar
%	-	Percent
$>$	-	Greater Than
$\leq$	-	Less Than and Equal to
$\geq$	-	Greater Than and Equal to
µg	-	Microgram
Cm	-	Centimetre
Ds	-	Solid Diffusion Coefficient
eg	-	For example
g	-	Gram
Hrs	-	Hours
K	-	Kelvin
k_L	-	Mass Transfer Coefficient
m	-	Meter
m	-	Mass
min	-	Minutes
ml	-	Millilitre
mm	-	Millimetre
N	-	Total Number of Run
ºC	-	Degree Celsius
P’	-	Polarity index
PS	-	Cohesion Energy Density
psi	-	Pounds Per Square Inch
RM	-	Ringgit Malaysia
s	-	Second
t	-	Time
Y	-	Response
Y	-	Yield
α	-	Alpha
β	-	Beta
γ	-	Gamma

δ	-	Solubility Parameter
ε	-	Random Error
η	-	Dynamic Viscosity
σ^2	-	Variance
Φ	-	Diameter
kg	-	Kilogram
kmol	-	Kilo mol

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CHAPTER 1

INTRODUCTION

1.1 General Background

Products from plants such as essential oils, rubbers, gums, gum-resins, resins, honey, fodder, wild fruits and some other materials are classified as minor forest products which are presently categorized as the Non-Wood Forest Products (NWFPs). Agarwood is a commodity of NWFPs with long documented history and widespread usage worldwide (Elias *et al*., 2017). The current global market for agarwood essential oil was estimated to be worth over US$ 201.03 million with the highest growth rate of 6.46% during the forecast period of 2019-2025 (MRFR, 2019). The demand for agarwood is rapidly increasing due to the growth in population, health awareness and affluence of agarwood-consuming market. In Malaysia, agarwood is being studied due to its potential in facilitating sustainable economic development. Malaysia is the 5^{th}-largest exporter of agarwood products in 2018 with the export value of RM 22 million for incense, RM 112 million for fragrances, and RM 9.93 million for agarwood chips (MRFR, 2019). The statistics show ample opportunities for local companies to be involved mainly in agarwood essential oil and its commercialisation products. Nowadays, aside from religious rituals, agarwood is widely used in cosmetics, medicine, perfume and aromatherapy industries due to its strong, unique and complex scent (Naef *et al*., 2011).

Agarwood is a dark resinous plant accumulated in the plants of the *Thymelaeaceae* family belonging to the *Aquilaria* genus (Nor Azah *et al*., 2013). The unique reaction of the trees towards damages caused by lightning strikes, fire, being cut by man, fungus or microorganism infection as well as insect disturbance results in the formation of agarwood resinous (Dai *et al*., 2010). Thus, agarwood rarely exists in naturally healthy, wild, and young trees, causing its resinous wood to be costly. Agarwood trees are large evergreen native to the South and South-East Asia. In Asia, there are 15 types of *Aquilaria* species recorded with the ability to produce agarwood (Nor Azah *et al*., 2008). Amongst them, the species of *A. malaccensis, A. microcarpa, A. hirta, A. rostrata* and *A. beccariana* can be mostly found in Peninsular Malaysia (Blanchette, 2006 and Barden *et al*., 2000). Locally known as Karas trees in Malaysia, agarwood had been long collected by the indigenous people of Peninsular Malaysia interior (i.e., Sarawak and Sabah) for domestic trade activities of these natural scented woods (Chua, 2008). It was reported that *A. malaccensis* is the most popular source in producing agarwood among the Karas trees and is extensively used for medicinal purposes (Barden *et al*., 2000; Lim and Anak, 2010).

The current price of agarwood products is costly due to the depletion of wild natural resources and concerns over future supplies. Agarwood trade primarily varies according to the geographical location sources (i.e., soil and climate) and cultural practices. Moreover, there is no valid standard exists. Criteria in determining the quality of agarwood products include chemical composition, fragrant, level of resins, level of essential oils, size, the form of flakes and fibres arrangements (Herlina and Siburian, 2017). Until now, essential oil quality, as well as its price, are arbitrarily determined by traders and buyers due to the numerous complexities of the agarwood trade nature (Ahmad *et al*., 2015). Theoretically, agarwood should be graded according to different classes based on its physical properties (i.e., colour and density), agarwood formation and unique scent while in reality, agarwood is graded according to the perceptions and experiences of individuals involved (Nor Azah *et al.*, 2013). There are four grades of agarwood, namely A, B, C and D in the descending order of quality and price. The price of grade A agarwood can reach up to RM16,000 - RM20,000 per kilogram. In Malaysia, agarwood of lower grades (i.e., C and D) with the physical appearance of natural dark yellow colour with stripes and whitish-yellow, respectively, are mostly used for its essential oil (Nor Azah *et al*., 2013).

Agarwood products in the market are usually in the form of chips, powders and incense sticks or manufactured extracts and oils. Agarwood extractives contain both semi-volatile (i.e., oleoresin) and volatile compounds (i.e., essential oils), which are highly demanded ingredient in perfumery due to its unique aromatic fragrance (Naef, 2011). Oleoresin content are the key compounds that make agarwood essential oils distinctive from other essential oils (Chetpattananondh, 2012). Aside from its oleoresin extraction, agarwood was mostly extracted for its expensive essential oil, which contains secondary metabolites such as sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, chromone derivatives etc (Nor Azah *et al*., 2013). There is a vast variety of methods available in extracting agarwood essential oil such as water or steam distillation, solvent extraction, percolation, carbon dioxide extraction, florasol or phytol extraction, supercritical fluid extraction, cold pressing, maceration, and others (Muhammad *et al*., 2013).

1.2 Problem Statement

Nowadays, there is no strict regulation that prevails in the processing of agarwood. Major Malaysian producers are still employing the traditional methods with an improvised system such as hydro-distillation and solvent extraction (e.g., Soxhlet) to extract the essential oils from agarwood (Nor Azah *et al*., 2008; Chua *et al*., 2016). Therefore, numerous studies on the enhancement or the exploration of the novel technique of agarwood extraction have been developed in order to improve the lack of current practice in the agarwood industry. Worse still is the fact that the quality and yield of agarwood extracts are woefully lacked as it arrives at the end-users. A study used Soxhlet extraction on *A. malaccensis* with a high grade of agarwood yielded high essential oil content, i.e., 14.5% (Muhammad *et al*., 2013). In the meantime, in a pilot study using supercritical fluid extraction (SFE) for agarwood *A. malaccensis* resulted in 0.6% essential oil yield within 2 hours, while using three days of hydro-distillation can produce only 0.2% oil yield (Che Daud and Hamdan, 2015). They discovered about eighteen to thirty-one compounds in the SFE extract of agarwood using GCMS. Obviously, agarwood essential oil yields obtained as presented in various literatures are different due to variances in terms of species and grades utilised during the extraction process.

Few efforts can also be found on the pre-treatment techniques before the extraction process in an attempt to investigate the cell wall breakage which was proven to improve the essential oil content. The developed pre-treatments also include the utilisation of water, ethanol, acid-alkaline, ultrasound, ionising, oxidation, steam explosion, and enzymatic hydrolysis in the soaking process prior to extraction. However, frustrating outcomes are still encountered by the agarwood trading market due to low product yield even with the utilisation of the proposed techniques. One study had revealed that the effectiveness of the soaking technique with water and three types of fungi on agarwood essential oil before hydro-distillation extraction demonstrated a higher average essential oil yield of 0.58% compared to the sample without soaking (i.e., only 0.19% essential oil yield) (Yusoff *et al*., 2015b).

In addition to process optimisation in extraction techniques, an approach of using other technology such as matrix solid-phase dispersion (MSPD) can also be explored in extracting agarwood. MSPD is a simple and flexible process for simultaneous disruption and extraction of mixtures of solid and semi-solid sample, which is very suitable for agarwood resin (Barker, 2000a). At present, there is limited study utilising MSPD in search of bioactive compounds in other plants or woods but even worse no information has been found on agarwood. Among the advantage of MSPD compared to the classical method of solid-liquid extraction is it provides less solvent usage for complete disruption or fractionation with shorter analysis time for sample preparation (Barker, 2007). In other words, MSPD eliminates several steps that do not need to be performed in order to reduce the interference compounds in the extract during analysis by chromatography based analytical methods. Thus, MSPD is deemed as an enhancement to the solid-phase extraction (SPE) technique, which offers efficient, fast, and straightforward implementation (Tu and Chen, 2018). In short, both techniques have similar principles but in different manners or mechanisms.

To address the issue of low product yield and inconsistent quality of agarwood resin and essential oils, the extraction technique used has to be improved and optimized in order to avoid high operating cost in the manufacturing process. The way to meet the high market demand and achieve the commercialised desires of agarwood essential oil is by doing optimization of current extraction techniques such as solvent extraction (i.e., reflux system) and conventional hydro-distillation (a comparison study). The best-operating conditions can be achieved via an experimental design that varies the significant parameters. By focusing only on lower grade of agarwood (i.e., grade D), a novel approach method of MSPD extraction was undertaken with the aim to improve the agarwood essential oil yield and quality of the extract. As the commercialisation of agarwood product multiplies, this work aims to develop an appropriate extraction method in producing high yield and standardised agarwood essential oil.

1.3 Objective of Study

The objectives of this study were as follows:

(i) To optimize the processing conditions for the solvent extractions of agarwood oleoresin and essential oil in order to obtain high yield and the best quality.
(ii) To establish the matrix solid-phase dispersion (MSPD) technique for standardising the agarwood essential oil production.

1.4 Scopes of the Study

In order to accomplish the objectives, the scope of the study is divided into five main parts:

(i) Screening and selecting the appropriate solvents for oleoresin and essential oil extractions, respectively.
   a. To study the effect of different type of solvents (i.e., polar solvents for extraction of agarwood oleoresin and non-polar solvents for agarwood essential oil extraction).

(ii) Investigating the effects of solvent extraction parameters on the extraction yield of agarwood oleoresin. The specific steps are:

a. To study the effect of extraction parameters such as solvent to raw material ratio, particle size and extraction duration on the yield of extraction.

b. To determine the optimum extraction conditions in achieving the desired oleoresin yield and quality.

(iii) Investigating the effects of MSPD extraction parameters on agarwood essential oil yield. The specific steps are:

a. To identify the most appropriate sorbent material.

b. To determine the effects of significant parameters such as MSPD extraction duration, oleoresin to sorbent material ratio, and oleoresin to solvent ratio.

c. To investigate the optimum extraction conditions in achieving the desired agarwood essential oil yield and quality.

(iv) Comparing the optimum extraction values with the conventional hydro-distillation technique in terms of,

a. Identification of chemical compounds for each sample.

b. Amount determination of each compound.

(v) Determining the mass transfer coefficient of agarwood essential oil extraction in MSPD extraction method through:

a. Characterisation of particle morphology of oleoresin sorbent powder using image analysis.

b. Determination of zeta potential value and particle size of oleoresin sorbent powder using different types of the sorbents.

c. Extraction curves and the rate of mass transfer coefficients analysis.

1.5 Significances and Original Contributions of the Study

This study offers several contributions in the field of processing technology and engineering, specifically in optimising and the production of enhancing agarwood extract associated with essential oil yield and end-product quality.

(i) To the best of the current knowledge, this study is the first to report on the specific establishment of MSPD extraction on agarwood through the implementation of solid-phase dispersion of the sample matrix with abrasive solid support to selectively elute the desired components. MSPD versatility and straight forward technique allows this extraction process to be applied to an extensive range of chemical compounds from an also wide range of agarwood grades in future. Basically, most studies reported in the literatures only emphasise on the optimisation and kinetic studies using the popular hydro-distillation method in the extraction of agarwood essential oil.

(ii) The extraction of agarwood has always been a challenging task for researchers due to unclear guidelines in preparing the final extract according to the application of the products. Non-standardized procedures of extraction may lead to degradation and variability of phytochemicals present in agarwood, thus governing to the lack of its reproducibility. Therefore, an effort to produce batches at approximate consistent quality can be replaced with a better way such as exploiting the MSPD. This is an attractive method towards the development of a standardised processing method in agarwood essential oil production with the best quality of its chemical compounds in the future.

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