OPTIMIZATION OF PRE-TREATMENTS OF EXTRACTION METHODS FOR QUALITY OF AGARWOOD (*AQUILARIA MALACCENSIS*) ESSENTIAL OIL

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Thesis submitted in fulfilment of the requirements
for the award of the degree of
Master of Science in Industrial Chemistry.

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LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Celsius
cm	Centimetre
δ	Delta
dbh	Diameter At Breast Height
ε	Epsilon
γ	Gamma
g	Gram
hr	Hour
kg	Kilogram
Kv	Kilo Volt
μ	Micro
mL	Millilitre
mm	Millimetre
$[\alpha]_D^{25}$	Optical Rotation (25°C)
%	Percentage
[n]D	Refractive Index
rpm	Rotation Per Minute
NaOH	Sodium Hydroxide

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometer
ETOH	Ethanol
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GC-Q-TOFMS	Gas Chromatography-Quadrapole-Time-of-flight/ Mass Spectrometry
HD	Hydrodistillation
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
KI	Kovats index
MAE	Microwave-assisted extraction
MS	Mass spectrum
m/z	Mass to ratio
NIST	National Institute of Standards Technology
NMR	Nuclear magnetic resonance
PDB	Potato dextrose broth
PDA	Potato dextrose agar
PH	Power of hydrogen
RI	Retention index
RT	Retention time
SEM	Scanning electron microscope
SFE	Supercritical fluid extraction
SHD	Standard hydrodistillation

SWE	Subcritical water extraction
TLC	Thin layer chromatography

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ABSTRACT

Agarwood tree is one of the source production of resin and known as fragrant wood that have a highly valuable product in the global market. However, the process of hydrodistillation efficiency is far from sufficient and affecting the lower product quality and oil yield. Therefore, the objectives of this study are to investigate the effect of pre-treatment techniques on agarwood essential oil yield and to obtain the effective way in the extraction process. There are four different techniques being studied in soaking process initiated by sample soaked with various sample sizes (0.5 cm, 0.75 cm and 1.0 cm), soaked with water, soaked at various shaking time (1, 3 and 7 days) and soaked with three types of fungi *(Phanorocaete crysosporium, Trichoderma reesei and Fusarium solani).* Agarwood without soak acted as a control in this research. The combination of the highest yield in pre-treatment technique was applied in two extraction methods, namely standard hydrodistillation and hydrodistillation coupled with stirrer. Then, the oil produced in all parameters studied was compared with two industrial oils. All analyses of hydrodistillation were performed in three replicate extractions to obtain an average oil yield. This research is not complete without doing the chemical analysis using Gas Chromatography with Flame Ionization Detector (GC-FID) and Gas Chromatography with Mass Spectrometry (GC-MS). The results in pre-treatment technique showed the highest oil yield extracted by agarwood size of 0.5 cm (0.44%), sample shaken for 7 days (0.34%) and soaked with fungi *P.chrysosporium* (0.59%). Agarwood oil extracted using hydrodistillation coupled with stirrer achieved the maximum average oil yield of 0.60% compared to standard hydrodistillation (0.48%) and two industrial oils at only (0.14% - 0.20%). This research proves that the essential oil yields were increased with the combination of optimum parameter in pre-treatment technique and the application of stirrer during hydrodistillation. The results showed that 4-phenyl-2-butanone, α-guaiene, α-selinene, α-muurolene, α-elemol, guaiol, agarospirol and n-hexadecanoic acid as marker for chemical compounds detected being studied.

ABSTRAK

Pokok gaharu adalah salah satu sumber pengeluaran resin dan dikenali sebagai kayu wangi yang mempunyai produk yang tinggi nilainya di pasaran global. Walau bagaimanapun, proses kecekapan penyulingan hidro jauh dari mencukupi dan memberi kesan kepada hasil kualiti produk dan minyak yang lebih rendah. Oleh itu, tujuan kajian ini adalah untuk mengkaji kesan teknik pra-rawatan pada hasil pati minyak gaharu dan untuk mendapatkan cara yang berkesan dalam proses pengekstrakan. Terdapat empat teknik yang berbeza telah dikaji dalam proses rendaman dimulakan oleh sampel direndam dengan pelbagai saiz (0.5 cm, 0.75 cm dan 1.0 cm), direndam dengan air, direndam di pelbagai tempoh masa (1,3 dan 7 hari) dan direndam dengan tiga jenis kulat *(Phanorocaete crysosporium, Trichoderma reesei and Fusarium solani).* Gaharu tanpa direndam digunakan sebagai kawalan dalam kajian ini. Gabungan hasil tertinggi dalam teknik pra-rawatan telah digunakan dalam dua kaedah pengekstrakan, iaitu penyulingan hidro piawai dan penyulingan hidro ditambah dengan alat pengacau. Kemudian, minyak yang dihasilkan dalam semua parameter yang dikaji dibandingkan dengan dua minyak industri. Semua analisis penyulingan hidro telah dijalankan dalam tiga mereplikakan perahan untuk mendapatkan purata hasil minyak. Kajian ini tidak lengkap tanpa melakukan analisis kimia dengan menggunakan Gas Kromatografi dengan Pengionan Nyala (GC-FID) dan Gas Kromatografi dengan Spektrometri Jisim (GC-MS). Keputusan dalam teknik pra-rawatan menunjukkan bahawa hasil minyak yang tertinggi diperolehi dengan saiz gaharu 0.5 cm sebanyak (0.44%), sampel digoncang selama 7 hari (0.34%) dan direndam dengan kulat *P.chrysosporium* (0.59%). Minyak gaharu diekstrak menggunakan penyulingan hidro ditambah pula dengan pengacau telah mencapai hasil minyak purata maksimum (0.60%) berbanding penyulingan piawai (0.48%) dan dua minyak industri hanya (0.14% - 0.20%). Kajian ini membuktikan bahawa gabungan parameter yang optimum dalam teknik pra-rawatan dan penggunaan alat pengacau semasa penyulingan hidro boleh meningkatkan pengeluaran minyak. Hasil kajian menunjukkan bahawa α-muurolen (0.23% -1,88%), α-elemol (0.23% - 1,32%), guaiol (0.79% -7,80%) dan agarospirol (0.29% -7.36%) adalah sebagai sebatian penanda kimia yang dikesan di semua parameter dikaji.

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