PROJECT TITLE:

EXTRACTION OF GAHARU OIL FROM AQUILARIA MALACCENCIS USING SUBCRITICAL EXTRACTION PROCES

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Abstract:

Aquilaria species from the family of *Thymelaeceae* are the main source of gaharu, which has been classified as one of the most highly valuable, non-timber products in the world market. Currently, the method used for extracting gaharu essential oil is by using hydrodistillation. However, this method is inefficient where it produced low yield of oil with long time of extraction and thus increasing the production cost. To overcome this problem, the extraction of gaharu essential oil using Supercritical Fluid Extraction (SFE) with pressure 20MPa, 30MPa and 40MPa at 65 °C for 2 hours was studied. Result obtained after 2 hours of experiment was 0.6% of yield, which was higher compared to 0.2% yield of gaharu essential oil obtained via 3 days of hydrodistillation. After an extraction process, the product will be analyzed by using Gas Chromatography-Mass Spectrometry (GC-MS) to determine the chemical compounds and composition in the Gaharu SFE's extract. Over eighteen to thirty-one compounds were identified in SFE extract compared with fifty-five identified in the hydrodistillated oil. The mutual occurrence of chemical compounds in all three samples in SFE was dimethyl phthalate, 8-epi-.gama.-eudesmol, diethyl Phthalate and 2-3-Butanediol. In comparison, the major compounds identified in hydrodistillated oil were 4-phenyl-2-butanone, jinkoh-eremol and α-guaiene (Saiful Nizam & Mashitah, 2010). There were certain aromatic sesquiterpenes compounds that were identified in this study, such as Globulol, Alloaromadendrene Naphthalene, 10s, 11s-Himachala-3(12), 4-diene, .delta.-Selinene and (-) - Aristolene.

Keywords: *Aqualaria*, *Thymelaeceae*, hydrodistillation, Supercritical Fluid Extraction (SFE), and Gas Chromatography-Mass Spectrometry (GC-MS).

Introduction

Agarwood also known as eaglewood or gaharu, is a valuable non- timber forest product from *Aquilaria spp*. The genus species occur mainly in South and Southeast Asia. As a result of a defense mechanism to fend off pathogenes, Aquilaria species develop agarwood that can be used for incense, perfume, and traditional medicines. The main markets for these products are in South and East Asia and the Middle East (Barden *et al*., 2000). This study examine the feasibility of Supercritical Fluid Extraction (SFE) as a better alternative method for gaharu essential oil extraction process at different pressure and determine the chemical compounds and compositions of SFE's gaharu extract that have similar characteristics with gaharu compound found in hydrodistillation were conducted. The characteristics of aroma between species may help in identification and authentication of agarwood as well as determination of its quality.

Background

Gaharu wood in Malaysia is often graded into different classes based on their colour, density, gaharu formation and unique scent. Currently, the grading of gaharu is based on individual perception and experience. Some gaharu wood has been graded into unspecified classifications, including according to origin or unique name in order to attract buyers. The price of gaharu is determined by its grade; the higher the grade the higher the price. The price of gaharu premium grade (grade A) may fetch RM16, 000 to RM20, 000 per kg. The price for supreme or deluxe oil may range from RM400–RM2000/tola (1 tola = 12 mL) (Husni, Mailina, Sahrim, Majid, & Faridz, 2013). One of the main causes for the relative rarity and high cost of agarwood is the depletion of the wild resource. The Malaysian example highlights the fact that there remain many challenges to effecting controls on the gaharu trade on the supply side of the industry. Challenges arise due to the fact that gaharu is not a uniform commodity: the substance has a highly variable chemical composition and is produced by a wide variety of tree taxa (Zich & Compton 2002). In the absence of standard grading rules or species identification procedures, effective regulation is often frustrated. Furthermore, small quantities of top grade gaharu are easy to hide and thus difficult to regulate in trade despite their high value. Due to the complexity of the nature of the species, the coordination among the various agencies involved in regulating the gaharu industry is mostly challenging. The challenges include coordination among the several agencies involved in regulating the various aspects of the gaharu industry (Gibson 1977 cited in Donovan & Puri 2004).

In Malaysia, these many challenges were combined with the fact that there was a significant slump in the gaharu trade between 1930 and 1970. When the trade picked up in the mid-1970s, gaharu was classified under "minor forest produce" and thus regulation of the harvest, trade and processing of gaharu was not given priority (Nik Hassan 1998). However, more recently several sectors of the Malaysian government, at Federal and State levels, have begun to put greater emphasis on developing industries related to biodiversity and biotechnology. In this regard, the government has recognized the significant potential for developing Malaysian gaharu in terms of downstream processing and added value industries such as perfumery (Chang *et al.* 2002).

Objective of the study

The objectives of the study are;

i. To examine the feasibility of Supercritical Fluid Extraction (SFE) as a better alternative method for gaharu essential oil extraction process.

ii. To determine the chemical compounds and compositions of SFE's gaharu extract.

Methodology

The extraction process was conducted using supercritical fluid extraction on the dried sample. An amount 430g dried Gaharu sample was extracted using the pilot scale SFE. The effects of pressure on the extraction yield were examined in this experiment. While the temperature was kept constant at 65 ^{0}C but the pressure was varied as Sample 1: P = 20 MPa, Sample 2: P = 30 MPa and Sample 3: P = 40 MPa. The extracted gaharu was then subjected to Gas Chromatography-Mass Spectometry (GC-MS). For this experiment, GCMS analysis was performed with Gas chromatography system; Agilent 7890A (Agilent Technologies) coupled with MSD quadrupole detector 5975 C. Separation of analytes by gas chromatography was carried out using a silica capillary column (30 m X 250µm X 0.25 µm) of HP-5MS (Hewlett Packard). The analytical conditions for GCMS are listed in Table 1.

Table 1: Analytical conditions for GC-MS analysis

Program	Conditions
Oven Program	80°C for 1 min
Carrier Gas	Helium
Gas flow	1.2mL/min
Split ratio	1:50
Injection Volume	1μL
Syringe Size	10μL
Heater	250°C
Pressure	11.5 psi
Total Flow	24.2 mL/min
Equilibrium Time	3 min

The peaks from GCMS were detected based on the total ion chromatography (TIC) and mass chromatograms.

Findings

The effect of pressure on the extraction yield of aqualaria malaccensis by supercritical carbon dioxide (CO2) and the chemical compounds present in the extract were examined in this study. The supercritical fluid extraction (SFE) was chosen in this study due to its advantages in giving a better characteristic of gaharu compound, more economical and requires a shorter extraction time. The parameter tested in this experiment is the pressure for the supercritical fluid extraction (SFE) process. In order to determine the pressure effect in the supercritical fluid extraction process, three values of pressure have been tested which were 20MPa, 30MPa and 40MPa. The collected data in this experiment is in terms of amount of essential oil at every 40 minutes. The amount of essential oils were calculated and translated in term of percentages of oil yield using the equation (1) below:

$$Percentage\ of\ Oil\ Yield\ (\%) = \frac{Amount\ of\ Gaharu\ Extract\ (g)}{Amount\ of\ Gaharu\ Raw\ Material\ (g)} \times 100$$

The data collected are tabulated in Table 2 below.

Table 2: Percentages gaharu of oil obtained from SFE at three different pressure

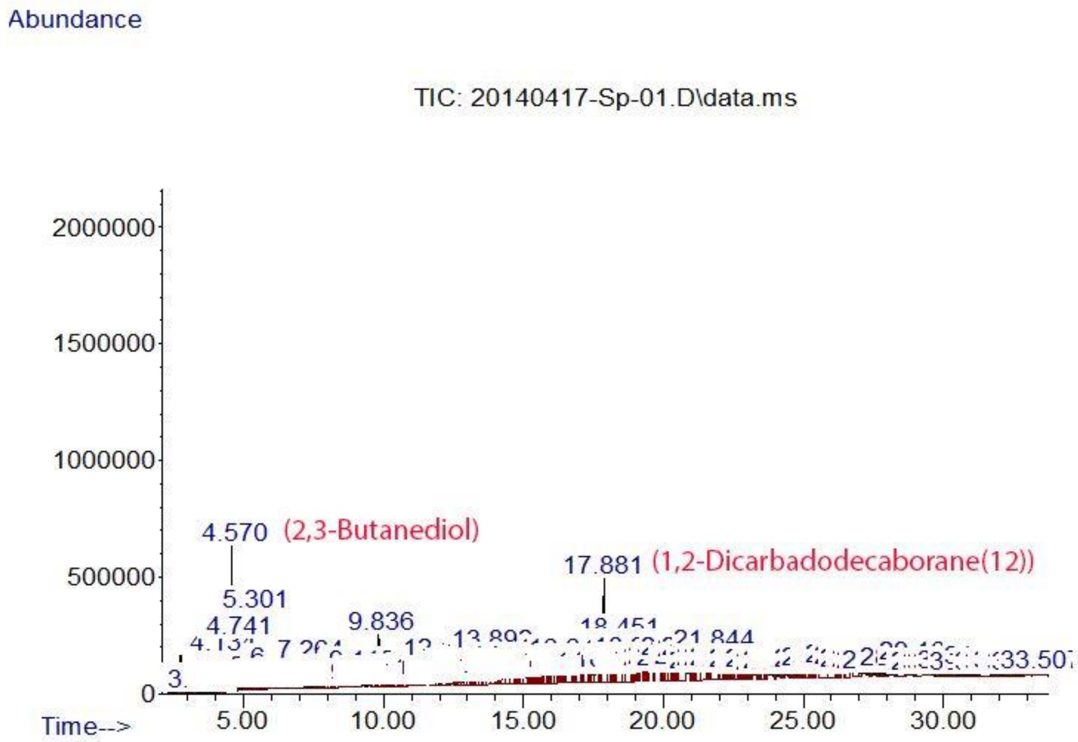
Types of sample	Pressure (MPa)	Time (min)	Temperature (^{0}C)	Amount of gaharu extract (g)	Percentages of oil yield (%)

S1	20	40	65	1.009	0.2
S2	30	40	65	0.523	0.1
S3	40	40	65	1.084	0.3

The effects of pressure on oil extraction operating at 65 ^{0}C and 3.0 ml/min CO2 solvent flowrate of percentages oil yield (%) against specific pressure. From figure above, it can be observed that from 20MPa to 30MPa there is slightly decline, the pressure reading may be fluctuating at the lowest pressure and it may affect the result during extraction process. Meanwhile, from 30MPa to 40MPa, it increase back.The highest percentages of oil yield was observed to be obtained at the highest pressure of 40 MPa and the lowest percentages oil yield occurred at 30MPa. This can be explained using the established principles of SFE technology for gases and fluids (De Castro et al., 1994). The pressure increase causes the density of CO2 solvent to increase. Furthermore, as the density of solvent increases, the inter-molecule interactions of solutes also rise. As a result, oil and solvent dissolution were promoted, thereby increasing the oil extracted (Machmudah et al., 2007). However, increase in the oil-solvent dissolution with pressure during the solubility-dependent stage was very slow as compared to the increase in the diffusivity dependent stage.

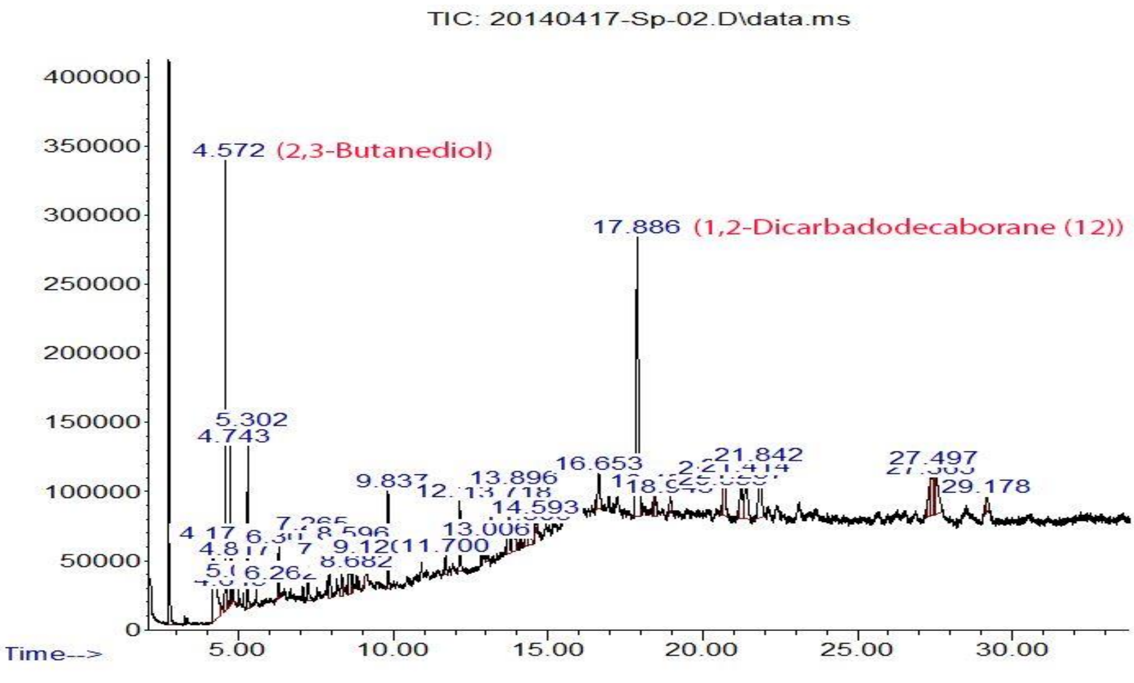
Examination of three samples of agarwood oils from different sources also showed some variations in GC profile and chemical components. The variations of GC profiles are shown in Fig. 2 (A-C).

A



Abundance
TIC: 20140417-Sp-01.D\data.ms
4.570 (2,3-Butanediol)
17.881 (1,2-Dicarbadodecaborane(12))
5.301
4.741
9.836
21.844
33.507
Time-->

B



TIC: 20140417-Sp-02.D\data.ms
4.572 (2,3-Butanediol)
17.886 (1,2-Dicarbadodecaborane (12))
5.302
4.743
9.837
13.896
16.653
21.842
27.497
29.178
14.593
13.006
11.700
8.682
6.262
Time-->

C

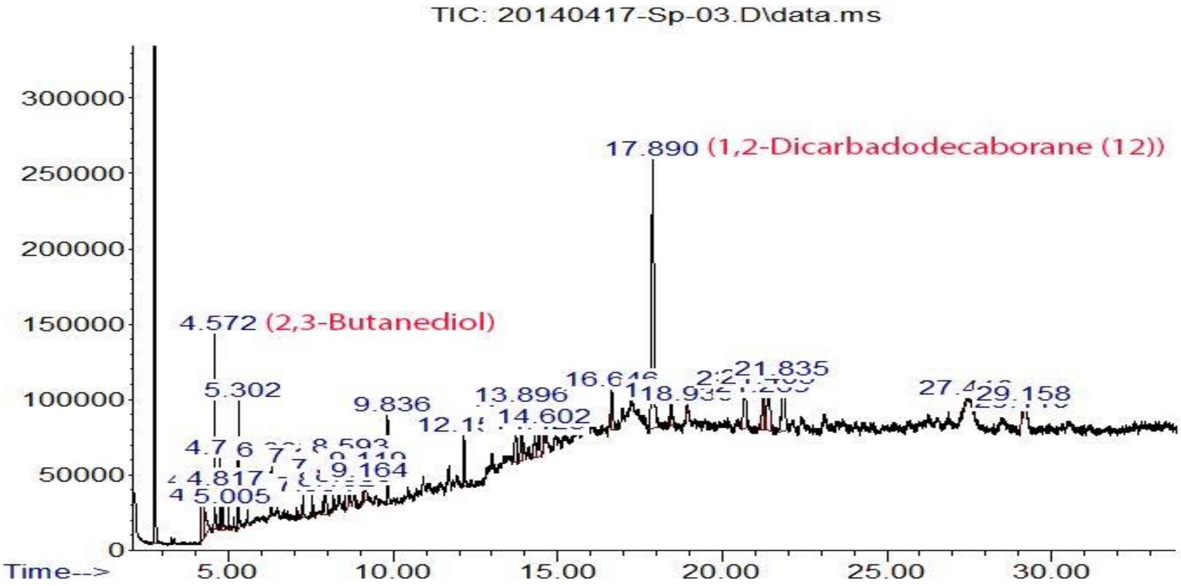


Fig. 1: Total ion chromatogram (TIC) for agarwood oil from different pressure. (A) Sample A (P = 20 MPa), (B) Sample B (P = 30 MPa) and (C) Sample C (P = 40 MPa)

Agarwood oil has been reported to contain sesquiterpenoids of the eremophilane, spirovetivane, eudesmane, nor-guaiane and prezizaane types, and 2-(2-phenylethyl) chromone derivatives (Yaacob & Joulain, 2000). GC-MS is the most commonly used analytical methods for compound identification in volatile oils. Based on the GC-MS analysis, it was found that there were 70 compounds listed for 20 MPa and 30 MPa condition. While for 40 MPa condition, only 36 compounds revealed. Meanwhile, thirteen (13) components were identified in Sample 1 and Sample 2 which contribute to the largest portion of the total compounds. In Sample 3, there were six (6) compounds identified respectively and only three (3) compounds were found in all samples. About 63.16% of the compounds identified were aromatic and sesquiterpenes which have been revealed to be the main active compounds of agarwood oils. The dominant compounds were 8-epi-.gama.-eudesmol, diethyl phthalate) and 1H-Cyclopropa[a]naphthalene. These main active components play important roles in giving the aroma and pleasant odor of agarwood. They might be used as the reference compounds in determining the quality of agarwood. While the other 36.84% of the compounds is monoterpenes hydrocarbons, fatty acids and others.

A compound named Azuleno[4,5-b]furan-2,9-dione, decahydro-6a-hydroxy-6,9a-dimethyl-3-methylene-,1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-1ar-,5(1H)-Azulenone and (1a.alpha.,4a.alpha.,7.beta.,7a.beta., 7b.alpha)] (also known as globulol) was identified to be present in both Sample 1 and Sample 2. There were two similar compounds identified from Sample 3 but not Sample 1 and Sample 2. One compound was from monoterpene which is 4-phenyl-2-butanone and another one is from sesqueiterpene which is acetohydrazide. The essential oil of S1 and S2 had profoundly different components from that of S3, which had abundant fatty acid and alkanes. For instance, a trace of n-hexadecanoic acid was found in the oils of S1 and S2, but it was totally absent in S3. As for the alkanes, S1 and S2 were very similar, and most of the alkanes identified in S2 were also identified in S1, whereas no alkanes were detected in S3. It has been reported that different artificial methods used to stimulate agarwood formation in Aquilaria result in different agarwood qualities. Tamuli and Bhuiyan both showed that the essential oils obtained from the plants (A. agallocha Roxb.) inoculated with fungus, i.e., Chaetomium globosum, for 30 days or from plants injected artificial screws showed similar component distributions with that of healthy trees according to GC-MS. Daniel (2009) found that the essential oils of the agarwood produced by nail inserting and holing for two years were full of sesquiterpenes and aromatic constituents, while the essential oil of agarwood formed through trunk breaking for two years was full of fatty acids. The composition of inoculum, natural variant and specific host-pathogen relationship also play great role in inducing the resin production in agarwood which relates to the quality and differential composition of chemical compounds in its resin and oil. Furthermore, the quality and chemical composition of the agarwood oil can be influenced by different extraction method. There are various techniques that have been used for agarwood oil extraction such as solvent extraction, water distillation (hydrodistillation), steam distillation and supercritical fluid extraction (SFE). Each technique has its own advantages and disadvantages.

Conclusion

In conclusion that Supercritical Fluid Extraction (SFE) is a promising approval for gaharu essential oil to obtain an optimal yield with good quality of oil based on the composition of compounds. Generally, different compounds have different retention times. For a particular compound, the retention time will vary depending on the boiling point of the compound, the solubility in the liquid phase and the temperature of the column.

Out Put

All the objectives of the project were successfully achieved. Comparing the SFE with the commonly used hydrodistillation method to extract gaharu, it was found that SFE consumes less time compared to hydrodistillation. It only took total 2 hours for one process of extracting using SFE from 430 gram of gaharu while compared to the study conducted by Ng and Azizol (1997), it took about 7 days to get complete extraction for 1kg of gaharu sample for hydrodistillation. The SFE resulted in 0.6% (wt/wt) as compared to hydrodistillation while was 0.2% (wt/wt). This study also showed this the supercritical extraction yield of *A. malaccensis* increases with increasing pressure and temperature. The number of chemical compounds were also found to increase with pressure increment up to 40MPa with temperature constant at 65 ^{0}C. The last 40 minutes, compound from SFE revealed almost similar to the gaharu compound by using hydrodistillation. This result demonstrates that pressure is an effective factor for the extracting gaharu compounds. In conclusion that Supercritical Fluid Extraction (SFE) is a promising approval for gaharu essential oil to obtain an optimal yield with good quality of oil based on the composition of compounds.

Future Plan For Research

For future research on this topic several recommendations may be considered.

1. Cost Evaluation of the Process

Cost evaluation is one of the essential factors in determining the optimize process. When evaluating the cost, the process with the least cost can be chosen since in manufacturing a process the less cost gives more advantages than high cost process.

2. Use to Subcritical R134a Refrigerant Gas

The use of R134a as the extraction solvent at sub-critical conditions can address the shortcomings of supercritical CO2 process. It is because R134a (1, 1, 1, 2-tetrafluoroethane) is non-toxic, non-reactive, non-flammable, and non-ozone depleting. It also has a high volatility and a boiling point at atmospheric pressure of -26.2 ^{0}C, which means that it leaves negligible solvent residues in the products.

3. Using the Standard for Each Compound of Gaharu

Basically, we refer to ion chromatogram (TIC) peak from the GCMS library to determine the compounds present for improvement and to get more accurate result, so it is recommended to buy standard for each compound that revealed and classified as gaharu compound.

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