Effect of Ultrasonic Pretreatment on Gaharu Essential Oil Using Supercritical Carbon Dioxide Extraction

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***Abstract*— Gaharu or commonly known as Agarwood, is a resinous heartwood in trees of *Aquilaria* genus and *Thymelaeaceae* family which is widely known as one of the most highly precious fragrant woods as it producse essential oil that is economically significant. However, conventional extraction method without the pretreatment produced small oil yield. Therefore, this experiment was carried out to determine the effect of ultrasonic pretreatment on gaharu essential oil using supercritical carbon dioxide extraction. Ultrasonic pretreatment was done at 30°C, 40°C and 50°C for 1 hr. The extraction was carried out at temperature of 40°C, 45°C, 50°C and pressure of 3000 psi, 4000 psi and 5000 psi. Gaharu from the species of *Aquilaria Malaccensis* was used in this experiment. The optimum temperature and pressure for the extraction was 50°C and 5000 psi respectively at which the highest oil yield was obtained (0.1867%) for the pretreated gaharu. The chemical compounds present in the essential oil were analyzed by using Gas Chromatograph Mass Spectrometry (GC-MS).**

Keywords*— Gaharu, *Aquilaria Malaccensis, Ultrasonic, Supercritical Carbon Dioxide Extraction, GC-MS

I. INTRODUCTION

Gaharu essential oil which is also be known as Agarwood essential oil comes from a tree called Agarwood tree. Agarwood tree is classified as *Aquilaria* trees as it grows up to 40 metres high and 60 centimetres in diameter and can be mostly found in the jungle of Malaysia like in Terengganu, Kelantan, Pahang and Perak (Atikah et al., 2015). *Aquilaria* species usually grow in locations where the average daily temperatures are between 20°C to 22°C with altitudes of 0 until 850 metres (Barden *et al.,* 2000) . Agarwood trees produce aromatic resin called Aloeswood or Oud when they become infected by mould (Yusoff *et al.,* 2015). Oil can be extracted from the trees and is usually used in the application of fragrance commonly perfumes as it has a strong smell. Gaharu has been used worldwide for its multipurpose applications. There are there principle uses of gaharu which is perfume, medicine and incense (Barden *et al.,* 2000). During the past, the bark of the tree has been used as a source to make paper and twine while the wood are reported to be used for making boxes (Blanchette *et al.,* n.d.). However in Khagrachari, the bark is used for heart disease (Begum, 2016). The essential oil which can be yielded from gaharu has been widely used in perfume industry (Schmidt & Nguyen, 2004)

The extraction of the essential oil from the wood can be implemented in many ways. The most popular methods for the oil extraction are hydrodistillation, microwave, accelerated solvent extraction, supercritical fluid extraction and ultrasound assisted extraction (Atikah et al., 2015). The conventional method by hydrodistillation to extract essential oil from the gaharu wood takes a long period to be completed and high energy is required (Wetwitayaklung *et al.,* 2009). Not only that, the amount of oil yield can be extracted is too small which is around 0.2% (Yoswathana *et al.,* 2012). On the other hand, the selling price for the essential oil and the resinous gaharu wood are extremely high due to low yield of oil extraction, high demand and the resinous woods are hard to get from wild and young trees (Sulaiman *et al.,* 2015). Another problem that occurs during hydrodistillation is that it demolishes the heat-sensitive essential oil compounds (Sulaiman *et al.,* 2015). Therefore, many researchers have come up with an alternative by using supercritical fluid extraction (SFE) method to extract oil from the resinous wood. Supercritical fluid extraction (SFE) has many advantages over hydrodistillation method. It is able to provide satisfactory yields of oil and solvent-free extracts without changes of characteristics (Andressa *et al.,* 2016). The carbon dioxide used in this method is inert and is a non-polar fluid which can dissolve any type of hydrophobic compounds or even slightly polar compounds (Yoswathana, 2013). Supercritical carbon dioxide has high diffusivity, low viscosity, good transport properties, gives faster extraction and leads to high yields of essential oil (Wetwitayaklung *et* al., 2009). Other than that, carbon dioxide are an inert, non-flammable, safe, and inexpensive (Yoswathana, 2013). Furthermore, it is a non-polar fluid (dipole moment = 0) which capable to dissolve any kind of hydrophobic compounds or slightly polar compounds (Yoswathana, 2013). In order to maximize the yield of extraction, ultrasonication is used as a pretreatment method. This pretreatment method has an ability to penetrate the cellular walls, decrease the particles size and increase the mass transfer between the cell walls and solvent (Yoswathana et al., 2012). Hence, it makes it easier for the extraction process to occur despite the strong woody structure of gaharu wood (Yoswathana et al., 2012). The aim of this research is to study the effect of ultrasonic pretreatment on gaharu essential oil using supercritical carbon dioxide extraction.

II. METHODOLOGY

A. Materials

The species of Gaharu used in this experiment were *Aquilaria Malaccensis (A.malaccensis)* and gaharu trunks were obtained from a plantation located in Kuala Krai, Kelantan in Malaysia. The gaharu trunks were chopped into small pieces of gaharu chips. The chips were then air-dried overnight to eliminate the moisture content. Then, the chips were ground into smaller size of 0.5 cm by using a crasher (SCP Automation (M) Sdn. Bhd.), (model: SLM-20PI/D). The ground gaharu was kept in a sealed bag. Carbon dioxide with a purity of 99% used as a fluid for the extraction was purchased from Poly Gas Sdn. Bhd.

B. Ultrasonic Pretreatment

Approximately 100 grams of ground gaharu were filled in a cotton fabric pouch and the pouch was immersed in the ultrasonic cleaning bath (Nexxion Science & Technologies Sdn. Bhd), (model: NS-A-18H). The operating temperature was set at 30°C, 40°C and 50°C. The ultrasonic pretreatment was performed for 24 hours. After the ultrasonic pretreatment was completed, the gaharu was taken out and dried completely under ambient temperature prior to extraction. After it has entirely dried, the plant material was then further ground by using a cutting mill (Interscience Sdn. Bhd.), (model: SM 2000) into a powder form with a size of 0.25 mm.

C. Supercritical Fluid Extraction (SFE)

The extraction was performed by using a supercritical fluid extraction system (Supercritical Fluid Technologies, Inc.), (model: SFT-400). About 6 grams of ground gaharu with a size of 0.25 mm was placed in the vessel. The extractions were carried out at different temperatures and pressures. The extractions temperature were done at 40°C,45°C and 50°C while the extractions pressure were performed at 3000 psi, 4000 psi and 5000 psi. The flow rate of carbon dioxide and extraction time were kept constant at 1 ml/min and 60 minutes respectively. After the extractions were completed, the extracted oil was kept in a pre-weighted glass vial and the oil yield was calculated. The glass vials containing the extracted oil were kept in the refrigerator at -20°C prior to analysis.

D. Gas Chromatograph Mass Spectrometry (GC-MS)

GC-MS analysis was carried out in order to identify the active compounds present in the extracted gaharu essential oil. The analysis was carried out by using a GC-MS system (Varian, Inc.), (model:450-GC, 240-MS). The analytical conditions of this analysis were stated in Table 1.

Table 1.1: Conditions for GC-MS (Yumi *et al.,* 2014)

Program	Condition
Oven Program	80°C for 2 min, then 10 °C/min to 250°C for 10 min
Carrier Gas	Helium
Gas Flow	2 ml/min
Split Ratio	1:50
Injection Volume	1µm
Mode	Splitless
Interface Temperature	250°C
Electron Impact	70 eV
Scan Range	32 to 500 amu

III. RESULTS AND DISCUSSION

A. Effect of Operating Pressure and Temperature on Oil Yield

The extracted oil from both unpretreated gaharu and pre-treated gaharu was weighed and the oil yield (%) for each sample from unpretreated gaharu and pretreated gaharu by ultrasonic pretreatment was calculated by using Equation 1.0 as follows:

$$\text{Oil yield} = \left(\frac{weight\ of\ extracted\ oil, g}{weight\ of\ feeding\ material, g}\right) x\ 100\% \qquad (1.0)$$

Where,

Weight of feeding material = 6 g

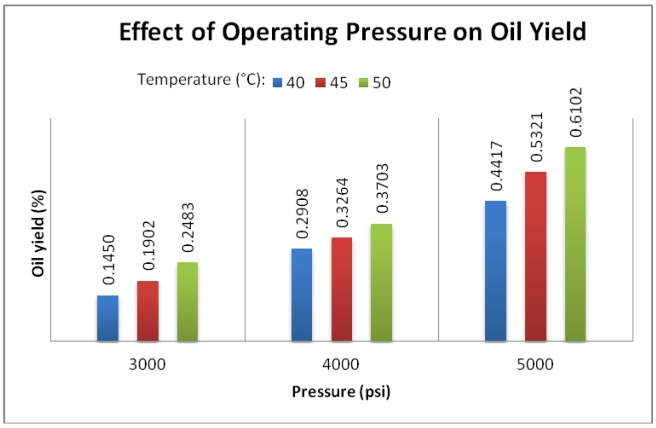
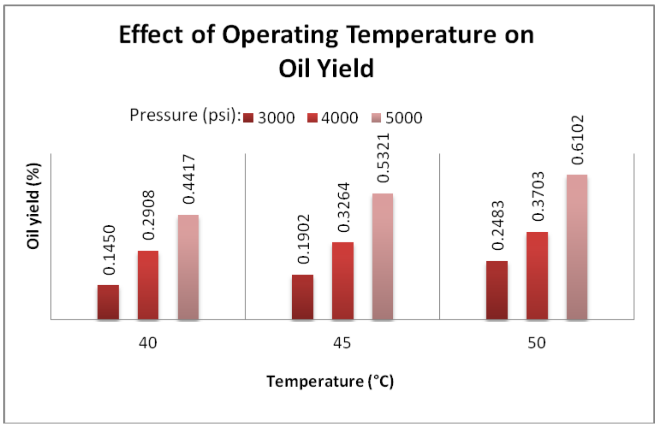


Figure 1.1(a) Effect of Operating Pressure on the Oil Yield of Gaharu without Pretreatment by using SFE



. **Figure 1.1(b) Effect of Operating Temperature on the Oil Yield of Gaharu without Pretreatment by using SFE**

The effect of operating pressure and temperature on the extraction yield of *A.malaccensis* by SFE was examined. The operating pressure and temperature of SFE determines the efficiency of oil extraction (Chen *et al.*, 2010). Gaharu essential oil was extracted by using SFE at 3000 psi, 4000 psi and 5000 psi with temperature setting of 40°C, 45°C and 50°C. The extraction time was done for 60 minutes based on preliminary study. This alternative gives faster extraction and leads to high yields of essential oil (Wetwitayaklung, *et* al., 2009) Figure 1.1(a) presents the effect of operating pressure on the oil yield of gaharu without pretreatment by using SFE. It can be seen from the bar chart in Figure 1.1(a) that the oil yield increased with increasing pressure. The highest oil yield was found at 5000 psi, 50°C which was 0.6102%. From Figure 1.1 (b), the trend of the bar chart is alike which shows an increasing result of the oil yield as the temperature increased. At 50°C, highest oil yield was obtained at 5000 psi with 0.6102% similarly. From these two results, it can be concluded that the oil yield increased with increasing pressure and temperature. The results achieved agreed with previous work carried out by many researchers who found that oil extraction by SFE can increase the oil yield. As compared to hydrodistillation method, the oil yield achieved was only about 0.2% (Yoswathana, 2013). The difference in the oil yield obtained for these two methods were quite obvious.

B. Effect of Ultrasonic Pretreatment on Oil Yield

The ultrasonic pretreatment was run for 24 hr at 30°C, 40°C and 50°C prior to extraction. Ultrasonic pretreatment was said as an effective pretreatment method because the extracted oil yield was higher than the one without pretreatment (Yoswathana *et al.,* 2012). From previous research, it was found that the oil yield for gaharu with ultrasonic pretreatment increased up to 0.20% as compared with the oil yield without ultrasonic pretreatment which was only 0.08% (Yoswathana *et al.,* 2012). However, the results obtained contradict with the previous studies. As shown in Figure 1.2(a), at the same pressure and temperature which was at 5000 psi and 50°C respectively, the oil yield with ultrasonic pretreatment at 30°C decreased to 0.1767% as compared to oil yield without pretreatment. This is believed to occur due to higher resin content in the ground gaharu without pretreatment. Nonetheless, the result for the oil yield with ultrasonic pretreatment at 30°C, 40°C and 50°C shows the same trends in which the higher the pressure and temperature, the higher the oil yield. The highest yield for gaharu with ultrasonic pretreatment of 30°C occurred at pressure 5000 psi and temperature 50°C which gave 0.1767% oil yield as shown in Figure 1.2(a) and Figure 1.2(b). As the temperature of the ultrasonic pretreatment increased to 40°C, the oil yield also increased. It can be seen in Figure 1.3 (a) and Figure 1.3 (b) that the oil yield has slightly increased at all temperature and pressure as compared to oil yield of gaharu with ultrasonic pretreatment at 30°C. Similarly, the highest oil yield for ultrasonic pretreatment at 40°C with 0.1867% was seen at the highest temperature and pressure of 50°C and 5000 psi respectively. This is thought to be due to the increase of mass transfer attributed by the collapse of bubbles on the cell wall of the *A. malaccensis* during ultrasonic pretreatment which consequently improved the extraction (Yoswathana *et al.,* 2012).

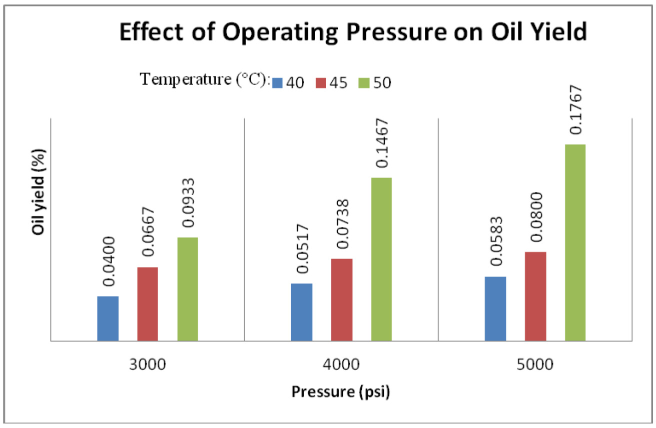


Figure 1.2(a) Effect of Operating Pressure on the Oil Yield of Gaharu with Ultrasonic Pretreatment at 30°C by using SFE

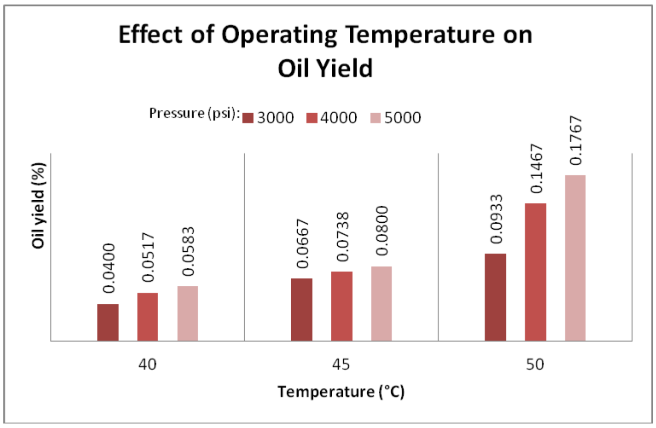


Figure 1.2(b) Effect of Operating Temperature on the Oil Yield of Gaharu with Ultrasonic Pretreatment at 30°C by using SFE

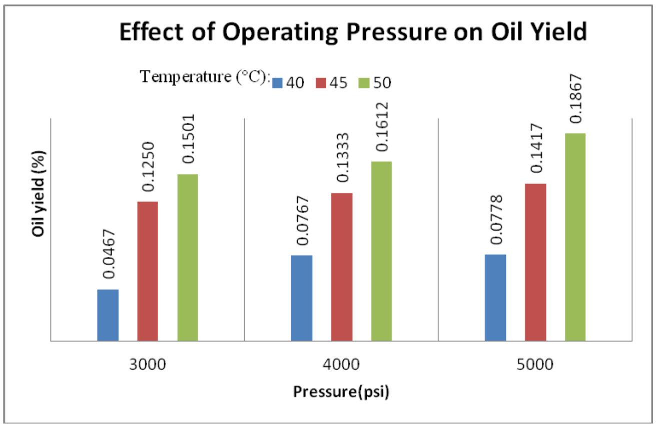


Figure 1.3(a) Effect of Operating Pressure on the Oil Yield of Gaharu with Ultrasonic Pretreatment at 40°C by using SFE

Nevertheless, the oil yield slightly decreased when the temperature of the ultrasonic pretreatment was increased to 50°C. From Figure 1.4 (a) and Figure 1.4 (b) we can see that at all temperature and pressure, there was a slight decrease in the oil yield as compared with ultrasonic pretreatment at 30°C and 40°C. This can be explained by the degradation reactions that occurred at high temperature of ultrasonic pretreatment on the cell walls or oil bodies of the gaharu (Porto *et al.,* 2013).

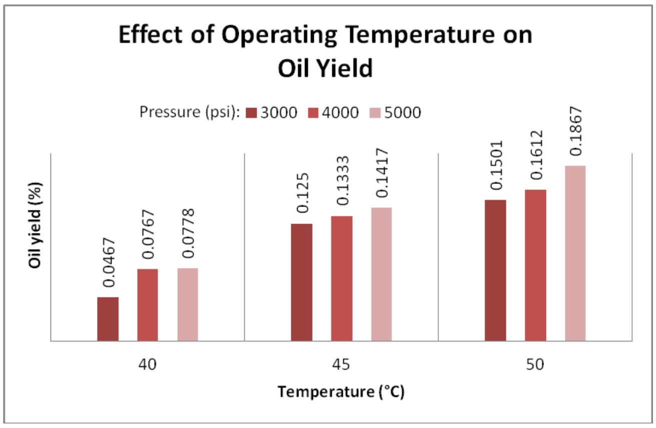


Figure 1.3(b) Effect of Operating Temperature on the Oil Yield of Gaharu with Ultrasonic Pretreatment at 40°C by using SFE

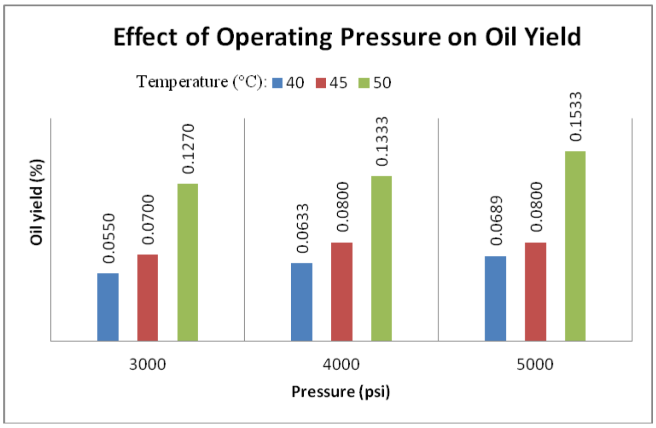


Figure 1.4(a) Effect of Operating Pressure on the Oil Yield of Gaharu with Ultrasonic Pretreatment at 50°C by using SFE

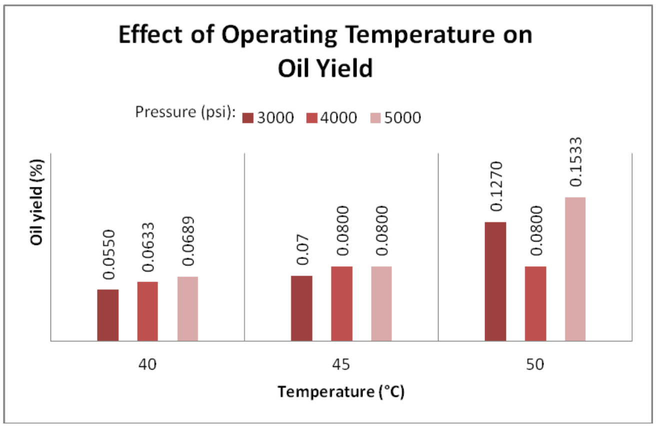


Figure 1.4(b) Effect of Operating Pressure on the Oil Yield of Gaharu with Ultrasonic Pretreatment at 50°C by using SFE

For the effect of the operating temperature and pressure of SFE on the oil yield, extraction of gaharu with ultrasonic pretreatment at 50°C showed similar result in which the oil yield increased with increasing temperature and pressure and the highest yield obtained was 0.1533% at 50°C, 5000 psi as can be seen from Figure 1.4 (a) and Figure 1.4 (b). Altogether, we can say that the higher the pressure and temperature of the SFE during the extraction, the higher the oil yields. The results are in agreement with the previous work done by Ibrahim et al., (2011).

Table 1.2 The Chemical Constituents of Gaharu Essential Oil with Ultrasonic Pretreatment of 40°C at 50°C and 5000 psi

Retention time, (min)	Identification	Amount (%)
3.1111	2,4-dimethyl-pentane	0.42
7.9144	Heptane, 2,2,4-trimethyl	0.09
11.9855	trans-2,2-Dimethyl-4-heptenal	0.03
12.4179	Diethyl Phthalate	0.04
25.0482	2-Propenal	0.04
26.6056	Oxalic acid, allyl heptyl ester	0.03
27.5823	Phthalic acid, isobutyl octyl ester	0.04
29.0935	Propane, 2-cyclopropy	0.03
27.5823	Phthalic acid, isobutyl octyl ester	0.02
31.4311	Oxirane, 2-methyl-3-propyl-, trans	0.03

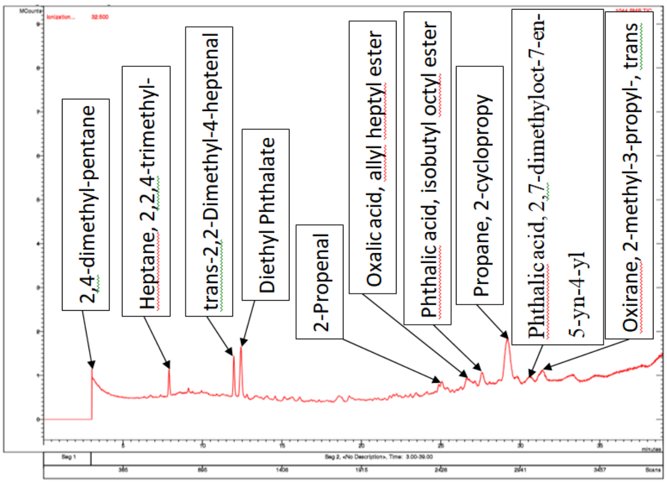


Figure 1.5 GC-MS Profile of Extracted Gaharu Oil with Ultrasonic Pretreatment of 40°C at 50°C and 5000 psi

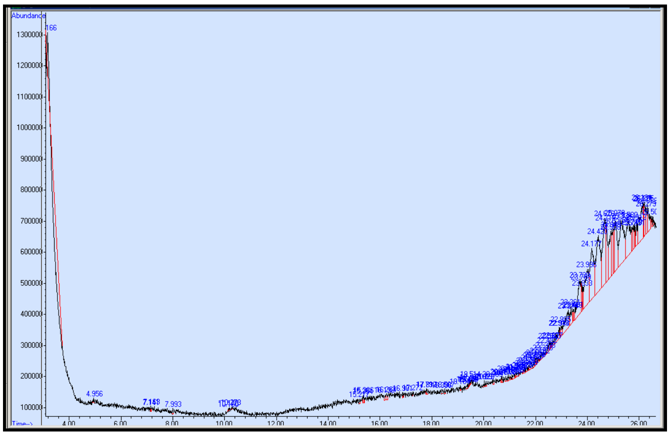


Figure 1.6 GC-MS Profile of Extracted Gaharu Oil without Pretreatment at 45°C and 5000 psi

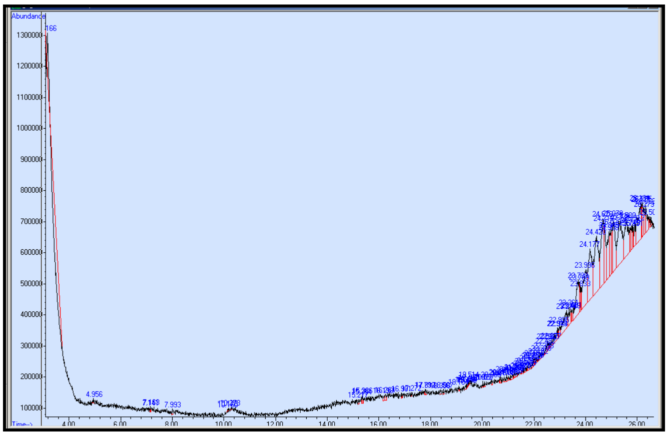


Figure 1.7 GC-MS Profile of Extracted Gaharu Oil without Pretreatment at 50°C and 5000 psi

C. *Chemical Compositions of Extracted Gaharu Oil*

The chemical compositions of the extracted gaharu oil were identified by using GC-MS. A total of 82 compounds were identified from the samples of gaharu with ultrasonic pretreatment of 40°C at operating extraction temperature and pressure of 50°C and 5000 psi respectively. Table 1.2 shows the list of compounds present with the percentage of amount (%). Ten major compounds out of 82 compounds were identified. The series of ten major compounds were 2,4-dimethyl-pentane (0.42%), heptane, 2,2,4-trimethyl (0.09%), trans-2,2-Dimethyl-4-heptenal (0.03%), diethyl phthalate (0.04%), 2-Propenal (0.04%), oxalic acid, allyl heptyl ester (0.03%), phthalic acid, isobutyl octyl ester (0.04%), propane, 2-cyclopropy (0.03%), phthalic acid, isobutyl octyl ester (0.02%) and oxirane, 2-methyl-3-propyl-, trans (0.03%). These chemical constituents are from aromatic compounds. The GC profile was shown in Figure 1.5.

IV. CONCLUSION

In this study, it was shown that the extraction of gaharu *(A. malaccensis)* essential oil using supercritical carbon dioxide extraction is more efficient in terms of time, energy and yield as compared with the conventional method. The oil yield of gaharu was found to be dependent on the operating temperature and pressure of the supercritical carbon dioxide extraction. From the results obtained, it can be concluded that the percentage of oil yield increased with the increment of temperature and pressure. It was found that the optimum condition for the SFE was at 50°C and 5000 psi which gave up to 0.1867%. For the effect of ultrasonic pretreatment on the oil yield, the results revealed that the optimum temperature for the ultrasonic bath was at 40°C. However, further studies are required in order to increase the oil yield of *A.*

malaccensis with ultrasonic pretreatment by supercritical carbon dioxide extraction. Therefore, for future works, the use of the same grade of gaharu should be taken into account during the extraction of all samples to ensure more accurate results can be achieved.

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