Agarwood Essential Oil: Study on Optimum Parameter and Chemical Compounds of Hydrodistillation Extraction

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A B S T R A C T

Agarwood tree is one of source resin production 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 to the lower product quality and oil yield. The aims of this research are to identify the effective extraction method conducted by standard hydrodistillation and re-design hydrodistillation. In order to increase the oil production, we optimize the extraction processing parameters tested by various particle sizes (0.5cm, 0.75cm, 1.0cm) and shaking time (1,3, 7 days). The results indicate that the high oil yield obtained by the oil extracted with sample size of 0.5cm (0.44%) and shake for 7 days (0.34%). Agarwood oil extracted using re-design hydrodistillation coupled with stirrer was achieving the maximum oil yield of 0.78% compared to standard hydrodistillation only 0.68%. Analysis of chemical compounds showed that 4-phenyl 2-butano, α-muurolene, α-elemol, agarospirol, selina-3,11-dien-9-ol and 2-hexadecanone as the major compounds detected.

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INTRODUCTION

Malaysia is one of the countries that produce fragrant wood and the famous name called as “Karas”. Agarwood tree or karas can be found in the jungle of Kelantan, Perak, Pahang and Terengganu jungle even though it is a rare species (Hilary, 2005). There are five species of *Aquilaria* are recorded in Peninsular Malaysia; *A. malaccensis*, *A. microcarpa*, *A. hirta*, *A. rostrata* and *A. beccariana*. All these species are believed to be able to produce oleoresin (Blanchette et al., 2006 and Barden, A., et al., 2000). The grading of agarwood oil is separated based on oleoresin content (Chetpattananondh, 2012). Usually, agarwood from high grade is more quality and expensive compare to low grade. Thus, agarwood are widely used with regards to their wood and oil in a number of countries around the world and makes agarwood one of the highly valuable products.

Currently, agarwood tree issues especially in benefit of agarwood oil have receive a lot of attention in the mass media. New technology extractions have been used such as using microwave (Ramly, 2006), supercritical fluid extraction (Mendiola et al., 2013), accelerated solvent extraction (Pingret et al., 2013) and ultrasound-assisted extraction (Esclapez et al., 2011). Each technique has particularly high in operating cost. Hence, the famous extraction process among the local trader is obtained by the standard hydrodistillation method. However, recent studies show that the process of hydrodistillation efficiency is low and analyses of chemical constituents are very rare (Jutarut et al., 2011).

Therefore, the objectives of this study were to increase the oil extraction efficiency obtained from different extraction method and to identify the optimum parameter of agarwood extraction. In this study re-design hydrodistillation coupled with stirrer was used to improved oil production. Whereas, study on chemical constituents of agarwood oil is done by using Gas Chromatography with Flame Ionization Detector (GC-FID) and Gas chromatography with Mass Spectrometry (GC-MS). Percentages of yield were calculated to evaluate the significant of oil quality and compared for all parameters studied.

MATERIALS AND METHODS

Plant material:

The chip woods of *Aquilaria malaccensis* used in this study were obtained from Rompin, Pahang. The sample was verified from herbarium of Institute of Bioscience, UPM and voucher specimen No SK 2422/14.

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Optimization technique:

Two parameters technique was selected, namely sample sizes and shaking time as control to get the optimum parameters for achieving maximum yield. The agarwood samples were initially ground into three various sizes at 1.0 cm, 0.75 cm and 0.5 cm by using grinder machine (FRITSCH). Then, the shaking time in 1, 3 and 7 days were harvested from automated shaker at 150 rpm (Arsat, 2008). Agarwood sample was soaked with water in a round bottom flask for 7 days (Bhuiyan et al., 2009 & Liu et al., 2008) and conducted by hydrodistillation I in triplicates.

Hydrodistillation (HD) extraction:

Hydrodistillation I: Approximately 300 g agarwood chip was performed with Clevenger type apparatus (Bhuiyan et al. 2009; Liu et al. 2008) for 9 hours in a round-bottom flask 5 L at a ratio of 1:10 (Tajuddin & Yusoff, 2010 and Fazila & Halim, 2012) and the temperature applied was 100 °C. Hydrodistillation II: HD II was modified from HD I by coupled with stirrer as depicted in Figure 1. The conditions of both extraction methods are same and carried out only at the optimum parameter. The extractions were conducted in triplicates and the average oils were calculated. The oils were then stored in sealed containers under refrigeration prior to analyze by chromatographic technique.

Chemical analysis:

The chemical constituents of agarwood oil obtained by two chromatographic technique of GC-FID (Agilent 7890A) and GC-MS (Agilent 7890A). GC-MS were attached to a mass spectrometer (Agilent 5975C) using a DB-1MS capillary column (30 x 0.25 mm I.D.; 0.25 μm film thickness). Both chromatographic techniques are same in operating condition. Temperatures of the injector and detector were set at 250 °C. The oven temperature was programmed at 60 °C for 3 min, ramped at 3 °C/min to 240 °C and then held for 10 min. The helium as a carrier gas was set at a flow rate of 1.2 mL/min. The volume of the sample injected was 1.0 μL. The components were identified on the basis of comparison of their retention indices and mass spectra with published data (Joulain & Konig, 1998) and matching with the National Institute of Standards Technology (NIST) libraries. The GC-FID instrument was equipped with a flame ionization detector (FID) and detector in full scan mode under electron impact ionization (EI, 70eV) was used in GC-MS.

RESULT AND DISCUSSION

Oil yield of two optimization parameters:

Fig. 1: Schematic diagram of hydrodistillation coupled with stirrer.

Fig. 2: Graph of oil yield (%) vs optimization parameter.
Two parameters technique was selected for enhanced oil production. The effect of sample size and shaking times on oil yield were present in Figure 2. The result shows that the size of 0.5cm is highest in producing oil with 0.44% compared to other parameter. Sample size of 1.0cm and 0.75cm gave a lowers of 0.35% and 0.30% respectively. This finding was supported by a study of Behrend & Schubert (2001) that when decreasing the particle size, increasing amount surface area and became the particle more to react each other. Thus, the oil production is faster to evaporate to the condenser.

The effects of shaking times on oil yield were investigated by different shaking time 1, 3 and 7 days. Time play an important role to describe the effect of the kinetics and mechanism of the soaking process. Herein, shake for 7 days gave the maximum percentage of yield about 0.34% followed by 3 and 1 day with 0.33% and 0.19% respectively. A possible explanation for this might be due to increasing time of soaking can help to promote activity process of cell wall wood degradation and making the oil yield will be increased. This cases supported by Allison et al., (2009) mentioned that activity process of cell wall is caused by a few organisms have the potential to degrade all the available plant cell wall materials. As expected extraction at shaking 1 day give the minimum in oil production because of the shorter time of the degradation process of agarwood. Therefore, the optimum duration time of shaking was found to be 7 days.

Oil yield of hydrodistillation method:

Fig. 3: Graph of oil yield (%) vs different type of extraction.

The optimum parameters will be chosen to run again by using standard hydrodistillation and hydrodistillation coupled with stirrer to investigate the effect of oil yield. Each extraction method has particular advantages and disadvantage was observed. The oil collected in both extraction methods is represented in Figure 3. The results indicated that HD II gave higher oil production of 0.99% compared to HD I at only 0.78%.

As expected, HD I is lower in oil production compared hydrodistillation coupled with stirrer. It occurred due to the oil separation incomplete and extraction process also not efficiency. Whereas, redesign hydrodistillation method assists in oil production due to the effect of stirrer and kinetic reaction of each particle increased during the process. This process also one of a new development technique that can be applied in the extraction process. The mechanism of this process involved the water as a good polar nature. Once supplied the heat source to the system, the water easier to evaporate with presence the stirrer. The vapor water is then attracted the oil as a non polar and brought to the condenser. Thus, the oil productions became faster and maximum yield can be obtained by the effect of stirrer during the process of extraction.

The more effective hydrodistillation method may be developed to improve the recovery of agarwood oil extracted in future and more knowledge about the efficiency process should be studied. Nevertheless, both extraction methods are the same result observed in solubility properties that are insoluble in water but soluble in alcohol. Besides, the oils collected showed yellowish color and sweet woody odor.

Chemical composition of agarwood oil:

The chemical compounds of oils extracted from varying method and two optimization parameters were identified by using GC-FID and GC-MS. The lists of components are shown in Table 1 and 59 compounds were identified in this analysis. From the results, there are several similar and varying of components can be observed. Based on the analysis by GC, oxygenated sesquiterpenes showed the highest constituents than other terpenes compounds.

In particle sizes, the oils extracted from sample 0.5cm shows the largest amount of hydrocarbon identified consists of 84.46% compared to oil extracted with sample size 0.75cm and 1.0cm lower consist of 66.97% and 77.64% respectively. Conversely, 26 number of chemical compound are same were detected by sample size 0.75cm and 1.0cm. In shaking time, all the parameter is not much difference in amount of hydrocarbon for 7, 3 and 1 day were 77.64%, 81.65% and 78.25% respectively. Apparently, the greatest of total hydrocarbon was observed from HD II consist of 80.67% compared to HD I only 63.02%.
There are several major compounds found in those studied namely 4-phenyl 2-butaneone, α-muuroene, α-elemol, agarospori, selina-3,11-dien-9-ol and 2-hexadecanone. Most of the present study reported that agarospori one of the marker compounds founds in agarwood oil extracted (Bhuiyan et al., 2009; Tajuddin & Yusoff, 2010; Jutarat et al., 2011; Azah et al., 2008). Agarospori was used in pharmaceutical industries for brain test, nervous system and sedative (Takemoto et al., 2009; Okugawa et al., 1996).

**Table 1: Chemical compositions of essential oil obtained by optimized parameter and two extraction method agarwood oil.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DB-1</th>
<th>Particle sizes (nm)</th>
<th>Shaking times (day)</th>
<th>Hydrodistillation</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monoterpene hydrocarbons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-pinene</td>
<td>93.07</td>
<td>0.07</td>
<td>2.36</td>
<td>4.07</td>
<td>4.26</td>
</tr>
<tr>
<td></td>
<td>2.15</td>
<td>1.09</td>
<td>0.91</td>
<td>0.56</td>
<td>0.40</td>
</tr>
<tr>
<td>Sesquiterpene hydrocarbons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Boswellic</td>
<td>93.07</td>
<td>0.07</td>
<td>2.36</td>
<td>4.07</td>
<td>4.26</td>
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<tr>
<td></td>
<td>2.15</td>
<td>1.09</td>
<td>0.91</td>
<td>0.56</td>
<td>0.40</td>
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</table>

**Conclusions:**

In this study, the optimum parameter were obtained by the highest oil yield identified from a sample size of 0.5cm and performed by shaking times for 7 days compared to others parameters. Agarwood performed with hydrodistillation coupled with stirrer shows the great results of percentage oil compared standard
hydrodistillation. It’s clear that this method can improve the extraction efficiency process. In chemical analysis, six major chemical compounds found in all extracted oils were 4-phenyl 2-butanone, α-muurolene, α-elemol, agarospirol, selina-3,11-dien-9-ol and 2-hexadecanone.

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