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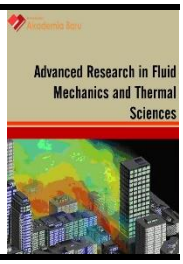

Microwave-Assisted Hydrodistillation of *Aquilaria Subintegra*

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ABSTRACT

Alternative extraction method by using the microwave-assisted hyrodistillation (MAHD) method is used to extract essential oil from agarwood. The effect of microwave power of 300, 400, 600, and 800 watt (W) and irradiation time of 5 hours on the extraction of agarwood essential oil were studied. The quality of the oil and the identification of its compounds were further analysed using GC-MS. The highest yield obtained from MAHD is at 600 W which is 0.13 %, while the yield for HD is 0.135 %. At 600 W, the time required for the sample to boil is within 20 – 25 minutes and oil started to produce within 15 – 20 minutes after boiling. Two compounds commonly identified in the extracted oil includes 6-Octen-1-ol, 3,7-dimethyl-,propanoate or knowns as Citronellyl propionate which have a floral scent and Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene) or γ-Gurjunene which have a musty-woody odour. The experimental results show a shorter operation time and a higher yield result from higher extraction rates for MAHD which is equivalent to 7 hours HD extraction.

Keywords:
Agarwood; essential oil; GC-MS; microwave-assisted hydrodistillation; extraction

1. Introduction

Essential oils (EO) are aromatic, natural and complex mixture of volatiles extracted from different plants parts such as flowers, roots, barks, leaves etc. EO contains a few dozen to several hundred constituents, especially hydrocarbons terpenes and sesuiterpenes and oxygenated compounds (alcohols, aldehydes, ketones, acids, oxides, lactones, acetals, ethers and esters) [1]. For centuries, these oils were mainly used primarily for medicine but have since expanded into fragrance, food and pharmaceutical [2]. Aquilaria is known as gaharu locally and also known as oudh, jin-koh, chen xiang and eagleswood [3]. Agarwood is a fragrant wood produced primarily by the Aqualaria species in the Thymelaeceae family and is high in demand globally. The resin obtained from these species are

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produced as a defense reaction when the trees become unhealthy in which it can occur both naturally and or through artificial wounding such as through inoculation.

Agarwood essential oil is a highly prized type of oil due to its unique aroma. The quality of the oil depends on several factors: its region of origin, botanical species, age of the tree and the section of the tree from which the agarwood stems from [4,5]. There are different grades of agarwood, and the highest quality wood is extremely expensive. According to The Rainforest Project [6] the first-grade wood has a price range of between a few dollars for the lowest quality to tens of thousands per kilo for the finest one. Mohd. Ali *et al.,* [7] studies show that the price of premium grade agarwood (grade A) is around RM16,000 to RM20,000 per kg and RM400 – RM 2000 per tola (1 tola = 12 ml) for supreme agarwood oil.

The highest grade of resin can only be obtained from wild Aquilaria trees, and the occurrence of the resin in this context is quite rare and happens slowly. The trees were chopped down indiscriminately as it is almost impossible to recognize whether the tree contained the resin. Jung [5] stated that it is important to raise public awareness of the possible threat of extinction of wild agarwood trees to ensure their existence in the future and also highlighted the importance of significant improvement in the identification of the species.

Essential oils can be extracted by several methods which include hydrodistillation (typically using water or steam), solvent extraction [8–10], supercritical fluid extraction [11,12], and cold pressing [13]. However, the most common approach used in extracting essential oil from plants is the hydrodistillation (HD) method [8,9] and is the method mostly applied by the agarwood industry in Malaysia. It is a simple method however ineffective because of the long extraction hours and the low yields [14–16]. These common methods can also influence thermal degradation and loss of some fragrance constituents [14,15,17]. The oil obtained through solvent aided extraction tends to contain residues that pollute food fragrances [18,19]. As a mean to overcome this sort of drawbacks, alternative and green methods in the extraction of natural products from plants such as ultra-sound-assisted [20], ohmic-assisted [14,21], and microwave-assisted extraction [19,22] have been investigated.

Microwave-assisted hydrodistillation (MAHD) is a green procedure that isolates essential oils and has become attractive for use in laboratories and extraction industry due to its effective heating, fast energy transfer, high extraction rates, shorter extraction times and environmental-friendly extraction technique in comparison to the conventional hydrodistillation (HD) method [17,19,23,24]. MAHD has been employed in the extraction of some essential oils from orange peel [25], herbs (lavender, rosemary, sage, fennel seeds and clove buds [26,27], wastes such as from pineapple for its phenolic contents [28] and antioxidant activity, and also in the extraction of organic components from coal [29].

Its acceptance as a prospective and powerful alternative for the traditional extraction techniques has been verified by several research works [17,30–34]. Moreover, the low content of essential oil in plant materials means that high efficiency technique is required to achieve higher yield. Thus, parameter optimization of the technique is the most vital process for achieving this [35]. Kusuma *et al.,* [36] in their study mentioned that during MAHD a pressure difference occurs between the inner and outer side of the plant cells easing the release of contained compounds to the surrounding solution hence resulting in a higher mass transfer coefficient. The MAHD technique has been described as time- and energy-saving and highly efficient [19].

Thus, this research has two objectives: (i) to employ the microwave-assisted hydro-distillation (MAHD) method for extraction of agarwood essential oil (*Aquilaria subintegra*) and (ii) to compare the extraction time and yield of agarwood essential oil between conventional hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) method.

2. Methodology

2.1 Material Preparation

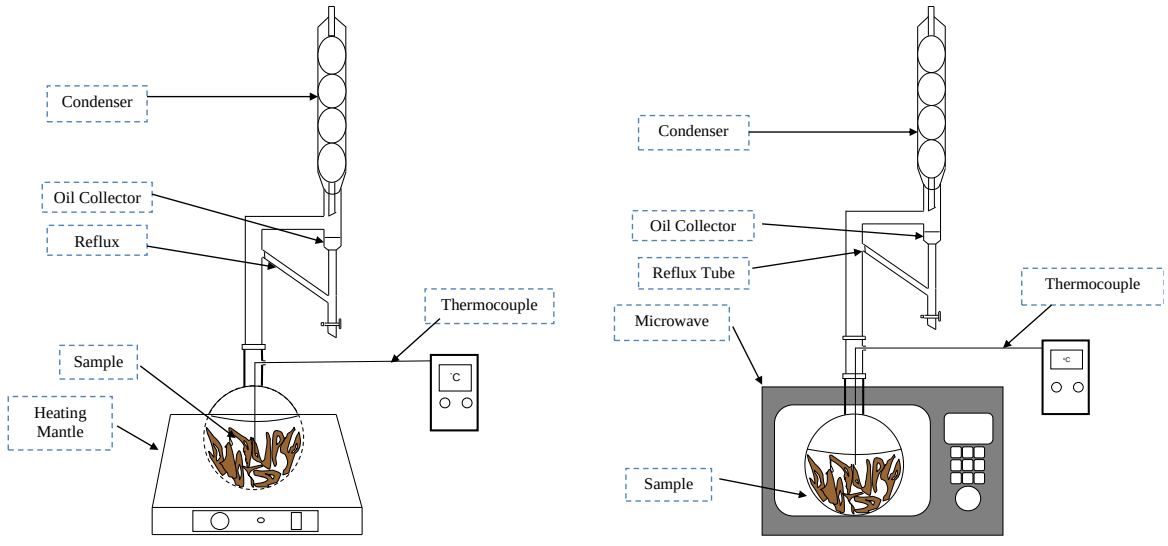
The process for the standard preparation of sample was adopted from a local agarwood processing company, Biobenua Lojistik Sdn. Bhd. where 200 g of the grounded agarwood chips were first weighed and mixed with deionized water at a 1:10 (ratio of volume of water to weight of sample). The sample was left to soak for 14 days at room temperature. A similar preparation process was made for 6 days of soaking.

2.2 Hydrodistillation (HD)

The sample prepared in 2.1 underwent hydrodistillation using the Clevenger-type apparatus paired with a heating mantle (Figure 1(a)). The temperature during the process was kept at 100±0.1 ℃. Oil formation was observed every hour for a duration of 7 hours. The oil extracted was collected and the moisture removed by using anhydrous sodium sulfate (Na_2O_4S, ≥ 99.0 %, Merck) and stored at 4 ℃ prior further analysis. The oil from sample for HD that are soaked for 14 days is known as A14 while oil from the 6 days of soaking is B6.

2.3 Microwave-assisted Hydrodistillation (MAHD)

In applying MAHD, a commercial microwave oven (NN-ST651M, Panasonic, 32 L, 1000W, 2.45 GHz) with a cavity dimension of 355 mm x 251 mm x 365 mm (W x H x D) was used. The microwave oven was modified in order to allow the access of the round bottom flask to the Clevenger apparatus (Figure 1(b)). Temperature within the reactor is observed and recorded using a digital thermocouple (Center DL306).



(a) (b)

Fig. 1. Schematic diagram of (a) Standard Hydrodistillation, and (b) Microwave-assisted Hydrodistillation

The 14 days-soaked sample material was placed in the flask in the microwave with different irradiation power of 300 W, 400 W, 600 W and 800 W which will be named as sample C14, D14, E14, and F14 respectively. The oil extracted was collected, dried with anhydrous sodium sulfate and stored

at 4 ℃. The dried extract was dissolved with n-hexane (C_6H_{14}, 98 %, R&M Chemicals) and then analysed using GC-MS. Meanwhile, the oil extracted from the 6 days-soaked sample which is exposed to irradiation power of 600 W is known as G6. The oil extraction times are kept at 5 hours.

2.4 Characterization of Agarwood Oil

The analysis is performed using a GC-MS (QP 2010) equipped with a VF-5 ms fused silica capillary column with film thickness of 0.25 µm. An electron ionization system with an ionization energy of 70 eV was used for the GC-MS detection. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1 mL/min. Injector temperature was set at 230 ℃ and the temperature for the oven was set at 50 – 230 ℃ with an increasing rate of 5 ℃/min. The peaks from GC-MS were identified using the National Institute of Standards and Technology (NIST) mass spectral library.

3. Results

3.1 Effect of HD and MAHD on Extraction Yield

The oil extracted from the standard hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) with 14 soaking days were tabulated in Table 1. The amount of agarwood essential oils extracted from both techniques shows similar yield throughout different parameters. During MAHD, for samples soaked for 14 days and the lowest yield obtained is from sample D14 which is 0.105 % while the highest yield is from sample E14, 0.13 %, which use microwave power of 400 W and 600 W respectively. The yield for the rest of the samples, C14 and F14 are 0.1125 % and 0.1075 % respective of microwave power of 300 W and 800 W correspondingly as visualized in Figure 2. Sample A14 yielded 0.135 % of oil which is similar to E14 with 0.13%. This is supported by Mahfud *et al*., [16] where MAHD offers a shorter time for the extraction of the essential oil compared to HD with less than 3 hours when HD takes more than 6 hours for the same oil yield.

Table 1
Tabulated Data for Oil Extracted from HD and MAHD

Type of Sample	Yield of extraction (%)	Standard Deviation (S)
A14	0.135	0.0212
B6	0.0625	0.0071
C14	0.1125	0.02121
D14	0.105	0.02828
E14	0.13	0.01414
F14	0.1075	0.0071
G6	0.0875	0.0071

E14 extracted using MAHD with a microwave power of 600 W showed the highest yield of 0.13 % where this shows that the MAHD technique can produce the similar amount of oil in 5 hours as compared to HD of 7 hours. As mentioned by Li *et al.,* [37], where MAHD can be completed in a shorter time compared to standard hydrodistillation and also supported by Golmakani *et al.,* [38]. The experiment was continued with reduced soaking days from 14 to 6 days which is B6 for HD and G6 for MAHD technique and the results showed that even with a reduced soaking preparation time, MAHD offers better yield than HD.

Taking E14 as precursor, G6 which was soaked for 6 days produced a yield of 0.0875 % while B6 with 0.0625 % showed that sufficient soaking days are required to have a high oil yield extraction. Jok *et al.,* [39] in their study stated that with a sample size of 25 kg of agarwood chips, 14 days of soaking produced the highest oil yield for untreated sample which is 2.5 g. This proves that it is

necessary to have a sufficient soaking time, however the oil yield is further reduced with extended soaking time of more than 14 days. Their study was previously supported by Bayram and Kaya [40] where the acidity of the soaking medium may increase with the increase of soaking time which may cause excessive cell wall breakage and the unnecessary release of oil into the surrounding. Alternatively, higher yield of agarwood oil extract is possible with the introduction of pre-treatment such as enzymatic [9,15], dilute acid or alkali [41–43], and organosolv pretreatment [44] etc.

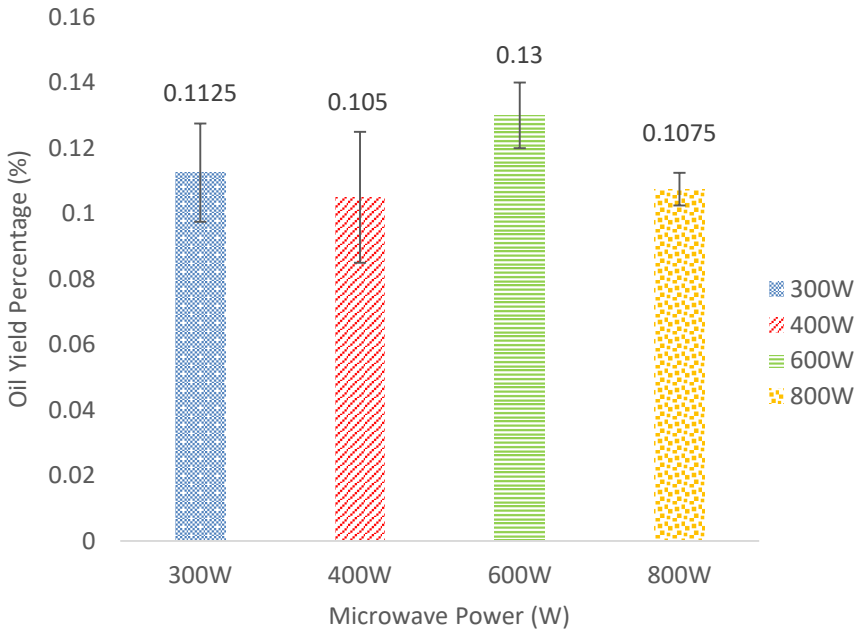


Fig. 2. Comparisons on The Percentage of Oil Yield Employing MAHD with Different Power

The MAHD method offers better advantages compared to HD such as shorter extraction time and better yield [16,38,45–48]. This could be due to the synergy of two transfer phenomena: mass and heat transfer which act at the same direction compared to mass transfers for HD where the mass and heat transfer occur counter-currently [32,48,49]. The release of essential oil starts from inside the particle outward for both HD and MAHD. However, during HD, the heat is transferred from the surface to the inside of the particle while for MAHD, heat is transferred outward of the particle which resulted in a synergistic transfer for MAHD easing the release of the essential oil.

According to Golmakani *et al.,* [38], the striking differences between HD and MAHD is that during the MAHD process, the raise of the extraction yield was quick and notably, achievable within a short time. It was also suggested that the extraction mechanism during MAHD is partly due to the internal heating of in situ water under microwave radiation for the MAHD heat transfer facilitating the oil diffusion [38]. Noelia *et al.,* [50] stated that heat is transferred by convection and conduction for HD meanwhile MAHD penetrated into the solid causing direct generation of heat within the material simultaneously increasing the temperature. MAHD also provides localized heating which acts as a driving force and with increase in power, the yield and extraction time will be shortened [22]. This is also supported by Elyemni *et al.,* [51] recent study where the internal overheating during MAHD will efficiently cause the rupture of cell walls rapidly (embrittlement). Hence from Table 1, sample A14 and E14 shows comparable oil extraction percentage.

3.2 Temperature Profile for MAHD Extraction

Figure 3 shows the temperature profile for the first sixty minutes during the extraction for samples from both HD and MAHD with 14 days of soaking period which were A14, C14, D14, E14 and F14. The initial temperature was around 25 ℃ for all the extraction methods and the final extraction temperature was equal to the boiling point of water (100 ℃). The yield obtained in Table 1 show that variation of microwave power from 300 W to 800 W had similar effect on the extraction yields where sample C14 to F14 which employs MAHD have an oil yield of 0.1125 %, 0.105 %, 0.13 % and 0.1075 % respectively. Figure 3 also shows that the time required for the samples to reach boiling point for HD and MAHD at 300 W and 400 W was almost the same where the boiling time required was around 40 – 45 minutes. Meanwhile, for microwave power of 600W and 800W, the time required for boiling for both powers were reduced to around 20 – 25 minutes. The condensate for all the MAHD starts to produce at 92 – 95 ℃.

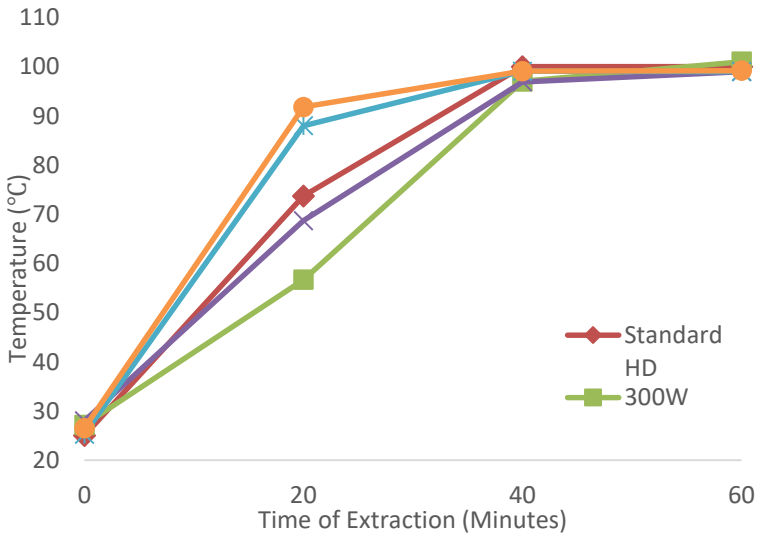


Fig. 3. Temperature Profile of HD and Different Power of MAHD for The First 60 Minutes

For A14, C14 and D14, oil started to produce 25 to 30 minutes after it started to boil. For microwave power of 600W, the time required for oil to start to form is around 15 – 20 minutes after boiling and for microwave power of 800W, the time needed is around 10 – 15 minutes. Thus, from the five powers, when the power of the microwave is high, the time for the sample to produce essential oil will become shorter. Golmakani *et al.,* [38] stated that the important reason for this is that MAHD applied three ways of heat transfer which are conduction, convention and irradiation and the rapid increase of the temperature added to the reduction of extraction time of agarwood essential oil.

The oil yield obtained from G6 at different soaking time of 6 days while employing MAHD is shown in Figure 4. The yield shows and incremental value for each hour of extraction up to 4 hours from when the sample started boiling. The oil yield percentage are 0.02 %, 0.03 %, 0.06 % and 0.075 % for extraction time of 1hr, 2hr, 3hr and 4hr respectively. From observation, the oil was harvested during the prolong boiling instead of affected by rate of heating. As shown in Figure 4, the oil yield is very small for first hour into extraction.

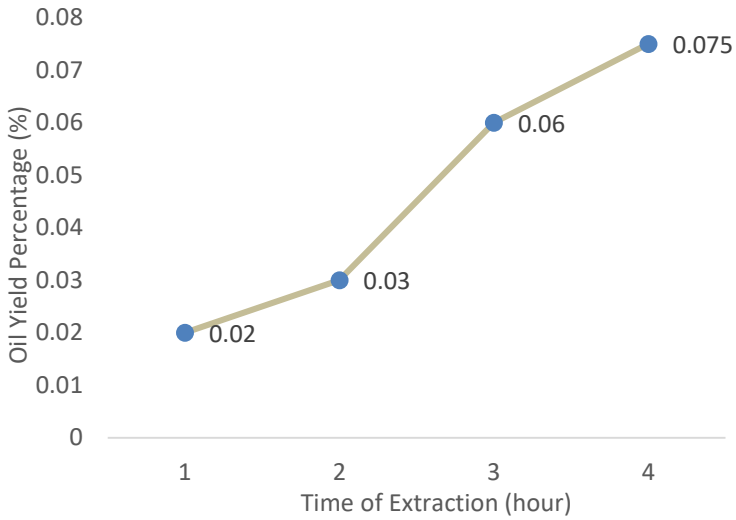


Fig. 4. Oil Yield with Different Extraction Time Obtained from MAHD

3.3 GC-MS on Chemical Compounds in Agarwood Oil Extracted from HD and MAHD Temperature Profile for MAHD Extraction

Essential oil from agarwood was extracted using the standard hydrodistillation (HD) and the microwave-assisted hydrodistillation (MAHD) and then analyzed by gas chromatography-mass spectrometry (GC-MS). Each sample gave similar total compounds as identified from the GC-MS analysis. All five samples of agarwood oils from the different extraction methods and power showed some variations in GC-MS profile chemical compounds. Some of the variations in the GC-MS profiles analyzed are shown in Table 2.

A total of 282 compounds were identified from the six samples of agarwood oils. 48 components were identified in sample A14 which is from standard hydrodistillation method. In samples C14, D14, E14 and F14 with 48, 47, 49 and 49 compounds were identified respectively and 41 compounds in sample G6. About 15 % of the compounds identified were aromatic, and terpene (monoterpene and sesquiterpenes) which are known to be the main active compounds of agarwood oils [4]. These main active components play important roles in giving the aroma and pleasant odor of agarwood [36]. Some of the sesquiterpene compounds found in the samples were Gurjunene and Valencene.

Table 2
Some of The Compounds Identified in Agarwood Essential Oil via GC-MS

Chemical Compound	% of Total Area				
	A14	C14	D14	E14	F14
Citronellyl propionate	-	0.893	0.768	1.434	0.624
γ-Gurjunene	8.081	3.017	7.001	19.155	0.422
Valencene	1.603	0.673	2.102	9.393	2.412
Caryophyllene	-	0.8	0.502	0.042	0.422
Aristolene	1.884	1.197	2.104	-	4.982
Alloaromadendrene	2.531	1.185	1.139	6.189	3.347

In contrast to a study done by Pripdeevech *et al.,* [52] on the same species of *Aquilaria subintegra*, some of the main compounds such as jinkoh-eremol, epoxybulnesene and β-agarofuran are absent from the results. According to Jong *et al.,* [53] , where the environment and genetic factors may play significant roles which cause the varying of aromatic compounds in agarwood essential oil. This is

further supported by Chen *et al.,* [54] and Hashim *et al.,* [4] where different artificial methods used in the stimulating the formation of agarwood and species/origin may produce and affect different compositions in agarwood essential oil.

Table 2 shows some of the compound identified from samples A14, C14, D14, E14 and F14. Two of the compounds identified were 6-Octen-1-ol, 3,7-dimethyl-,propanoate or knowns as Citronellyl propionate which have a floral scent and Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene) or γ-Gurjunene which have a musty-woody odor. Gurjunene is one of the highest compositions of compounds found in the samples and found to be significant in all MAHD samples and the percent total were notably higher in sample E14. Based on the data obtained from GC-MS analysis, Aristolene and Alloaromadendrene was identified in both sample E14 and F14 but the % of total area were too small making it negligible.

As previously mentioned, the aromatic compounds identified in Table 2 provide the woody aroma to agarwood oil. Caryophyllene contributes to the spicy-wood aroma and alloaromadendrene for the woody scent. Studies done by other researchers [17,27,32,33] showed that that extraction method between HD and MAHD on different type of plants doesn't have substantial effect on the concentrations of the compounds. This show that MAHD, even with accelerating the extraction process doesn't cause significant changes towards the EO composition but in our study, it showed quite an increase in most of the compounds while some others might have a decrease in percentage.

As for sample G6 which employs the same method but with reduced soaking days of 6 days, similar compounds can be identified. A total of 41 compounds were identified as compared to sample E14 with 49 compounds. The percentage of total area for the same compound from Table 2 were tabulated in Table 3. From Table 3, almost all compounds were present, but the compositions are lesser than the compounds identified in sample E14. The composition of Citronellyl propionate is similar for both sample E14 and G6, 1.434% and 1.982% respectively but for γ-Gurjunene there is a significant different.

Table 3
Comparison on The Compounds Identified in Agarwood Essential Oil via GC-MS from different soaking days for MAHD

Chemical Compound	% of Total Area	
	E14	G6
Citronellyl propionate	1.434	1.982
γ-Gurjunene	19.155	5.29
Valencene	9.393	1.096
Caryophyllene	0.042	0.216
Aristolene	-	0.481
Alloaromadendrene	6.189	-

This showed that with reduced soaking time, less compounds can be extracted. This is in contrast with a research done by Yusoff *et al.,* [55] employing HD where there were no significant changes between the compounds composition found with shaking time of 1, 3 and 7 days. There were no reported effects for agarwood compounds composition on soaking time less than 7 and Jok *et al.,* [39] did a research comparing the effect of different soaking time of 0, 7, 14, 21 and 28 days with no pre-treatment employing standard HD. It can be concluded that the different microwave power used, will affect the composition of agarwood oil that is present in its essential oil. It can increase and reduce the total number of compounds in the oil extracted. The insignificant number of compounds may also be affected due to sample preparation or extraction time and the microwave power used.

4. Conclusions

From the results obtained, it can be concluded that MAHD offers better benefits than HD, which are shorter extraction time and comparable yields. From the MAHD method, the microwave power of 800 W takes a lesser time to extract the oil, but the microwave power of 600 W produces the highest yield of 0.13 % as compared to 800 W with a yield of 0.1075 %. Microwave power of 600 W takes 20 – 25 minutes to boil and another 15 – 20 minutes to start producing agarwood oil. Among the similar components identified are Citronellyl propionate, γ-Gurjunene, Valencene, and Caryophyllene. Oil extracted using both methods must contain a large amount of high volatile compounds and their composition should be same for them to be considered on par, but microwave irradiation may improve or decrease the release of the volatile compounds. Thus, microwave-assisted extraction offers better results with less extraction time and better yield and can likely be used in agarwood oil extraction.

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