NOTE:

Effect of Microwave Pretreatment on Gaharu Essential Oil Using Hydrodistillation Method

Nurhaslina Che Radzi* **and Filzah Anati Kasim**

Faculty of Chemical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

*** Corresponding author:**

email: nurhaslina483@uitm.edu.my

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Abstract: *Gaharu wood produces an expensive essential oil due to its fragrance and chemical compounds. Normally, the black color wood is used for the extraction of oil because it contains high resin. However, the cost of this resinous wood is very expensive. Therefore, in this study, the raw material with low resin content (identified by the white color of the wood) will be used for the extraction of oil. However, hydrodistillation alone will not produce satisfactory volume. Thus, pretreatment is needed to enhance the amount of essential oil. This paper aims to investigate the effect of microwave pretreatment on gaharu essential oil extraction using the hydrodistillation method for the lower grade wood. The gaharu wood was pre-treated with microwave irradiation power of 800 kW at three different durations which were 1, 2 and 3 min. Later, extraction was performed using the hydrodistillation method for 30 h. The chemical composition of the oil was analyzed using gas chromatography-mass spectrometry (GCMS). The results show that microwave pretreatment produced higher yield (0.0379 wt.%) of gaharu essential oil compared to the extraction of the non-pretreated wood (0.0286 wt.%) in which the increase was 24.55%. The three minutes pretreatment time produced the largest amount of oil (0.0877 wt.%). The outcomes of this study indicated the increase of chemical components of gaharu essential oils such as (+)-Epi-bicyclosesquiphellandrene, gurjunene, α-farnese, estragole, guaiene, valencene, spathulenol, α-cubebene and (-)-Spathulenol in the extraction of the pre-treated wood compared to the non-pretreated one.*

Keywords: *gaharu; microwave; hydrodistillation; essential oil; gas chromatography mass spectrometry (GCMS)*

■ INTRODUCTION

Gaharu is a resinous heartwood tree that belongs to the *Thymelaeceae* family. A few other names for Gaharu are agar, agarwood, eaglewood, and aloeswood. Gaharu types of *Aquilaria malaccensis, Aquilaria crassna, Aquilaria sinensis and Aquilaria filarial* are famously exploited worldwide [1]. *Aquilaria malaccensis* usually live and are cultivated in the habitat with a temperature between 22 to 24 °C and at the altitude range of 0 to 850 m, which is suitable to Malaysia's climate [2].

Essential oils can be obtained from the roots, stems, leaves, stalks, fruits, and flowers of plants and distilled from resins [3]. However, the uniqueness of gaharu comes from the development of the resinous gaharu in a natural process due to its response to the parasitic ascomycetous mold, *Phialophora parasitica*, a dematiaceous (dark-walled) fungus. The sweet-smelling pitch is normally formed in the bark, roots and the heartwood of the trees. Gaharu is a timeless tree that can be used in fragrance and perfumery products, medical purposes, religious rituals, etc. [4]. As early as the thirteenth century, gaharu has been playing an important role in lots of practices related to Buddhism, Hinduism, Islam, Christianity and Judaism for cultural, religious, and medical purposes. In the Old Testament, gaharu was used as perfume, while in Ayurvedic, Tibetan and East Asian literature, gaharu was mentioned as a medicinal medium [5]. Gaharu also plays a significant part in Buddhist religious ceremonies, where gaharu is burned around the Buddha statue in their rituals [2].

Nowadays, since the value of gaharu is increasing, researchers and entrepreneurs are inspired to find the best method and technique of extraction to get the optimum essential oil yield while reducing the cost and energy. The effectiveness of the extraction process from a natural plant is affected by several factors such as polarity and concentration of the solvent, solvent to feed ratio, extraction process time and thermal degradation as well as valid sample preparation to avoid the degradation of the plant extract. The price of essential oil from gaharu is solely depend on its quality which is graded by human experience from the old-age practice of each country [6].

Various extraction methods can be used to produce essential oils. Currently, hydrodistillation, steam distillation, water distillation, and solvent extraction are commonly used to extract essential oil from plants; however, hydrodistillation method is one of the simplest, oldest and most primitive processes for obtaining essential oil from plants and it is mostly used by small scale manufacturers of essential oils, but the process is slow because it takes 3-5 days to distillate, thus making it uneconomical due to the high consumption of fuel/gas [7].

From the author's view, there are two main constraints in this area. First, a direct heating source using a hydrodistillation extraction method will destroy a certain sensitive essential oil compound [8]. However, the process to change the hydrodistillation technique with other methods will be an issue to the practices of conventional industries due to additional costs that need to be invested. Therefore, an alternative approach combining conventional technology (hydrodistillation) with additional lower cost equipment may be an option. The hydrodistillation method aided with microwave pretreatment will help improve the efficiency of extraction of the gaharu essential oil by reducing the processing time, saving energy and cost as well as increasing extraction yield and quality of the essential oil produced. All this is possible because microwave radiation will induce the rupture of the cell membrane and improve its porosity, thus causing the oil to transfer better [8-9].

The second issue is the high price of the gaharu itself. The Grade A gaharu reaches as high as RM 16,000 per kilogram in Malaysia depending on the quality of the essential oil [8]. However, in one whole tree of gaharu, usually only the black woody part that represents the high quality resin, would be used, leaving the white woody part as waste. Therefore in this study, a method is proposed to fully utilize this waste by extracting the essential oil and study its chemical properties. Thus, the objectives of this research are to investigate the effect of microwave pretreatment on essential oil yield via the hydrodistillation process and the effect of microwave on the chemical composition of oil extract from lower grade gaharu.

■ EXPERIMENTAL SECTION

Materials

Gaharu stembark (*A. malaccencis*) grade C was collected and purchased from a plantation in Kuala Krai, Kelantan, Malaysia. Then the sample was ground using a crusher (model: SLM-20PI/D) to obtain the size of 0.5 cm and afterwards stored into zip plastic bags. Tap water was used to soak the gaharu sample.

Procedure

Microwave pretreatment

Two kilograms (2 kg) of the ground gaharu was prepared for each sample. The gaharu samples were placed on a 16 cm microwave plate (40.0 g on each plate). The microwave (Model R-397J(S), SHARP Malaysia) ran with the constant operation power of 800 kW and the processing time for this microwave pretreatment was set in three different durations of 1, 2 and 3 min. Gaharu samples were then cooled under ambient temperature.

Hydrodistillation

Two kilograms (2 kg) of gaharu with pretreatment and without pretreatment process was soaked indifferent containers, each containing 9 L of water for 72 h at room temperature. Then, the hydrodistillation process was carried out with the processing time set at 30 and 47 h. The obtained gaharu essential oil was collected and the existing solvent was removed using the rotary evaporator. Essential oil yield (wt.%) can be calculated using Eq. (1).

$$\text{Essental oil yield}(\text{wt.\%}) = \frac{\text{Mass of oil extracted}(g)}{\text{Mass of sample}(g)} \times 100\% \quad (1)$$

Gas chromatography mass spectrometry (GCMS)

The analysis of gaharu essential oil chemical compounds was carried out using GCMS. This analysis was required to identify the active compounds in the gaharu essential oil. Table 1 shows the summarized setting conditions of the GCMS analysis [10]. Fig. 1 demonstrate the flow of the extraction process and the GCMS equipment.

Table 1. Condition for GCMS analysis

Component	Condition
Ionization voltage	70 eV
Gas flow	2 mL/min
Split ratio	1:50
Injection volume	1 µL
Oven program	80 °C for 2 min, then 10 °C/min heating rate to 250 °C for 10 min
Carrier gas	Helium
Interface temperature	250 °C

Fig 1. Extraction Process flow and GCMS analysis of gaharu essential oil, (a) Coarse raw material, (b) Grinding process using crusher, (c) Ground gaharu, (d) Microwave pretreatment, (e) Hydrodistillation process, (f) Analysis using GC-MS

■ RESULTS AND DISCUSSION

The Effect of Microwave Pretreatment on Essential Oil Yield via Hydrodistillation Process

Microwave pretreatment provides the potential to induce stress reactions in the plant system. Fig. 2 shows the yield percentages of gaharu extracted oil at 30 h of hydrodistillation with and without microwave pretreatment for the duration of 1, 2 and 3 min. Results show that the pretreated gaharu with 3 min exposure to microwave produced a higher oil yield which was 0.0877 wt.% compared to the non-pretreated sample which only produced 0.0286 wt.%. For the treated gaharu, microwave pretreatment at 3 min had the highest percentage of extracted oil yield (0.0877%) followed by 2 min (0.0779%) and 1 min (0.0379%) of microwave pretreatment. This result is in line with a previous research [9] which stated that through microwave pretreatment, bioactive compounds can penetrate out from the cell wall and produce greater amounts of oil. Similar studies by other researchers [8] revealed that the extracted oil yield from hazelnut seeds increased after the application of microwave treatment before conducting extraction.

The increase in the extracted oil yield was due to the modification of the cellular walls which resulted in greater porosity. The electromagnetic wave produced by

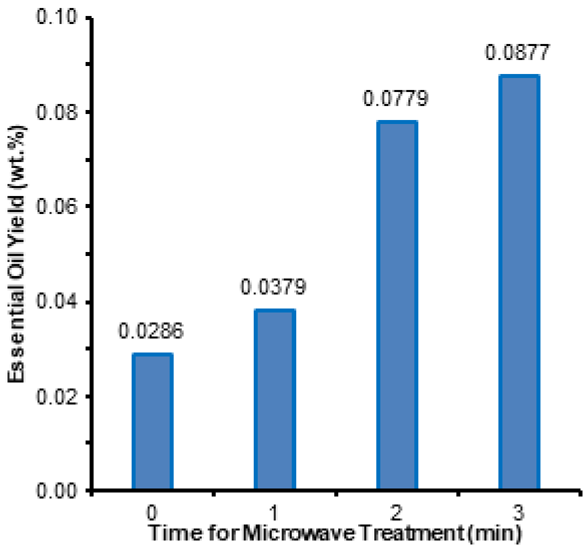

Fig 2. Effect of pretreatment on extracted oil yield weight percentage

microwave at high frequency can cause penetration to the plant and reach the inside of the materials and rupture the cell wall, thus causing damage to accelerate and simplify the cell wall and bring the gaharu oil out of the material [11]. Rupture of cells is essential before the extraction of the desired compound of plant tissue. Pretreatment of material by blanching and microwave treatment can influence product quality [12]. Thus, a higher quality of essential oil can be obtained with lower time and fuel consumption by performing microwave pretreatment. The results obtained was also in agreement with previous work that stated that the yield of essential oil of gaharu increased after 5-day distillation compared to 1-day distillation [13].

In addition, it can also be observed that the production of oil increased as the exposure time to microwave during pretreatment increased. This trend is found to be similar to the results obtained by a previous researcher which had proven that oil extraction yield can be increased by prolonging microwave treatment time [14]. This can be related to the mechanism of the heating source, which is the microwave that provides changes on the microstructure of the sample. The higher number of oil yield resulted from the interaction of the sample molecule with an electromagnetic field produced from microwave treatment that can cause energy delivery to the plant sample [8].

Chemical Composition of Gaharu Essential Oil

The list of the chemical composition of the oil obtained from the extraction of gaharu samples, with and without microwave pretreatment, are presented in Table 2. According to the results, the sample with a duration of 3 min microwave pretreatment showed a higher number of volatile components (64 compounds) compared to the non-pretreated samples (61 compounds). Similar trends have been observed by previous researchers [15-16] which highlighted that volatile components of gaharu essential oil depended on its species, pretreatment and extraction method. The research done by using microwave pretreatment on seed had supported the finding of a greater number of bioactive compounds that may have penetrated out from the grounded gaharu to the produced oil [15,17-18]. Conditioning of gaharu at 3 min microwave pretreatment and 30 h of hydrodistillation processing time increased the amount of gurjunene, α-Farnesene, estragole, guaiene, verrucarol, valencene, α-cargophyllene, spathulenol and α-cubebene in the essential oil yield.

As can be seen in Table 2, 21 active compounds were detected in the essential oil obtained from the non-pretreated sample, from a total of 61 chemical compounds with purity higher than 7%. The major compounds for non-pretreated gaharu are α-panasinsene, gurjunene (1,4-dimethyl-1,2,3,3α,4,5,6,7-octahydro-7-azulene) and α-farnesene. The compound α-panasinsene is known as a sesquiterpenoid that can be found in tea and ginseng oil. Meanwhile, gurjunene, spathulenol, verrucarol, spathulenol and guaiene were found to be the major components of oil extracted from the sample with 3 min microwave pretreatment. The results showed that the pretreated and non-pretreated samples produced oil that had a similar chemical composition where gurjenene is one of their major components. Gurjenene falls under the sesquiterpene hydrocarbon group that is known to contribute to the wood fragrant aroma in gaharu essential oil. Therefore, it can be confirmed that microwave pretreatment did not destroy the original aroma of gaharu essential oil. In addition, the amount of gurjenene in the sample with microwave pretreatment was found to behigher than the non-pretreated sample, thus the essential oil produced from the pretreated sample had a sweeter wood odor compared to the non-pretreated one.

Exposing the gaharu to microwave pretreatment increased the purity of most compounds including, (+)-Epi-bicyclosesquiphellandrene, gurjunene, α-farnese, estragole, guaiene, valencene, spathulenol, α-cubebene and (-)-Spathulenol. A similar study using *Nigella sativa* L. seeds showed that microwave pretreatment was an effective factor in oil quality [19-21]. However, for certain chemical compounds such as α-panasinsene, exposure to a high power microwave energy of 800 W had reduced its percentage of purity. This is due to the degradation or loss of material that occur when the temperature or pressure inside the microwave is high.

Table 2. Chemical composition for without pretreatment and microwave pretreatment (3 min) at 30 h of hydrodistillation processing time

Chemical compound	Untreated gaharu essential oil at 30 h hydrodistillation extraction			Microwave pre-treated gaharu essential oil at 30 h hydrodistillation extraction		
	Retention time (min)	Amount (%)	Purity (%)	Retention time (min)	Amount (%)	Purity (%)
α-Panasinsene	17.725	0.259	45	18.124	0.021	19
Gurjunene	19.523	0.186	37	19.495	0.541	54
α-Farnesene	22.828	0.162	33	22.611	0.261	42
Estragole	8.1349	0.154	49	8.1206	0.298	69
Guaiene	19.518	0.018	10	12.911	0.306	63
				25.852	0.158	36
α-Guaiene	12.957	0.110	38	-	-	-
Verrucarol	31.104	0.092	25	30.634	0.371	33
Aromadendrene	18.475	0.056	23	-	-	-
Valencene	21.325	0.047	20	21.219	0.133	35
Caryophllene	-	-	-	25.852	0.055	17
α- Caryophllene	14.619	0.046	21	21.8	0.066	12
Spathulenol	30.916	0.043	12	22.603	0.318	43
(-)-Spathulenol	22.173	0.026	10	22.603	0.383	37
β-Selinene	19.660	0.032	11	-	-	-
α-cubebene	17.249	0.028	8	18.986	0.044	25
Cis-Limonenen oxide	15.461	0.014	21	19.927	0.095	22
α-Vatirenene	22.750	0.026	12	-	-	-
α-Phellandrene	24.978	0.022	10	-	-	-
(+)-Epi-bicyclosesquiphellandrene	18.241	0.017	7.9	18.361	0.092	27
Germacrene	19.024	0.02	25	-	-	-
Citronellyl propionate	-	-	-	19.347	0.033	11
β-Cadinene	-	-	-	18.366	0.031	5.3
Citronellal	-	-	-	16.026	0.03	13
Squalene	19.711	0.014	21	-	-	-
Humulane-1,6-dien-3-ol	18.552	0.012	7.9	-	-	-
c-Elemene	-	-	-	19.317	0.026	10
Aristolene	-	-	-	22.640	0.075	16

Thus, to avoid losing valuable chemical compounds, the power of the microwave energy should be reduced and studied.

■ CONCLUSION

This study highlights the effect of microwave pretreatment on gaharu essential oil using the hydrodistillation method as well as the effect of microwave processing and hydrodistillation time on gaharu oil yield. From the results, several main findings can be deduced. The oil yield values extracted by microwave pretreated samples were higher than those obtained by the non-pretreated samples. The longest microwave pretreatment time increased the exposure time to the wall structure of gaharu causing it to break down. Microwave penetrates directly into biomass substrate and generate heat from within, therefore enhancing the extraction efficiency of the components contained in the plant bodies. In conclusion, microwave pretreatment produces high quality essential oil with a higher amount of chemical compositions. This technique can be used as a desirable pretreatment method prior to extraction. This study gives an alternative to the gaharu essential oil manufacturers to utilize thelow-grade gaharu to obtain a more valuable product.

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