Effect of Microwave Pretreatment on Gaharu Essential Oil Using Hydrodistillation Method

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Abstract— **Gaharu (*A.malaccensis*) is a valuable resinous heartwood trees that belong to Thymelaeceae family. The objectives of this research were to study the effect of microwave pretreatment on gaharu essential oil using hydrodistillation method, the effect of microwave processing time on gaharu's oil yield and to study the effect of hydrodistillation time on gaharu's oil yield. Gaharu sample were pre-treated with microwave irradiation at the power of 800 kW at three different times, which are 1, 2 and 3 minutes. After the pretreatment, gaharu sample were extracted using hydrodistillation method for 30 and 47 hours. The oil produced were weight to determine the percentage of oil yield. Effects of microwave pretreatment were determined using gas chromatography-mass spectrum analysis to study the chemical composition of oil. Hydrodisillation process with microwave pretreatment produce higher yield (0.0379%) of gaharu essential oil compare to non-pretreatment extraction (0.0286%). In addition, higher microwave time (3-min) during the pretreatment also will produce greater amount of oil (0.0877%). As it will break the structure of gaharu for better extraction. Hydrodistillation processing time also gives effect on the amount of produced oil as longer the extraction time, higher the oil yield. Major component of oil contains sesquiterpene such as gurjunene, α-parasinsene, spathulenol and guaiene that were known to offer the sweet wood fragrant in the oil.**

Keywords— Aquilaria malaccensis, microwave, hydrodistillation, essential oil, Gas chromatography mass spectrometry (GCMS), high performance liquid density (HPLC).

I. Introduction

Gaharu is a resinous heartwood trees that belong to Thymelaeceae family. A few of the other names for Gaharu are agar, agarwood, eaglewood, aloeswood etc. The scientific name of Gaharu is *Aquilaria malaccencis*. It is a large classic tree growing over 15-30 m tall and 1.5- 2.5 m in diameter, and has white flowers, (Barden et.al., 2000).

The development of gaharu is a characteristic procedure because of a parasitic ascomycetous shape, Phialophora parasitica, a dematiaceous (dark-walled) organism assaults (Junaidi, 2008) and the sweet-smelling pitch is normally formed in the bark and the roots and also the heartwood of the trees. Gaharu is a timeless tree that can be used in many difference ways such as fragrance and perfumery product, medical purposes, activity for religious ritual and many more, (Mat Yusoff et al, 2015). Since a long time ago, gaharu has been used as it plays important role in a lots of practices for Buddhist, Hindu, Muslim, Christian and Jewish including cultural, religious, medicine. In Old Testament, gaharu is used as a perfume, while in Sahih Muslim and Susruta Samhita have mentioned about gaharu as medicinal medium, (Barden et.al, 2002). In Buddhist religious ceremonies, gaharu incense is commonly used for their ritual as it plays significant part among people and their God whereby gaharu is burned around the Buddha statue, (Ismail et.al, 2015).

At the moment, since the value of gaharu is keep increasing, researchers and entrepreneurs are inspired to find the best method and technique of extraction in order to get the optimum yield number of gaharu essential oil while saving the cost and energy.

Hydrodistillation method is one of the simplest, oldest and primitive process known to man for obtaining essential oil from plants. For the extraction of gaharu oil, gaharu in powder or chips formed are immersed in water until boil. Oil from the gaharu will evaporate then lost in the water as a distillate. It is mostly used by small scale producer of essential oils but the process is slow and the distillation time is much longer thereby consuming more fuel/ gas making it not economical wise, (Tandon, 2008). Hydrodistillation method with aided of microwave pretreatment may contribute to the efficiency improvement of extracted gaharu essential oil. The time consume in the hydrodistillation process can be shorten to save energy and cost.

According to Uquiche et al. (2008), from microwave radiation, a higher yield of oil extraction can be achieved as a cell membrane of plant is ruptured, resulting to permanent pores which allow the oil to transfer through the cell walls. As stated by Wroniak et al., (2016), through microwave pretreatment, bioactive compounds can penetrate in producing oil in a greater amount. Resulting to a better extraction and will produce higher quality of oil and produce higher oil yield. The objectives of this research are to study the effect of microwave pretreatment on gaharu essential oil's yield extracted using hydrodistillation method; to study the effect of processing time differences of microwave pretreatment on essential oil and to study the effect of hydrodistillation processing time on gaharu essential oil yield.

II. Methodology

A. *Material*

Gaharu stembark (*A. malaccencis*) was collected and purchased from a plantation in Kuala Krai, Kelantan, Malaysia. The woods were then dried at room temperature under direct sunlight for about 12 hours to remove the moisture. Then the sample were ground using crasher (SCP Automation (M) SDN BHD), (model: SLM-20PI/D) into size of 0.5 ~ 0.8 cm and stored into zip plastic bag.

B. *Microwave pretreatment*

Two kilograms (2 kg) of the ground agarwood were prepared for each sample. The gaharu sample were placed on 16 cm microwave plate (40.0 g on each plate). The microwave (Model R-397J(S), SHARP Malaysia) run with the constant operation power of 800 kW and the processing time for this microwave pretreatment are 1, 2 and 3 minutes, (Nyam et al., 2015). Gaharu samples were then cooled under ambient temperature before soaking for 72 hours.

C. *Hydrodistillation*

Gaharu of 2 kg with pretreatment and without pretreatment were then soaked in a container with 9 L of water for 72 hours at room temperature to ensure all the gaharu chips are fully absorbed into the water. Afterwards, the hydrodistillation process was carried out at 100 °C. The processing time for this process are varied at 30 and 47 hours. The obtained gaharu essential oil, residue solution and hydrosol were taken and stored at ambient temperature until the process of chemical compounds analyzation.

D. *Gas Chromatography Mass Spectrometry (GCMS)*

The analysis of gaharu essential oil chemical compounds was carried out using GC-MS. This analysis is required to identify the active compounds in the gaharu essential oil. Chemical constituents of extracted gaharu essential oil were analysed by GCMS with mass spectrometer. Table 2 shows the summarize conditions of GCMS analysis (Hashim et al., 2014).

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 to 250 °C for 10 min
Carrier Gas	Helium
Interface Temperature	250 °C

III. RESULTS AND DISCUSSION

A. *The Effects of Microwave Pretreatment*

Figure 1 compares the yield percentages of gaharu extracted oil at 30 hours and 47 hours of hydrodistillation. Firstly, the result obtained from Table 2 presents higher oil yield for microwave pretreated gaharu (0.0379%) compare to untreated sample (0.0286%). This experiment was run for the second time using higher processing time of hydrodistillation extraction method which is 47 hours to compare the result with 30 hours extraction whether the result shows the same pattern or not. From this further analysis, it is confirmed that the percentage of extracted oil yield with microwave pretreatment is higher than without pretreatment similar with the first run's result.

It can be discussed that extracted oil yield with microwave pretreatment is higher than the yield of oil produced for without pretreatment. This result is similar with the previous study by Wroniak et al. (2016) which stated that through microwave pretreatment, bioactive compounds can penetrate in producing greater amount of oil. Another finding from Uquiche et al. (2008), revealed that the oil extraction yield from another plant which is hazelnut seeds is increased after the application of microwave pretreatment before pressing extraction. They also stated that increasing in extracted oil yield was due to the modification of cellular walls resulting in greater porosity.

Table 2: Yield percentage of gaharu extracted oil on pretreatment effect via 30 hours hydrodistillation

Sample	Yield (%)
Without microwave pretreatment	0.0286
With 1 min microwave pretreatment	0.0379

Table 3: Yield percentage of gaharu extracted oil on pretreatment effect via 47 hours hydrodistillation

Sample	Yield (%)
Without microwave pretreatment	0.0523
With 1 min microwave pretreatment	0.0737

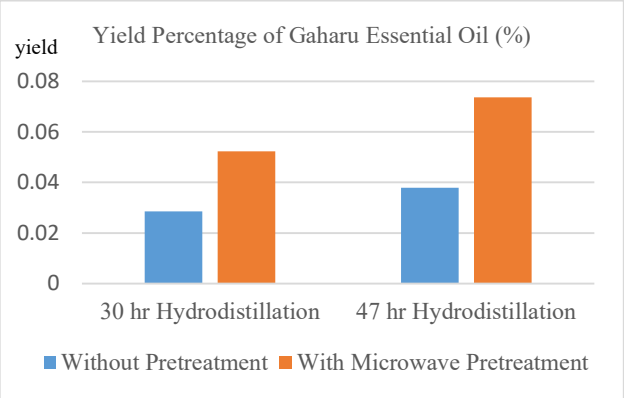

Figure 1: Effect of pretreatment on extracted oil yield.

B. *The Effects of Microwave Processing Time*

The effect of microwave processing time on the amount of extracted oil is presented in Figure 2, which showed that the longest microwave time (3 minutes) gave the most extracted oil. Based on findings (Table 4), microwave pretreatment at 3 minutes has the highest percentage of extracted oil yield (0.0877%). While the first sample with 1 min microwave pretreatment has the lowest oil yield percentage (0.0379%) compared to the sample with 2 min of microwave pretreatment (0.0779%) and sample with 3 minutes of pretreatment.

Table 4: Yield percentage of gaharu extracted oil on pretreatment processing time effect via 30 hours hydrodistillation

Sample	Yield (%)
1 min Microwave pretreatment	0.0379
2 min Microwave pretreatment	0.0779
3 min Microwave pretreatment	0.0877

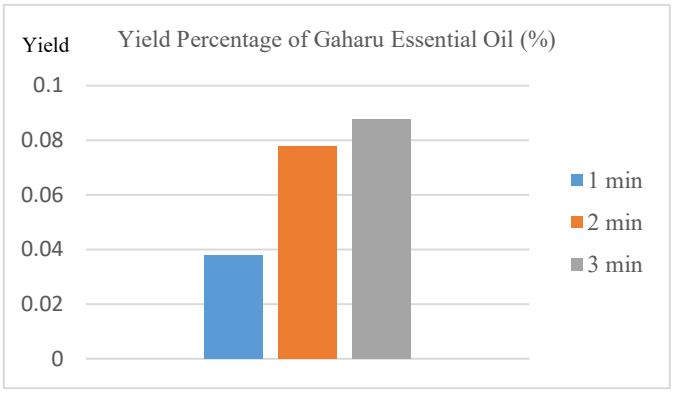

Figure 2: Effect of microwave processing time difference on extracted oil yield.

From this result, it can be observed that, the longer the microwave pretreatment time, the greater amount of oil will be produced. By comparing with the previous study done by Damirchi et al. (2011), a study was proved that oil extraction yield can be increased based on longer microwave treatment time which has similar findings with this study. The heating sourced by the microwave provides the changes on the microstructure of the sample and the factor of oil that being produced. According to Uquiche et al., (2008), the higher number of oil yield was resulted

from the interaction of molecule with electromagnetic field that can cause to energy delivery to the plant.

C. *The Effects of Hydrodistillation Processing Time*

Table.5 illustrates the yield percentage of gaharu extracted oil in two different conditions. Firstly, the extraction was done with the untreated sample. For 30 hours of hydrodistillation, the percentage of gaharu extracted oil shows significant changes with 47 hr processing time. The sample with 47 hour of extraction produce higher oil yield (0.0523%) compared to 30 hours of processing time's sample (0.0286%). Similar findings by Yoswathana (2013) stated that the yield of essential oil of gaharu obtained increased after 5-day distillation compared to 1-day distillation.

Table 5: Yield percentage of gaharu extracted oil without microwave pretreatment.

Sample	Yield (%)	
	Without Pretreatment	With 1-min Microwave Pretreatment
30 hr hydrodistillation	0.0286	0.0379
47 hr hydrodistillation	0.0523	0.0737

The second sample was run after 1 min of microwave pretreatment. The result trend was alike with the untreated sample where 47 hour of processing time has greater amount of oil (0.0737%) than 30 hours hydrodistillation extraction (0.0379%). This can be related that higher processing time, higher amount of essential oil that will be produced.

D. *Chemical Composition of Gaharu Essential Oil*

The lists of chemical composition obtained from the extraction of gaharu samples, one is without pretreatment and another one with microwave pretreatment are shown in Table 6 and 7. According to this analysis, sample with 3-min microwave pretreatment obtained the higher numbers of volatile components which is 64 compounds compared to non-pretreatment sample (61 compounds). As accorded with earlier result obtained of oil yield, which showed that the extracted oil of pretreated gaharu with microwave was much greater than without microwave pretreatment. This shows that there was a changes happened in the gaharu chemical composition when microwave radiation is applied to the sample before the extraction. Previous study stated that volatile components of gaharu essential oil are depends on its species, pretreatment and extraction method (Fadzil et al., 2013).

Essential oil from non-pretreatment sample detected 21 active compound from 61 chemical compund that has purity higher than 7%. The major compounds for non-pretreatment gaharu are α-panasinsene, gurjunene(1,4-dimethyl-1,2,3,3α,4,5,6,7-octahydro-7-azulene) and α-farnesene. The compound of α-panasinsene is known as sesquiterpenoids that can be found in tea and ginseng oil (Yannai & Shmuel, 2004). While, gurjunene, spathulenol, verrucarol, spathulenol and guaiene are the major components of oil extracted with 3-min microwave pretreatment.

The result shows that both sample still has similarity where gurjenene is one of their major components. Gurjenene falls under sesquiterpene hydrocarbon group that was known to the contribution of wood aroma fragrant in gaharu essential oil. It can be confirmed that, microwave pretreatment did not destroy the original aroma of gaharu essential oil. In addition, gurjenene is the most major component in the sample with microwave pretreatment, thus make the essential oil has sweeter wood odor compared to the non-pretreted gaharu oil.

Table 6: Compound of Untreated Gaharu Essential Oil at 30 hr hydrodistillation extraction.

Retention Time (min)	Chemical Compound	Amount (%)	Purity (%)
17.725	a-Panasinsene	0.259	45
19.623	gurjunene	0.186	37
22.828	a-Farnesene	0.162	33
8.1349	Estragole	0.154	49
12.957	a-Guaiene	0.11	38
31.104	Verrucarol	0.092	25
18.475	Aromadendrene	0.056	23
21.325	Valencene	0.047	20
14.619	a-Caryophyllene	0.046	21
30.916	Spathulenol	0.043	12
19.660	beta-Selinene	0.032	11
17.249	a-cubebene	0.028	8
22.173	(-)-Spathulenol	0.026	10
22.75	a-Vatirenene	0.026	12
24.978	a-phellandrene	0.022	10
19.024	Germacrene	0.020	25
19.518	guaiene	0.018	10
18.241	(+)-Epi-bicyclosesquiphellandrene	0.017	7.9
19.711	Squalene	0.014	21
15.461	Limonene oxide, cis-	0.014	6
18.552	Humulane-1,6-dien-3-ol	0.012	7.9

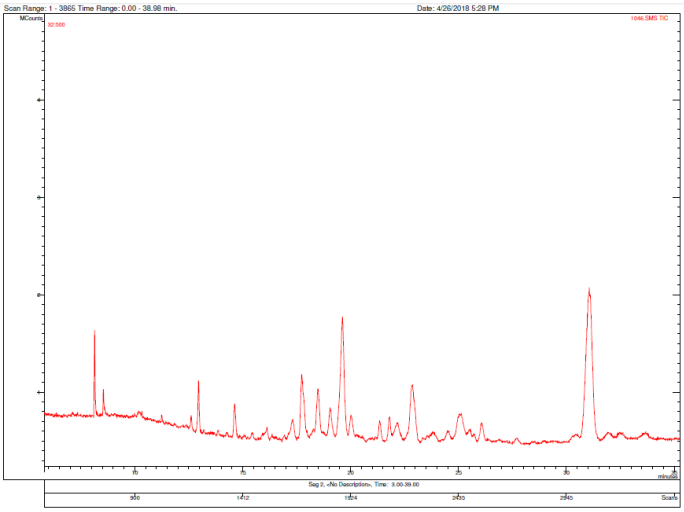

Figure 3: Gas chromatogram of gaharu essential oil compund without microwave pretreatment.

Table 7: Compound of Microwave Pre-treated Gaharu Essential Oil at 30 hr hydrodistillation extraction.

Retention Time (min)	Chemical Compound	Amount (%)	Purity (%)
19.495	gurjunene	0.541	54
22.603	(-)-Spathulenol	0.383	37
30.634	Verrucarol	0.371	33
22.603	Spathulenol	0.318	43
12.911	Guaiene	0.306	63
8.1206	Estragole	0.298	69
22.611	a-Farnesene	0.261	42
25.852	Guaiene	0.158	36
21.219	valencene	0.133	35
19.927	Limonene oxide, cis-	0.095	22
18.361	(+)-Epi-bicyclosesquiphellandrene	0.092	27
22.64	a-Vatirenene	0.075	16
21.800	a-Caryophyllene	0.066	12
25.852	Caryophyllene	0.055	17
18.986	a-Cubebene	0.044	25
19.347	Citronellyl propionate	0.033	11
18.366	(beta-Cadinene)	0.031	5.3
16.026	Citronellal	0.030	13
19.317	c-Elemene	0.026	10
18.124	a-Panasinsene	0.021	19
21.647	Aristolene	0.019	9.4

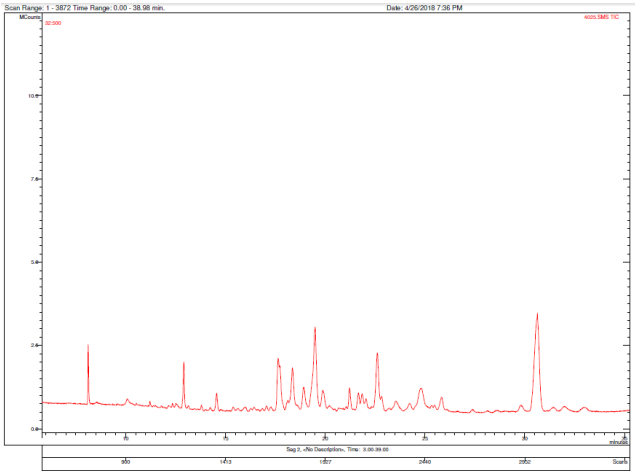

Figure 4.4 Gas chromatogram of gaharu essential oil compund with 3-min microwave pretreatment.

IV. Conclusion

Hydrodisillation process with microwave pretreatment produce higher yield of gaharu essential oil compare to non-pretreatment extraction. In addition, higher microwave time during the pretreatment also will produce greater amount of oil. As it will break the structure of gaharu for better extraction. Hydrodistillation processing time also gives effect on the amount of produced oil as longer the extraction time, higher the oil yield. Major component of oil contains sesquiterpene that was known to offer the sweet wood fragrant in the oil.

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References

A. Barden, N.A. Anak, T. Mulliken, and M.Song, (2000). Heart of the Matter: Agarwood Use and Trade, and CITES Implementation for Malaccensis, TRAFFIC x International.

Afiffudden, S. K. N., Alwi, H., & Hamid, K. H. K. (2015). Determination of 4'-hydroxyacetanilide in leaves extract of Aquilaria malaccencis by high pressure liquid chromatograph. Procedia - Social and Behavioral Sciences, 195(July), 2726–2733.

Azadmard-Damirchi, S., Alirezalu, K., & Achachlouei Fathi, B. (2011). Microwave Pretreatment of Seeds to Extract High Quality Vegetable Oil. World Academy of Science, Engineering and Technology, 5(9), 72–75.

Fadzil, A. H. M., Hamid, K. H. K., Rodhi, M. N. M., & Kamaruddin, L. M. (2013). Extraction of essential oil from biologically inoculated agarwood. BEIAC 2013 - 2013 IEEE Business Engineering and Industrial Applications Colloquium, 889–892.

Hashim, Y. Z. Y., Ismail, N. I., & Abbas, P. (2014). Analysis of chemical compounds of agarwood oil from different species by gas chromatography mass spectrometry (GC-MS). IIUM Engineering Journal, 15(1), 55–60.

Ismail, N., Hezri, M., Rahiman, F., & Malaccensis, A. A. (2015). A Review on Agarwood and Its Quality Determination, 103–108.

Junaidi, A., (2008). Extraction of Gaharu Essential Oil Using Ultrasonic Assisted Steam Distillation. Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang.

Mat Yusoff, N. A., Nizam, T. S., Hisyam, A., & Mohd Omar, N. A. (2015). SHORT COMMUNICATION Production of Agarwood Essential Oil Study on Effectiveness Pre-Treatment Technique of Hydrodistillation Extraction, 5, 62–69.

Nyam, K. L., Tan, C. H., & Long, K. (2015). Effect of microwave pretreatment on stability of kenaf (Hibiscus cannabinus L.) seed oil upon accelerated storage. International Food Research Journal, 22(5), 1898–1905.

Tandon, S. (2008). Hydro-distillation and Steam Distillation from Aromatic Plants. Chemical Engineering Division, CIMAP, Lucknow.

Uquiche, E., Jeréz, M., & Ortíz, J. (2008). Effect of pretreatment with microwaves on mechanical extraction yield and quality of vegetable oil from Chilean hazelnuts (Gevuina avellana Mol). Innovative Food Science and Emerging Technologies, 9(4), 495–500.

Wroniak, M., Rekas, A., Siger, A., & Janowicz, M. (2016). Microwave pretreatment effects on the changes in seeds microstructure, chemical composition and oxidative stability of rapeseed oil. LWT - Food Science and Technology, 68, 634–641.

Yoswathana, N. (2013). Extraction of agarwood (Aquilaria crassna) oil by using supercritical carbon dioxide extraction and enzyme pretreatment on hydrodistillation. Journal of Food, Agriculture and Environment, 11(2), 1055–1059.