

Effect of Ultrasound as Pretreatment on the Bioactive Compounds in the Hydrosol and Condensate from the Extraction of Agarwood Bark

Nawal Syakirah binti Abu Bakar, Nurhaslina bte Che Radzi

Faculty of Chemical Engineering, Universiti Teknologi Mara Shah Alam

Abstract— The purpose of this study was to identify the effect of ultrasound as pretreatment on the bioactive compounds of Agarwood bark's hydrosol and condensate. Most of the researches conducted on this plant only focused on the yield of essential oil of Agarwood, where the condensate and the hydrosol or plant water from the experiments were considered as wastes. However, the yield of essential oil obtained is little when compared to the total amount of Agarwood bark used for the extraction. To overcome this problem, a research is made on the hydrosol of Agarwood which was often disposed and discarded. This is to prove that the so-called waste is also as beneficial as the essential oils. The compounds in the hydrosol were identified to determine the benefits or uses for each compound. To enhance the transfer of compound from the wood to the solvent, a pretreatment was conducted on the wood. Ultrasound water bath equipment was used as pretreatment process while for extraction, hydro-distillation was selected. Pretreatment of grinded Agarwood with ultrasonic was done by adjusting the frequency between 12 kHz (Degas), 23 kHz (Pulse) and 46 kHz (Sweep), where ultrasonic for each frequency was done for 45 minutes, 60 minutes and 75 minutes. Hydro-distillation was performed after the pretreatment process. The effect of frequency from sonication towards the time taken to collect 200 ml of hydrosol was observed and the compounds from condensate and hydrosol were identified using Gas Chromatography Mass Spectrometry (GC-MS). The arsenic level for each hydrosol was also identified for its heavy metal using Induced Coupled Plasma- Mass Spectrometry (ICP-MS). The results indicated that the main compounds in the hydrosol and condensate are fatty acid and aromatic compounds. The mole percentage of each compound was also observed and evaluated. From the results and evaluation that has been done, it can be concluded that hydrosol and condensate contain beneficial bioactive compounds and can be used as by-product.

Keywords—Agarwood, hydrosol, ultrasonic, hydro-distillation, GC-MS

I. INTRODUCTION

Agarwood or its scientific name *Aquilaria malaccensis* has been used since ancient times for its remedial properties as well as for its aromatic properties. Its fragrant resin is obtained from the wounded trees caused by injury, cutting, chemical, pest or insect disturbance, fire or colonization [1]. Another use of this plant is its essential oil

which can be obtained by several methods such as hydro-distillation and solvent extraction. Essential oils are known for thousands years for its aromatherapy and medicinal properties. Even from the ancient Chinese, Egyptians and Greeks era, essential oils are in high demand for restoration of health [2]. Even in 10th century, the Arabians seek essential oil and used it to make medicine. Apart from being used as medicine, essential oils are widely used to make perfume.

Hydrosol, hydrolates, plant water or aromatic water is known as by-product obtained from the extraction of plant using hydro-distillation. The name derived from “hydro”, a Latin word which means water and “Sol” which means solution. Comes with aromatic properties, hydrosol contains some of constituents of essential oils and has pH around 4.5 to 5.5 [3]. They are presented many benefits such as anti-oxidants, anti-bacterial, and anti-fungal [4]. In Turkey, hydrosol has been used as drinking water since ancient time [5]. As the heater heats up the flask containing plant and water, the cell walls and glands of the plant breaks down to produce desired product such as oil and transfer constituent into the solvent. The oil and water then distilled and collected as essential oil and hydrosol. The compounds from the plant transferred into the essential oil as well as the hydrosol strongly depend on the method of extraction and presence of pretreatment process.

Nowadays, pretreatments are being applied on the plant to allow the oil glands of the Agarwood to open, hence can improve the oil yield during extraction. The medium of soaking have to be different in order to disrupt the cell's surrounding nature [6]. The purpose of soaking is to allow the moisture content of the dry wood increase to suitable value for extraction process. Compared to non-soaking, the size of the cell wall tends to be bigger when compared to non-soaking hence resulting to higher oil yield extraction. Soaking also increases the wood cell degradation which resulting to larger size of the pore [7]. The compounds found in the soaked wood are also differ compared to non-soaked wood. Examples of the compounds found in the Agarwood essential oil as well as in the hydrosol are sesquiterpenes, fatty acid and aromatic compounds.

Another pretreatment method that can be used is water bath ultrasound. This method required a water bath or ultrasonic probe, which both are used to supply vibration to the sample that results to reduction of material size. Energy from sonication interrupts the barrier of lignin, get rid of hemicelluloses and allow the solvent to access to the cellulose [8]. In conducting ultrasound pretreatment, parameters that can be manipulated are the frequency applied, the duration of the ultrasound and the temperature of the solvent which depends to the equipment limitation. This type of process only consumes time and energy that commonly needed in other conventional operations [9]. Therefore, it is not difficult to carry on.

The objective of this study is to investigate the effect of frequency and duration of ultrasound on the constituents and

compounds present in the hydrosol and condensate of Agarwood bark.

METHODOLOGY

A. Raw Materials

Agarwood was obtained and purchased from Kuala Krai, Kelantan before grinded into small size of 0.5 cm using grinder machine in Renewable Energy Laboratory, UiTM Shah Alam. Smaller size of Agarwood is required to ease the extraction process.

B. Soaking with water

Agarwood grind was fully immersed in distilled water for 5 days in 2 L closed containers under room temperature. Then the water was filtered and removed using sieve. After that, the soaked wood was let dry for 24 to 48 hours in room temperature on dry paper to remove the excess moisture. The dried wood then was collected and stored in plastic bag for further process.

C. Pretreatment with ultrasound

For ultrasound process, NEXXSONIC NS-A-18H water bath was used 100g of Agarwood and 1500ml of distilled water were inserted into the ultrasound water bath. The frequency of the water bath was adjusted to 12 kHz (degas), 23 kHz (wave) and 46 kHz (sweep). These three frequencies are used due to equipment limitation. For each frequency, the sonication duration was set to 45 minutes, 60 minutes and 75 minutes to investigate the effect of duration towards cell wall breakage of the wood. The temperature of the solvent was monitored and maintained around 60°C to 65°C. At the end of the sonication process, sample water from the water bath was removed the Agarwood was sieved and filtered before stored into closed container to be kept until further use and proceeding to hydro-distillation process. Table 1 shows the sample of hydrosol and condensate according to the frequency and duration of sonication pretreatment.

Table 1: Name of sample according to frequency and sonication duration

Name of sample	Frequency	Duration of Sonication
Sample A	12 kHz	45 min
Sample B	12 kHz	60 min
Sample C	12 kHz	75 min
Sample D	23 kHz	45 min
Sample E	23 kHz	60 min
Sample F	23 kHz	75 min
Sample G	46 kHz	45 min
Sample H	46 kHz	60 min
Sample I	46 kHz	75 min

D. Extraction Process by Hydro-distillation

For hydro-distillation process, Agarwood from the water bath was sieved before inserted into the 1000 ml round bottom flask used for hydro-distillation process. A Clavenger-type hydro-distillation set model HM 1000 was used in this experiment. 750 ml of distilled water was added into the flask and the wood was let soaked for one hour to allow the wood to be properly soaked and to open the oil gland. The power button was turned on and the power was adjusted between 4 until 6 with careful observance to prevent spillage of the solvent. The extraction process was continued until 150 ml to 200 ml distillate was obtained. From the distillate, there was no

essential oil produced, therefore only hydrosol was obtained. Along with the hydrosol, the condensate was also collected for further analysis. The collected samples were kept in closed container under room temperature until further used.

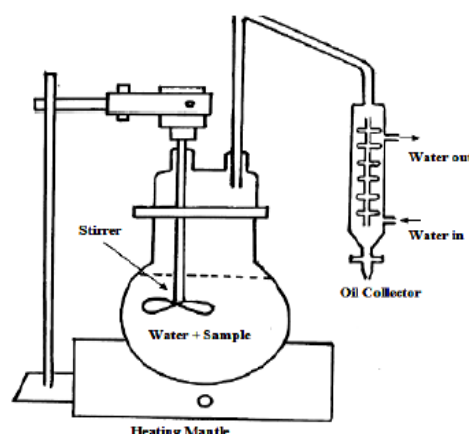


Figure 1: Hydro-distillation diagram

E. Analysis using GC-MS

Before sent to GC-MS analysis, 10 mL of the sample was mixed with 5 ml of methanol. The purpose of the mixing with methanol solvent was to separate the compound from the sample as the solvent acted as an absorber of the compound. Methanol is chosen instead of hexane because hydrosol sample contain mainly water. The mixture of solvent and the sample was done to allow absorption. After mixing, the mixture is injected into small tubes for further action.

For GC-MS analysis, Varian 450 gas chromatograph that connected to Varian mass spectrometer system was used and using a DB-1MS capillary column of 30 m length and 0.25 mm diameter with 0.25 μ m film thickness. The temperature was set as 230°C for the injector and also detector. The oven temperature was set to 50°C for 5 minutes with a ramp of 3°C per minutes to 220°C that is then held for 1 minute. The carrier gas chosen was helium with a flow rate of 1.2 ml/min and the volume of sample injected in the process is 1.0 μ L. The analysis was performed 3 times and the average values were recorded and reported [7].

F. Analysis using ICP

10 ml of Agarwood hydrosol for each parameter was sent to Inductively Couple plasma atomic emission spectroscopy (ICP-OES) model Thermo Scientific to identify the arsenic level or range of heavy metal content in the sample such as arsenic, iron and copper. The metal contained data is collected and evaluated.

II. RESULTS AND DISCUSSION

A. The effects of soaking

The Agarwood was grinded to 0.5 cm and soaked in distilled water for 5 days. The result shows that soaking could break the parenchyma cells which would boost the diffusion of oil from broken oil glands. 5 days of soaking duration was selected according to study made by [10]. Previous study proved that soaking helps to break down the parenchyma cells which led to diffusion of compound from the fractured cell walls [11].

B. Effect of ultrasound pretreatment on hydro-distillation duration

Sonication acts as pretreatment to enhance breaking of parenchyma cell walls. However, the duration of sonication is very important. Short duration might not be enough to break the cells which then lead to longer hydro-distillation time. Sonication helps to break down the external gland structure of the plant [12], hence ease the transfer of compound into the hydro-distillation solvent. The best frequency for pre-treating Agarwood prior to hydro-distillation

could also be identified from the result obtained. Hydro-distillation works to extract the oil from the Agarwood which has been pretreated by sonication beforehand. For each sample, time taken to collect 200 ml of hydrosol has been taken and the results shows that the usage of sonication helps to shorten time taken for 200 ml of distillate to be collected. Table 2 shows the time taken for each frequencies and sample to extract precisely 200 ml of distillate.

Table 2: Time taken for hydro-distillation

Sample	Duration of Sonication (min)	Time taken for hydro-distillation
Sample A	45 minutes	6.5 hours
Sample B	60 minutes	6.5 hours
Sample C	75 minutes	5.5 hours
Sample D	45 minutes	4 hours
Sample E	60 minutes	4 hours
Sample F	75 minutes	3 hours
Sample G	45 minutes	3.5 hours
Sample H	60 minutes	3 hours
Sample I	75 minutes	3 hours

Applying sonication for pretreatment contributes to shorter time taken to yield hydrosol compared to the one without ultrasound [13]. From the same study also indicates that by applying ultrasound, the time required to obtain hydrosol was only 20 minutes which is shorter than normal hydro-distillation that needed 80 minutes to obtain the same amount of hydrosol. This is due to the reason that ultrasound prior hydro-distillation facilitates the breaking down of the cells and reduce the size of particles increase the surface area in the solvent [14]. According to the results, 23 kHz and 46 kHz are found to contribute to shorter hydro-distillation time compared to 12 kHz. Time required in extraction for 12 kHz required longer time which is averagely 5 to 6 hours. 23 kHz and 46 kHz in other hand shows promising result by required only averagely 3 to 4 hours. This result can be compared to research made by Seidi and co-researchers (2016) [12] that claimed that applying higher frequency of sonication resulting to faster extraction process.

C. Analysis using GC-MS

Each sample beforehand was dried using ROTAVAP until only about 2 to 3 ml of sample left. As the sample is not suitable to be used with hexane for GC-MS analysis, methanol was used instead as solvent due to polarity. However, for GC-MS analysis, only samples for 23 kHz and 46 kHz frequencies were selected since only these two frequencies has thin oil layers in the hydrosol while 12 kHz frequency has not shown any oil layer. 12 kHz frequency in ultrasonic pretreatment is considered as too low hence does not cause any changes to the cell wall. Figure 1, figure 2, figure 3 and figure 4 show that the result of GC-MS analysis where the retention time represents the type of compound whereas the peaks indicate the area of the compound. From the graphs obtained, the type of compounds existed in every sample is extracted according to its retention time and the amount of the compound is depending on the percentage of area. Bigger area percentage indicates higher quantity of the compound. The lack of peaks shown was assumed due to the short time of ultrasound pretreatment. The compounds detected in each sample for both condensate and hydrosol is recorded into table 3, 4, 5 and 6 as shown below.

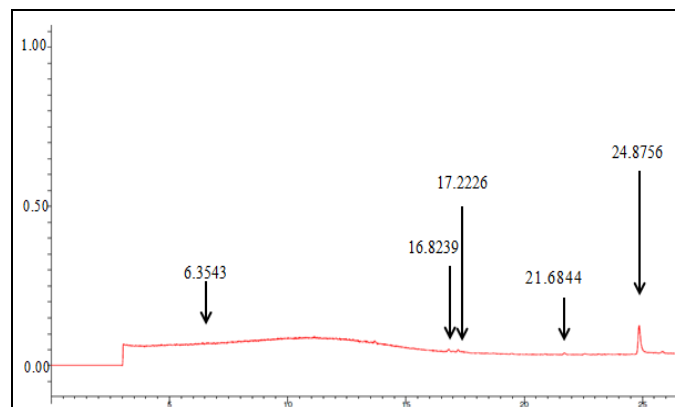


Fig. 1: GC-MS for 23 kHz in 75 minutes in condensate sample of Agarwood

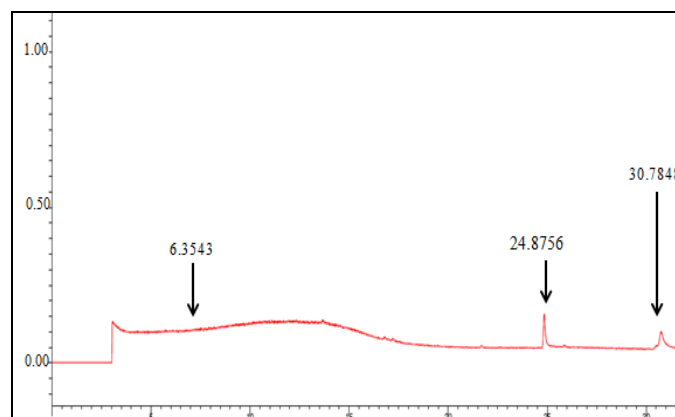


Fig. 2: GC-MS for 46 kHz in 75 minutes in condensate sample of Agarwood

Table 3: The chemical compositions of Agarwood for pulse frequency (23 kHz) in the condensate of Agarwood hydro-distillation

No	Compound	Retention time (min)	% Area	
			60 min	75 min
1.	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	16.8239	1.33	0.57
2.	Hexadecanoic acid, methyl ester	24.8756	56.9	66.8
3.	Phenol, 2-methoxy-	6.3543	39.9	27.79
4.	Iron, acetyl dicarbonyl ((1,2,3,4,5- η)-1,2,3,4,5-pentamethyl-2	17.2226	1.6	1.75
5.	2-(2-Hydroxyphenyl) benzothiazole	21.6844	0.21	0.29

Table 4: The chemical compositions of Agarwood for sweep frequency (46 kHz) in the condensate of Agarwood hydro-distillation

No	Compound	Retention time (min)	% Area	
			60 min	75 min
1.	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	16.8239	17	-
2.	Hexadecanoic acid, methyl ester	24.8756	82.95	50.85
3.	Phenol, 2-methoxy-	6.3543	-	46.87
4.	Mebendazole	30.7848	-	2.27

From the result of the condensate sample, most of the constituents found are aromatic compounds or fatty acid. There were several types of compounds found; Hexadecanoic acid, methyl ester, 2-(2-Hydroxyphenyl) benzothiazole, Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester, Iron, acetyl dicarbonyl ((1,2,3,4,5- η)-1,2,3,4,5-pentamethyl-2, Mebendazole

and Phenol, 2-methoxy-. Hexadecanoic acid, methyl ester or also known as palmitic acid is an organic compound from fatty acid methyl ester group. Palmitic acid is a type of saturated fatty acid that can be found in plants, animals and microorganisms. It is known for its anti-inflammatory, anti-androgenic flavor and antioxidant properties [15] For Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester also known as methyl dihydrojasmonate is from ester group and is an aromatic compound. Phenol, 2-methoxy- or guaiacol is an organic compound also with aromatic characteristics. This compound can be extracted from the occurrence of pyrolysis of lignin.

From the tables, it can be seen that for frequency 46 kHz, lesser number of constituents can be detected by the analysis compared to 23 kHz. Sample I has the highest area percentage of compound Hexadecanoic acid, methyl ester (0.72%) meanwhile only 0.41% for sample H. Meanwhile for frequency 23 kHz, sample E and sample F have higher percentages (1.56% and 0.81%). Higher percentage of this acid proves that the main constituent in the condensate is fatty acid. A study also indicated that pretreating Agarwood with sonication effect to higher percentage of fatty acid [10]. Phenol, 2-methoxy-, in other hand, shows increment in percentage as the frequency increases. For 23 kHz, both sample E and sample F show area percentage of 0.46% and 0.59%, slightly lower than the value for sample I which is 0.64%. Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester shows the lowest area percentage on sample H which is 0.089% where sample E and F are 0.6% and 0.57%. Other compounds such as Iron, acetyl dicarbonyl ((1,2,3,4,5- $\bar{u}$)-1,2,3,4,5-pentamethyl-2 and 2-(2-Hydroxyphenyl) benzothiazole or HBT only shown in graphs of sample E and sample F (1.08% and 0.57% for the former and 0.53% and 0.4% for the latter). Higher frequency (46 kHz) shows no result for these two compounds.

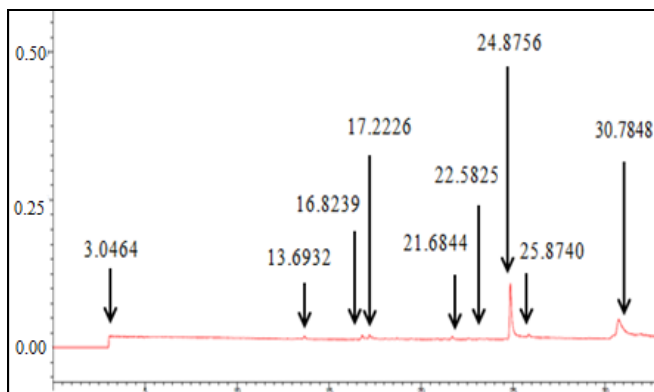


Fig. 3: GC-MS for 23 kHz in 75 minutes in hydrosol sample of Agarwood

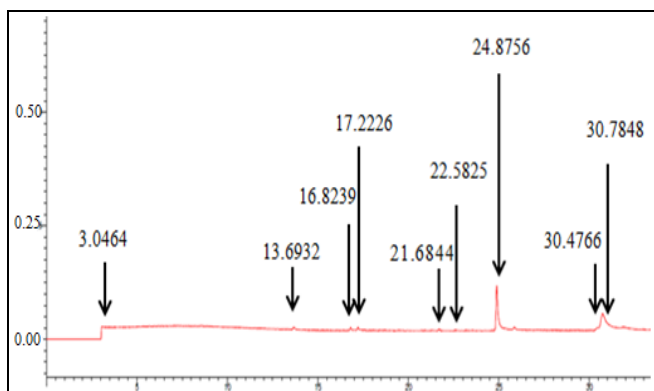


Fig. 4: GC-MS for 46 kHz in 75 minutes in hydrosol sample of Agarwood

Table 5: The chemical compositions of Agarwood for sweep frequency (46 kHz) in the hydrosol of Agarwood hydro-distillation

No	Compound	Retention time (min)	% Area	
			60 min	75 min
1	Phenol, 2-methoxy-	3.0464	0.09	0.14
2	Lilial	13.6932	0.03	0.03

3	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	16.8239	0.08	0.08
4	Iron, acetyl dicarbonyl ((1,2,3,4,5- $\bar{u}$)-1,2,3,4,5-pentamethyl-2	17.2226	0.03	0.02
5	2-(2-Hydroxyphenyl) benzothiazole	21.6844	0.03	0.02
6	Galaxolide 2	22.5825	0.003	0.002
7	Benzoic acid, 2-hydroxy-, phenylmethyl ester	23.1832	0.014	-
8	Hexadecanoic acid, methyl ester	24.8756	2.57	2.45
9	1,6- Octadien-3-ol, 3,7-dimethyl-	25.8740	-	0.022
10	9,15-octadecadienoic acid, methyl ester	30.4766	0.03	-
11	Mebendazole	30.7848	0.56	0.55

Table 6: The chemical compositions of Agarwood for pulse frequency (23 kHz) in the hydrosol of Agarwood hydro-distillation

No	Compound	Retention time (min)	% Area	
			60 min	75 min
1.	Phenol, 2-methoxy-	3.0466	-	0.32
2.	Lilial	13.6932	0.10	0.11
3.	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	16.8239	0.09	0.06
4.	Iron, acetyl dicarbonyl ((1,2,3,4,5- $\bar{u}$)-1,2,3,4,5-pentamethyl-2	17.2226	0.11	0.11
5.	2-(2-Hydroxyphenyl) benzothiazole	21.6844	0.02	0.06
6.	Galaxolide 2	22.5825	0.0016	0.0075
7.	Hexadecanoic acid, methyl ester	24.8756	4.61	4.03
8.	9,15-octadecadienoic acid, methyl ester	30.4766	0.04	0.07
9.	Mebendazole	30.7848	0.36	0.75
10	Cyclopentane, 2-methyl-1-methylene-3-(1-methylethenyl)-	25.8590	0.03	-

From table 5 and table 6 show the constituents collected from the hydrosol can be analyzed. Some of the constituents are similar to the ones in the condensate; Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester, Hexadecanoic acid, methyl ester, Mebendazole, Phenol, 2-methoxy- and Iron, acetyl dicarbonyl ((1,2,3,4,5- $\bar{u}$)-1,2,3,4,5-pentamethyl-2). There are also some other compounds that only found in the hydrosol such as lilial, Galaxolide 2, 9,15-octadecadienoic acid, methyl ester, 1,6-Octadien-3-ol, 3,7-dimethyl- and Benzoic acid, 2-hydroxy-, phenylmethyl ester. Galaxolide 2 is an aromatic compound from Galaxolide species where is for its sweet musky floral woody odor. 1,6- Octadien-3-ol, 3,7-dimethyl-galaxide has been used as fragrance since earlier times and human are most likely to exposed to galaxolide through dermal contact and self-care product which could bring harm especially to young children's skin. Lilial, in other hand also known as butylphenyl methylpropional is a colorless compound with mildly floral odour which is widely used in large number of industries as fragrance especially in cosmetics, toiletries and household cleaners. [16]. Another constituent found is 9,15-octadecadienoic acid come from methyl lonolenate group which is a polyunsaturated fatty acid wit anti-melanogenesis activity and can be used for skin whitening agent.

From the results, the highest percentage of constituents come from fatty acids which is Hexadecanoic acid, methyl ester and 9,15-octadecadienoic acid, methyl ester. Hydrosol from sample E shows the highest content of Hexadecanoic acid, methyl ester (4.61%) which followed by sample F (4.03), sample H (2.57%) and sample I (2.45%). With this, it can be proved that ultrasonic treatment helps to improve the extraction of fatty acid from the plant cell wall [17]. From the pattern, it can be concluded that as the cell wall undergo longer sonication pretreatment and higher frequency, lesser mole percent of fatty acid was transferred into the solvent. The reason is probably due to stronger force from the pretreatment which broken down the cell wall hence ease the excretion of fatty acid compound. However, longer sonication time might cause loss of activities [18].

In other case, for liliac compound, 23 kHz shows increment in percentages where Sample E consists of 0.10% and sample F 0.11%. 46 kHz frequency however shows constant values of 0.03% for both sample H and I. This proves that rather than condensate, hydrosol contains more aromatic compound where results to the woody odor of the sample. For Phenol, 2-methoxy-compound, the data of the area only available when the duration reached 75 minutes for frequency 23 kHz. Compared to condensate data, hydrosol has lower Phenol, 2-methoxy- percentage which only 0.32% for sample F, 0.09% for sample H and 0.14% for sample I. Lower area percentage means lower mole percent which probably due to higher number of constituents exist in the hydrosol compared to condensate.

For compound mebendazole, this constituent has higher percentage in hydrosol than in condensate. All sample shows positive result for mebendazole while for condensate, only one sample has positive result. From the hydrosol, the highest percentage of mebendazole comes from sample F (0.75%) followed by sample H (0.56%), sample I (0.55%) and sample E (0.36). When we relate the percentage to the duration of the sonication, sample E has lower percentage likely because the lower pretreatment duration at lower frequency.

Another aromatic compound that can be analyzed is Galaxolide 2, where all hydrosol samples show positive result. However, at higher frequency of 46 kHz, both samples contain very small mole percent (0.003% and 0.002%). Frequency 23 kHz at 75 minutes (sample F) has slightly higher value (0.0075). Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester compound demonstrates that only small percentages are obtained from all samples which are 0.09% for sample E, 0.06% for sample F, 0.08% for both sample H and I.

D. Analysis using ICP-MS

10 ml of hydrosol or for each sample have been sent to ICP test using Inductively Coupled Plasma mass spectrometry (ICP-MS) equipment to figure out the range of metal content in each sample. It is necessary to determine the arsenic level of the hydrosol because arsenic can be harmful to in exceed the intake limit. Three types of metal are selected; Arsenic (As1890), copper (Cu3247) and Iron (Fe2599). The result is as stated in the table 7. The result is compared with other experiment using oil yield from extraction of 10kg of Agarwood using microwave hydro-distillation method. This Agarwood in the other experiment was soaked in water for 7 days.

The data in table 8 shows that sample J from microwave hydro-distillation method has a very small amount of arsenic content which is 0.000740586 whereas for all sample weighed 100 g have not shown any metal content as can be seen from the negative values as the highest value obtained is -0.435534. This shows that not all hydrosol from Agarwood extraction is arsenic-free. Some of the metal might have resulted from the longer soaking time or because of the mass of Agarwood is higher which 10 kg. Different result may also come due to different extraction method since the hydrosol from microwave hydro-distillation content extraction oil while the hydrosol from normal hydro-distillation method with sonication as pretreatment does not content oil.

Furthermore, the duration taken for microwave hydro-distillation time is 10 hours which is long when compared to normal hydro-distillation which only 45 to 75 minutes which might be the cause of the absence of arsenic content. Longer extraction time allows the Agarwood to be heated and boiled in longer time. However, even from 10 kg of Agarwood, the arsenic content is very small and not exceeding the limit intake for human. This proved that the hydrosol of Agarwood is not contaminated and safe for consuming.

Table 7: Result of arsenic test on Agarwood hydrosol

Sample	Arsenic (As1890) content (ppm)	Copper (Cu3247) content (ppm)	Iron (Fe2599) content (ppm)
Sample A	-0.218047	-0.197863	-0.045109
Sample B	-0.210594	-0.213852	-0.121536
Sample C	-0.463652	-0.435534	-0.122125
Sample D	-0.042567	-0.122948	-0.111701
Sample E	-0.009779	-0.009905	-0.002274
Sample F	-0.011244	-0.009897	-0.002307
Sample G	-0.010234	-0.009977	-0.002356
Sample H	-0.009779	-0.009905	-0.002274
Sample I	-0.011245	-0.009897	-0.002307

Table 8: Arsenic level for hydrosol of microwave hydro-distillation method

Sample	Weight	Extraction duration	Arsenic, As1890 content (ppm)
Sample J	10 kg	10 hours	0.000740586

III. CONCLUSION

In this study, it can be concluded hydrosol and condensate from hydro-distillation of Agarwood bark contain beneficial constituents that can be used in various areas. Fatty acid and aromatic compounds were the major compounds found from the extraction. This proved that instead of considering hydrosol and condensate as waste they can be converted into useful by-products. Besides, pretreating Agarwood with sonication helps to reduce required time for hydro-distillation as higher frequency and longer sonication duration results to shorter hydro-distillation duration. Lastly, from the arsenic test, Agarwood hydrosol obtained from hydro-distillation method does not contain heavy metals such as arsenic, iron and copper. Therefore if it is consumed it will not cause harmful effect to human health.

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References

- [1] Nor, M. A., Husni, S. S., Mailina, J., Sahrim, L., Majid, J. A., Mohd, Z., & Science, F. (2015). Classification of Resin Content, 25(2), 213–219.
- [2] Liu, Y., Chen, H., Yang, Y., Zhang, Z., Wei, J., Meng, H., Chen, H. (2013). Whole-tree agarwood-inducing technique: An efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis* trees. *Molecules*, 18(3), 3086–3106.

- [3] Aazza, S., Lyoussi, B., & Miguel, M. G. (2011). Antioxidant activity of some Moroccan hydrosols, 5(30), 6688–6696. <https://doi.org/10.5897/JMPR11.1176>
- [4] Hamdi, A., Majouli, K., Vander, Y., & Flamini, G. (2017). Phytotoxic activities of essential oils and hydrosols of *Haplophyllum tuberculatum*. *Industrial Crops & Products*, 97, 440–447. <https://doi.org/10.1016/j.indcrop.2016.12.053>
- [5] Tornuk, F., Cankurt, H., Ozturk, I., Sagdic, O., Bayram, O., & Yetim, H. (2011). International Journal of Food Microbiology Efficiency of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157: H7 and *Salmonella* Typhimurium on fresh cut carrots and apples. *International Journal of Food Microbiology*, 148(1), 30–35. <https://doi.org/10.1016/j.ijfoodmicro.2011.04.022>
- [6] Alexander Jok, V., Che Radzi, N., & Ku Hamid, K. (2014). Effect of Soaking on the Temperature and Ph Profiles in Agarwood Extraction, 3(6), 111–113.
- [7] Fazila, K. N., Halim, K. H. K., Journal, S., Science, F., October, N., Science, F., & Fazila, K. N. (2015). Effects of Soaking Agarwood Oil on Yield and Quality, 24(4), 557–564.
- [8] He, Z., Wang, Z., Zhao, Z., Yi, S., Mu, J., & Wang, X. (2017). Influence of ultrasound pretreatment on wood physicochemical structure. *Ultrasonics Sonochemistry*, 34, 136–141. <https://doi.org/10.1016/j.ultsonch.2016.05.035>
- [9] Koubaa, M., Mhemdi, H., Barba, F. J., Roohinejad, S., Greiner, R., & Vorobiev, E. (2016). Oilseed treatment by ultrasounds and microwaves to improve oil yield and quality: An overview. *Food Research International*, 85, 59–66. <https://doi.org/10.1016/j.foodres.2016.04.007>
- [10] Yoswathana, N., Eshiahi, M. N., & Jaturapornpanich, K. (2012). Enhancement of Essential Oil from Agarwood by Subcritical Water Extraction and Pretreatments on Hydrodistillation. *World Academy of Science, Engineering and Technology*, 65(5), 832–838.
- [11] Rashid AMA & Zuhaidi YA (eds). 2011. Tapping the Wealth From Karas (*Aquilaria malaccensis*) Tree. Forest Research Institute Malaysia, Kepong
- [12] Seidi, M., Niakousari, M., & Jamal, M. (2016). Ultrasound pretreatment impact on *Prangos ferulacea* Lindl. and *Satureja macrosiphonia* Bornm. essential oil extraction and comparing their physicochemical and biological properties. *Industrial Crops & Products*, 87, 105–115. <https://doi.org/10.1016/j.indcrop.2016.04.025>
- [13] Pingret, D., Pingret, D., & Chemat, F. (2014). An Improved Ultrasound Clevenger for Extraction of Essential Oils An Improved Ultrasound Clevenger for Extraction of Essential Oils, (October 2016). <https://doi.org/10.1007/s12161-013-9581-0>
- [14] Gokce, Y., Cengiz, B., Yildiz, N., Calimli, A., & Aktas, Z. (2014). Ultrasonication of chitosan nanoparticle suspension: Influence on particle size. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 462, 75–81. <https://doi.org/10.1016/j.colsurfa.2014.08.028>
- [15] Mancini, A., Imperlini, E., Nigro, E., Montagnese, C., Daniele, A., Orrù, S., & Buono, P. (2015). Biological and Nutritional Properties of Palm Oil and Palmitic Acid: Effects on Health, 17339–17361. <https://doi.org/10.3390/molecules200917339>
- [16] Correia, P., Cruz, A., Santos, L., & Alves, A. (2015). Risk of Children's Dermal Exposure to Galaxolide through Personal Care Products, 93–109. <https://doi.org/10.3390/cosmetics2020093>
- [17] Gutte, K. B., Sahoo, A. K., & Ranveer, R. C. (2015). F LAX AND HEMP Effect of ultrasonic treatment on extraction and fatty acid profile of flaxseed oil, 22(6).
- [18] Wang, H. N., Dong, W. H., Huang, S. Z., Li, W., Kong, F. D., Wang, H., Dai, H. F. (2016). Three new sesquiterpenoids from agarwood of *Aquilaria crassna*. *Fitoterapia*, 114, 7–11. <https://doi.org/10.1016/j.fitote.2016.07.014>