UNIVERSITI PUTRA MALAYSIA

COMPARISON STUDY OF ULTRASOUND AND MICROWAVE PRE-TREATMENTS ON YIELD OPTIMIZATION OF AGARWOOD OIL

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DEDICATION

Dedicated to my beloved late father, mother, brother and sister ... .

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ABSTRACT

Agarwood is regarded as one of the foremost expensive plant and highly valuable within the world due to the scent from its essential oil produced. Agarwood refers to dense, heavy and fragrant resinous wood that came from the family of *Thymelaeaceae* and which is formed in the tree of *Aquilaria* species. The resin derived from the Aquilaria tree makes it as a high valuable tree. The essential oil itself used in varies purpose for many countries to fulfil cultural, religious, medical and pharmaceutical purposes in these centuries. Agarwood farming in Malaysia is reportedly reaching a huge amount of plantation area and causing many amounts of tree are being unexploited due to limitations of efficient method to process an agarwood.

The agarwood that was used in this research is grade C that came from the peninsular of Malaysia and known as 'karas'. Nowadays, the most method used to extract agarwood essential oil is the traditional hydrodistillation method. However, this method required a long extraction times that consume a lot of heat energy. This extraction method also has a low efficiency and higher production cost. In order to improve the method itself, hydrodistillation extraction method assisted with the ultrasound and microwave pre-treatment is focused on this study to determine the effects of ultrasound and microwave pre-treatment on yield of essential agarwood oil.

The important parameter that has been studied in this research are three different agarwood size which are 1cm, 0.2cm and powder, time of microwave which are from 2, 4, 6, 8 and 10 minutes, time of sonication which are 10, 20 and 30 minutes. The time of extraction for hydrodistillation is 5 hours. Solvent been used in this research are water. From the result, it showed that the agarwood oil yield is increasing along with the increase of pre-treatment time. The highest percentage of oil yield for ultrasound pre-treatment is 0.2342 % for agarwood powder at sonication time is 30 minute while the lowest percentage of oil yield is 0.1328 % for agarwood size of 1cm at 10 minute. While for the microwave pre-treatment, the highest percentage of oil yield is 0.2580 % for agarwood powder at time of microwave is 10 minute and the lowest percentage of oil yield is 0.1838 % at time of microwave is 2 minute. Relatively, the microwave pre-treatment give the better result as the percentage of essential oil yield is higher compared to the ultrasound pre-treatment.

ABSTRAK

Agarwood dianggap sebagai salah satu tumbuhan yang paling mahal dan sangat berharga di dunia kerana aroma dari minyak pati yang dihasilkan. Agarwood merujuk kepada kayu resin padat, berat dan wangi yang berasal dari keluarga Thymelaeaceae dan yang terbentuk di dalam pokok spesies Aquilaria. Resin yang berasal dari pokok Aquilaria menjadikannya sebagai pokok berharga tinggi. Minyak pati sendiri digunakan dalam pelbagai tujuan untuk banyak negara untuk memenuhi tujuan budaya, agama, perubatan dan farmaseutikal di abad-abad ini. Ladang gaharu di Malaysia dilaporkan mencapai kawasan perladangan yang besar dan menyebabkan banyak pokok yang tidak dieksploitasi disebabkan oleh batasan kaedah yang cekap untuk memproses kayu gaharu.

Kayu yang digunakan dalam penyelidikan ini adalah gred C yang berasal dari Semenanjung Malaysia dan dikenali sebagai 'karas'. Pada masa kini, kaedah yang paling banyak digunakan untuk mengekstrak minyak pati gaharu ialah kaedah hidrodistilasi tradisional. Walau bagaimanapun, kaedah ini memerlukan masa pengekstrakan panjang yang menggunakan banyak tenaga haba. Kaedah pengekstrakan ini juga mempunyai kecekapan yang rendah dan kos pengeluaran yang lebih tinggi. Untuk meningkatkan kaedah itu sendiri, kaedah pengekstrakan hidrodistilasi yang dibantu dengan pra-rawatan ultrasound dan gelombang mikro tertumpu kepada kajian ini untuk menentukan kesan pra-rawatan ultrabunyi dan gelombang mikro terhadap hasil minyak agarwood.

Parameter penting yang telah dikaji dalam kajian ini adalah tiga saiz kayu gaharu yang berbeza iaitu 1cm, 0.2cm dan serbuk, masa gelombang mikro yang terdiri daripada 2, 4, 6, 8 dan 10 minit, masa ultrabunyi iaitu 10, 20 dan 30 minit. Masa pengekstrakan untuk hidrodistilasi adalah 5 jam. Pelarut yang digunakan dalam kajian ini adalah air. Dari hasilnya, ia menunjukkan bahawa hasil minyak gaharu semakin meningkat seiring dengan peningkatan masa pra-rawatan. Peratusan tertinggi hasil minyak untuk pra-rawatan ultrabunyi adalah 0.2342% untuk serbuk agarwood pada masa ultrabunyi adalah 30 minit manakala peratusan terendah hasil minyak ialah 0.1328% untuk ukuran kayu agar 1cm pada 10 minit. Walaupun untuk pra-rawatan gelombang mikro, peratusan tertinggi hasil minyak ialah 0.2580% untuk serbuk agarwood pada masa gelombang mikro adalah 10 minit dan peratusan terendah hasil minyak ialah 0.1838% pada masa gelombang mikro adalah 2 minit. Secara relatifnya,

pra-rawatan microwave memberikan hasil yang lebih baik kerana peratusan hasil minyak yang penting lebih tinggi berbanding pra-rawatan ultrasound.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Agarwood also known as eaglewood is respected as one of the foremost expensive natural items within the world due to the scent actuating compounds it contains. It is from the family of *Thymelaeaceae*. Agarwood refers to dense, heavy and fragrant resinous wood which is formed in the tree of *Aquilaria* species. The resin derived from the Aquilaria tree makes it as a high valuable tree. This species can grow up to 40m height in middle-size of tree and can be defined by its physical appearance such as thin, light gray rind, thick-tanned leaves and has a white flower (Fazila & Halim, 2012).

Agarwood is considered to be the finest natural incense and has been used in many countries to fulfill cultural, religious, medical and pharmaceutical purposes in these centuries. It has been known in a few different types of names for different country such as 'gaharu' in Malaysia and Indonesia, 'jin-koh' in Japan, 'chenhsiang' or 'chenxiang' in China, 'agar' in India, 'chim-hyuang' in Korea, 'kritsana noi' in Thailand and 'tramhuong' in Vietnam (Zuhanis, Hashim, Kerr, & Abbas, 2016).

Figure 1.1: Agarwood wood tree of Aquilaria Sp.

A. Malaccensis is distributed and can be found throughout Peninsular Malaysia except for the States of Kedah and Perlis (Radzi, Che, & Hamdan, 2015). Malaysia has a long history in the trade in gaharu wood, which has long been collected by the indigenous peoples of the interior of Peninsular Malaysia, Sabah and Sarawak to supplement their income. In Peninsular Malaysia, the gaharu products in domestic trade are woodchips and powder or sawdust. Some use has been recorded locally for medicinal purposes, but it appears that the majority of *A. malaccensis* harvested is exported (Barden et. al., n.d.).

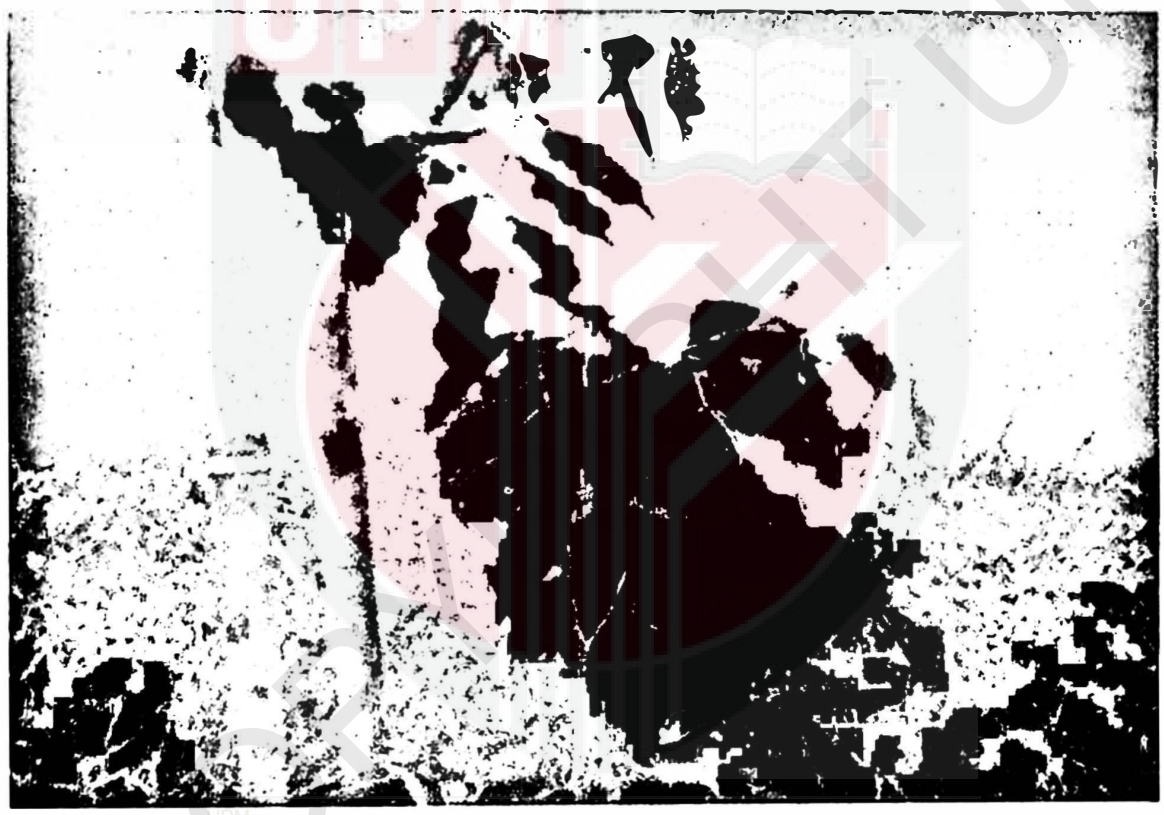

Figure 1.2: Tree of Aquilaria growth (6 months)

Figure 1.3: Aquilaria farm at Jerantut, Pahang

Gaharu wood or Agarwood have been widely used in many purposes in many countries since a long time ago. It plays an essential role in traditional medicine, cultural and religious practices in Buddhist, Hindu, Muslim, Jewish and Christian. In Buddhist religious ritual, incense plays an important medium between human and God whereby it is burned to purify the space surrounding the Buddha statue. Agarwood incense also is applied in Islamic culture where it is used in mosques, shops and houses during ceremony or ritual. They have a wide use in medical treatment which is pain reducer, dental pain, and kidney and rheumatism medicine, as venom repellent, in perfume and as incense raw material.

Gaharu contains more than 12 chemical components that can be extracted. In addition, agarwood also is used in medicine including as an adaptogen and anti-aging cure in Ayurveda medicine. Moreover, in the pharmaceutical industry, it is employed as an anticmetic, tranquilizer and digestive agent in oriental medical treatment. Nowadays, the range of agarwood products and their uses are apparently expanding. The essential oils and perfumes are very popular and high in demand especially in the Arab world.

1.2 Problem Statement

Gaharu wood known as the expensive trees due to human harvest of the wood to extract valuable resins. Nowadays, gaharu is used in the manufacture of the products such as perfume, soap and shampoo. These products are marketed at prices about ten times more expensive than the common brands of toilet soaps and shampoo. Currently, new products that derived from gaharu expected to be more appear on the market with advancing technology.

Agarwood farming in Malaysia is reportedly reaching a huge amount of plantation area. It is estimated that many private companies have a total of 1,068 ha with an estimation 256,080 to 1,169,028 trees (Elias, Ibrahim, & Wan, 2019). Due to limitations of efficient method to process an agarwood, many amounts of tree are being unexploited.

Nowadays, the most method used to extract agarwood essential oil is the hydrodistillation method. This method required a long extraction times that consume a lot of heat energy. This extraction method has a low efficiency due to the yield of essential oil from plant material is lower and need a lot of raw material to be used in the experiment. Thus, it will increase the cost to conduct the processing of essential oil from agarwood.

In order to improve the hydrodistillation method itself, some enhancement is needed to be made and to be study to reduce the extraction time and produce a high quality and high yield of the oil. Therefore, hydrodistillation extraction method assisted with the microwave and ultrasound pretreatment is focused on this study to determine the effects of ultrasound and microwave pretreatment on yield of essential agarwood oil. This study also investigated the effects of parameters such as different time to do the ultrasound and different time in microwave pre-treatment of the agarwood plant materials affected the hydrodistillation process in order to improve production of essential agarwood oil.

1.3 Research Objectives

The main objectives of this study are:

a) To determine the yield of Agarwood oil extraction via ultrasonic pre-treatment
b) To determine the yield of Agarwood oil extraction via microwave pre-treatment.

1.4 Research Design and Design of Experiment

In this research design, the objectives for this experiment is to determine the yield of Agarwood essential oil by using pre-treatment which is microwave and ultrasound. After the pre-treatment, the Agarwood undergo the hydrodistillation process to extract the essential oil.

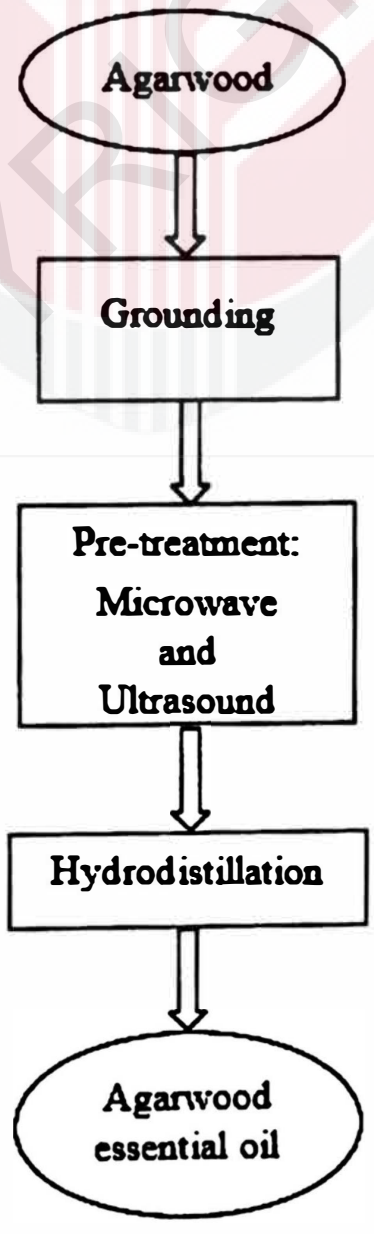

Figure 1.4: Research design of experiment

1.5 Scope of Research

In order to achieve the objective, these following scopes have been identified and to be applied:

i) The effect of ultrasound time on the yield of agarwood essential oil.

ii) The effect of microwave treatment towards the amount of agarwood oil produced in the hydro distillation process.

CHAPTER 2

LITERATURE REVIEW

2.1 Agarwood

Agarwood has been recognized by the local Malaysian since a long time and its valuable oil has been collected and extracted traditionally as a 'backyard industry' by the local people. Nowadays, local people extracting the essential oil of agarwood by using distillation unit made from stainless steel as a container that contains ground-up agarwood that will undergo a 96-hour distillation process to get its essence. Generally, process of extract oil is started from the inoculation of gaharu tree until extraction process and bottles it to be sell in the market.

Gaharu wood may be classified into several grades in the market. The grade codes which is Super A, A, B, C, D, E was varies among the buyer. Super A grades was really expensive compared to other grades due to its darkest appearance and have strong odor when producing the perfumes. Grading of gaharu wood can be complicated process usually based on its physical properties, formation and unique scent. It also includes evaluating the size,color, odour, weight and flammability of the wood.

In Malaysia, oil is mostly produced from grade C gaharu wood using Hydrodistillation method. Depending on the grade and quality, the price for a best quality of gaharu can reach up to RM10, 000 per kg depends on the grade of resinous wood. According to (Sulaiman et al., 2015) different qualities of gaharu oil are sold at RM50 to RM200 for every 12 g Oil extracted from gaharu wood obtained from different locations vary in quality (Nor Azah et al. 2008).

Table 2.1: Grading of Gaharu

Grade	Characteristics
A	Dark, dense, concentrated, heavy
B	Dark, purple, less dense, small hole
C	Dark yellow stripes, dark yellow
D	Whitish yellow

Figure 2.1: Pieces of Gaharu from Gaharu trees

2.2 Formation of Gaharu Resin

Agarwood formed when the gaharu tree produce a resin as a mechanism of defense against fungi infection of injury causing soft and white wood to become hard and dark in color. This hard and dark wood also known as resin. The formation of resin or fragrant wood was naturally produces by randomly inject with some parasite, fungi or molds and the microbe starts to produce resin inside the tree due to immune system of tree response to the injections of parasites.

Generally, resin or gaharu formation is caused by the response of tree towards mechanical or natural injury associated with the wood. Agarwood tree has two response mechanisms to injury. The first line of defense is for the phloem cells to produce callus growth over the injury. If the formation of callus prevented, the tree will produce resin as a chemical defense to the injury. The degree which resin produced in the phloem fibers will determines the value of resin in the market at Malaysia or globally.

This biological process develops very slowly over several years and there has limitations where not all *Aquilaria* species trees produce resin (Zubair, 2008) only approximately 10 % of mature Aquilaria tree that age from 20 years onwards and with above 40 cm diameter can naturally produce resin. Therefore, it really take a long time for one tree to produce resin after the injections of parasite and fungi.

Figure 2.2: Formation of Resin

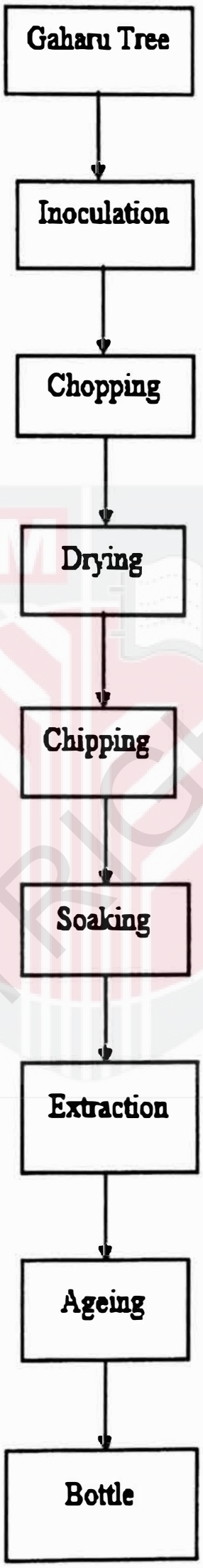

Figure 2.3: Processing flow of extraction essential oil from Gaharu

2.3 Essential Oil

Essential oil are fragrant volatile materials and products produced by the metabolism of plant consisting of complex mixtures of hydrocarbons and oxygenated materials. Another common constituents include phenyl propanoids from the Shikimic acid pathway, and their biotransformation products and other compounds from the metabolism of fatty acids and amino acids. Large number of other types of chemical components are also found including nitrogen and sulphur compounds. Essential oil can be derived from variety types of plant materials part such as leaves, bark, stems, flowers, wood, and stigmas. Oil is usually contained in specialized secretory structures which include secretory cells, ducts, cavities and glandular trichomes. The yield of essential oils from seeds can be high but majority of other materials is range in 0.1 % to 1 %.

Essential oil provides a targeted dose of nature's large medical specialty active ingredients in an exceedingly single drop of oil. Hundred percentage of oil are distinguished by outstanding diversity of substances that solely nature might manufacture. Essential oil conjointly volatile, they simply evaporate into air. Additionally, essential oil is sensitive to heat and light weight. There also are sure that essential oil may be cyanogenic and public awareness and proper use is needed once mistreatment it for the primary time or experiment it with alternative oil.

2.4 Hydrodistillation

The results of the extraction are either essential oils, absolutes, concretes or butters depending on the amount of waxes in the extracted product. The most frequently methods have been for gaharu oil extraction are hydro distillation and solvent extraction. The extraction method employed is of central interest because it determines the quality of the oil produced. An incorrect or wrongly carried out extraction procedure would produce inferior quality oil as it would change the chemical signature of the original gaharu oil.

Based on Jutarut et. al. (2011), the traditional extraction gaharu oil distillations are reported low in process efficiency and data of chemical analysis of gaharu oil distillation are very rare. Additionally, the extraction process is not always complete and the heat is not stable to control.

Hydro distillation is used in the manufacture and extraction of essential oil from gaharu oil. This is the simplest and usually the cheapest process of distillation. The main advantages of this method are that less steam is used, shorter processing time and a higher oil yield. In distillation, the plant material is heated, either by placing it in water which is brought to the boil or by passing steam through it.

The process mechanism for this process is started when heat and steam cause the cell structure of the plant material to burst and break down, thus freeing the essential oils. The emerging liquid is a mixture of oil and water, and since essential oils are not water soluble, they can be easily separated from the water and essential oils which are lighter than water will float on the surface. According to Liu et al. (2008) stated that hydro distillation extraction is safe to operate and environmentally friendly but its disadvantage is a time-consuming process and needs a large amounts of plant material.

Table 2.2: Hydrodistillation on different pre-treatment of Agarwood

Pre-treatment	Experimental Parameter	Yield	Reference
Soaking with Lactic and Sulphuric acid	Hydrodistillation: 200 g of agarwood, 1.5 L of water, 12 h Soaking: 0.1 M of Lactic & Sulphuric acid, 168 h;	Agarwood soaked in Lactic acid had more potential to enlarge the pore size of wood compared to Sulphuric acid. More yield and chemical constituents obtained.	(Fazila & Halim, 2012)
Soaking in ethanol solvents	Hydrodistillation: 200 g of ground agarwood, 1 L of water, 30 h Soaking: 50% ethanol (v/v), 80% ethanol (v/v), 5 days	Oil yield is 0.20 % after soaking in 50% aqueous ethanol and 0.21% after soaking in 80% aqueous ethanol. Increasing of ethanol percent did not significantly affected to the higher oil yield after 50% ethanol.	(Yoswathana, Eshiaghi, & Jaturapornpanich, 2012a)
Soaking with fungi	Hydrodistillation: 300 g of agarwood chips, 3 L of water, 9 h Soaking: soaked with three various fungi	Total of constituents for sample soaked with water and without soaking is lower than sample soaked with fungi at 72.58 % and 66.24 % respectively. Oils extracted from sample mixed with fungi *P. crysosporium* consists of number of hydrocarbon is 80.13% compared to the *T.reesei* (72.13 %) and *F. solani* (76.38%).	(Atikah et al., 2015)

2.5 Microwave

The application of the microwave assisted extraction where the use of microwave as one of the pretreatment to accelerate the production of essential oil gained broad popularity as a favored environment-friendly process. The theoretical study on the microwave heating mechanism is based on the dielectric properties, volume rate of heat generation and penetration depth. Microwave extraction has been recognized as a technique with several advantages compared to other extraction methods, such as reduction of costs and extraction time, less solvent consuming, environmental friendly and green technology (Nitthiyah, Nour, Kantasamy, & Akindoyo, 2017).

The mechanism of microwave is related to the irradiation of microwave energy which then turns into heat energy that used for heating the solvents and plant materials during extraction which extraction process became faster (Nitthiyah et al., 2017). The principle of heating using the microwave depends on the direct interaction with polar solvents.

The moisture inside the cells of plant materials is evaporate when heat is apply via microwave, thus the cell expansion occur and subsequently generation of high pressure on the oil gland and cell wall. The generated internal pressure pushes out the oil gland cell wall and stretches the cell wall until it will ruptured causing the process of release of essential oil from plant material to the water. The high temperature produced by the microwave absorbed by the plant cell wall because the dehydration process of cellulose became efficient and decrease the mechanical strength of the plant.

Table 2.3: Method of microwave pre-treatment on essential oil yield

Method	Parameter	Yield	Reference
Microwave assisted extraction (MAE) of orange dermis essential lubricant	Microwave power: 300, 600, 800 W	The extraction using MAE AT 800 W stopped after 20 min and produced 8.63% yield, while the one using HD stopped after 73 min and produced 7.03% yield.	(Selvia, Budi, & Hisyam, 2019)
Microwave assisted hydrodistillation of extraction of essential oils of Ferulago angulata	Microwave power: 300, 400, 500, 600, 700 W Extraction time: 3 hours	The results stated that MAHD of sample produce the highest essential oil yield which is 6.50 % as compared to the essential oil prepared with hydrodistillation which is 2.65 %.	(Mollaei, Sedighi, Habibi, Hazrati, & Asgharian, 2019)

2.6 Ultrasound

Ultrasound is an alternative method to prevent the utilization of solvents during the extraction of essential oil and decrease the time required for the extraction process. Ultrasound where it used an acoustic energy to produce a mechanical wave with a frequency rate over than 16 kHz. The ultrasound waves generated from transducer magneto strictive which are send to an electric field resulting in a conversion from electric to mechanical energy. The use of ultrasound in low frequency to extract compounds mainly through molecular agitation, heating, micro-jets formation and cavitation phenomenon (Michelon, Nora, & Borges, 2017).

Table 2.4: Method of ultrasound pre-treatment on essential oil yield

Method	Parameter	Yield	Reference
Ultrasonic bath assisted hydrodistillation	Extraction time: 1 hour, 3 hour, 6 hour, 9 hour Solid to solvent (water) ratio:1:8, 1:12, 1:16,1:20	Highest oil percentage for extraction time which is 9 hour is 0.169% while for solid to solvent ratio which is 1:20 is 0.139%.	(Zubair, 2008)
Ultrasonic cleaning bath assisted hydrodistillation	Frequency: 44-48 KHz Operation time: 30 hours Solid to solvent ratio: 1:9	Essential oil yield increased up to 0.20% and distinct higher than the oil yield without ultrasound pre- treatment.	(Yoswathana et al., 2012a)

CHAPTER 3

METHODOLOGY

3.1 Sample Preparation

Agarwood used in this study is grade C and were obtained form Best Agriventures SDN BHD's company. The plant material was dried, cut into small pieces and grounded to a size of 1 cm, 0.2 cm and powder size.

3.2 Preparation sample with ultrasound

Fifty grams (50 g) of ground agarwood were placed in the 1000 ml flask and 450 ml of distilled water were added into the flask. Then, the soaking of sample was placed under the ultrasonic probe and operated at 50 % amplitude. The sonification was performed for 10 min followed by using hydrodistillation about 5 hours. The same steps were repeated for different agarwood size which is 0.2cm and agarwood powder and different time of sonication which is 20 and 30 minute.

3.3 Preparation sample with microwave

Fifty grams (50g) of the ground agarwood for size 1 cm were mixed with 450 ml of distilled water and put the sample in the microwave with power 600 W about 2 minute followed by using hydro distillation about 5 hours. The same steps were repeated for different agarwood size which is 0.2cm and agarwood powder and different time of microwave which is 4, 6, 8, and 10 minute.

3.4 Hydrodistillation

Fifty grams (50g) of the agarwood were soaked with distilled water in beaker with 450 ml. Hydrodistillation was carried out at boiling temperature of water for 5 hours. The obtained agarwood oil in Clevenger-type apparatus was taken and stored at 5°C until analyze the chemical constituents (Yoswathana, Eshiaghi, & Jaturapornpanich, 2012b).

3.5 Data Collection

The extracted agarwood oil was stored at ambient temperature until the oil analyzed by a Gas Chromatography. Gas chromatography used to identify the chemical components extracted from the sample.

CHAPTER 4

RESULT AND DISCUSSIONS

4.1 Introduction

Hydrodistillation known as a popular traditional extraction technique used in obtaining the essential oils from plant materials. However, the traditional agarwood oil Hydrodistillation are reported by Jutarut et. al. (2011) are low in process efficiency, take a long time, the extraction process is not always complete and difficult to control the heat of distillation.

Pre-treatment techniques are important way in extraction process to improve the production of essential oil yield and improve the quality of Agarwood oil. Some studies have been carried out by previous researcher to investigate the effect of cell wall breakage during pre-treatment technique before undergo the Hydrodistillation process.

The result obtained from this study which is the mass of agarwood oil tabulated on the Table 4.1 and 4.2 and the percentage of oil yield were calculated.

4.2 Pre-treatment Time of Ultrasound

The effects of pre-treatment techniques was crucial to obtain the efficient method in order to get the more production of oil yield and good quality of essential oil of Agarwood. The essential oils extracted by using Hydrodistillation process and undergo Ultrasound pre-treatment were compared between three types of Agarwood size which is 1cm, 0.2cm and powder size. Three different time of sonicate which is 10, 20 and 30 minute has been used to determine the effects of sonication towards the breakage of lignocellulose plant wall of Agarwood. The amplitude of ultrasound used is constant parameter which is 50 %. According (Solanki, Desai, & Parikh, 2018), yield of oil was found to increase up to 50 % amplitude.

Table 4.1: Data obtained for ultrasound pre-treatments

Size of Agarwood (cm)	Time of Sonicate (min)	Mass of Agarwood Oil (g)	Percentage of Oil Yield (%)
1	10	0.0664	0.1328
	20	0.0733	0.1466
	30	0.1003	0.2006
0.2	10	0.0843	0.1686
	20	0.1108	0.2216
	30	0.1166	0.2332
Powder	10	0.0903	0.1806
	20	0.1116	0.2232
	30	0.1171	0.2342

Table 4.1 showed the mass of agarwood oil obtained and the percentage of oil yield was calculated from the pre-treatment of ultrasound for three different size of Agarwood which is 1cm, 0.2cm and powder. For Agarwood size of 1cm, the highest mass of agarwood oil obtained is 0.1003 gram and the percentage of oil yield calculated is 0.2006 % for sonication time is 30 min while the lowest mass of Agarwood oil obtained is 0.0664 gram and the percentage of oil yield calculated is 0.1328 % for sonication time is 10 min.

The result obtained showed that the longest time of sonication causing more essential oils being extracted from the Agarwood in Hydrodistillation process. According to cavitation's phenomena, the collapse of bubbles produced by tip of sonicator on the cell wall was expected to induce cell disruption together with a good penetration of the solvent into the cells (Yoswathana et al., 2012a). Thus, mass transfer of essential oil from the cell wall of Agarwood was increased and the yield of essential oil was improved.

Based on the Table 4.1, the highest mass of agarwood oil for Agarwood size of 0.2 cm obtained is 0.1166 gram and the percentage of oil yield calculated is 0.2332 % for sonication time is 30 min while the lowest mass of Agarwood oil obtained is 0.0843 gram and the percentage of oil yield calculated is 0.1686 % for sonication time is 10 min. The percentage of oil yield for agarwood size of 0.2 cm which is 0.1686 %, 0.2216 % and 0.2332 % is more higher compared to the percentage of oil yield for agarwood size of 1 cm which is 0.1328 %, 0.1466 % and 0.2006 % for sonication time is 10 min, 20 min and 30 min respectively. The data value obtained showed that the size of agarwood can affect the production of essential oil as the heat produced by boiling the mixture of solvent used which is distilled water and agarwood can penetrate more on smaller molecule of Agarwood cell wall and freeing more oil from Agarwood. This also showed that the longest time of sonication which 30 min is causing more essential oils being extracted from the Agarwood in Hydrodistillation process.

For Agarwood powder, the highest mass of agarwood oil shown in Table 4.1 is 0.1171 gram and the percentage of oil yield calculated is 0.2342 % for sonication time is 30 min while the lowest mass of Agarwood oil obtained is 0.0903 gram and the percentage of oil yield calculated is 0.1806 % for sonication time is 10 min. The percentage of oil yield for agarwood powder which is 0.1806 %, 0.2232 % and 0.2342 % was higher compared to the percentage of oil yield for agarwood size of 0.2 cm which is 0.1686 %, 0.2216 % and 0.2332 % for sonication time is 10 min, 20 min and 30 min respectively. This is because the smaller size of the wood, the highest percentage of oil content will be produced (Nasardin et al., 2018).

Figure 4.1 showed the line of data value for agarwood size of 0.2cm and agarwood powder has not much different in terms of percentage of oil yield. In the beginning of line data between agarwood size of 0.2 cm and agarwood powder, the percentage of essential oil yield increased up by 0.012 % for sonication time 10 min. It is significant because the smaller size of particle of Agarwood has a potential to enhance the extraction process (Michelon et al., 2017).

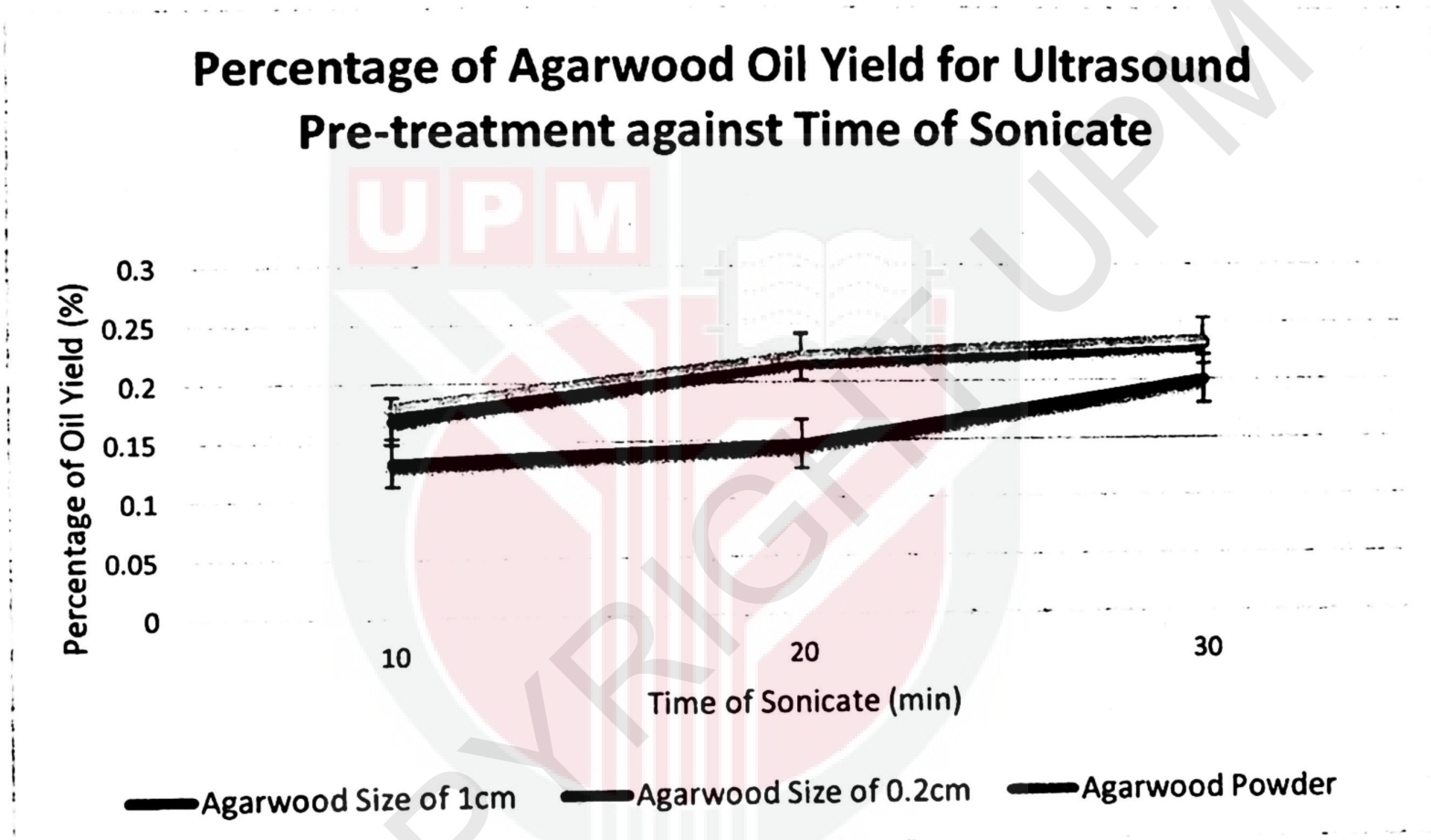

Figure 4.1: Graph of percentage of agarwood oil yield against time of sonicate For ultrasound pre-treatment

4.3 Pre-treatment Time of Microwave

Hydrodistillation extraction through microwave pre-treatment has been done and observed in order to increasing the potentials for producing good quality and high yield of essential Agarwood oil. The effects of these pre-treatment method has been seen in the experiment where the essential oils extracted by using Hydrodistillation process. Three types of Agarwood size which is 1cm, 0.2cm and powder size has been used as a sample and different time of microwave which is 2, 4 6 8 and 10 minute. The constant parameter is microwave power which is 600 W.

The factors that affects the performance of microwave pre-treatment is the microwave power and the time used to do the pre-treatment. Based on the previous research, more yield of essential oil obtained when the microwave power is high. However, according the (Selvia et al., 2019) less essential oils were obtained at the power of 800 W and more essential oils were obtained at the power of 600 W. This is because at the high power, more components of oil were degrade.

Table 4.2: Data obtained for microwave pre-treatment

Size of Agarwood (cm)	Time of Microwave (min)	Mass of Agarwood Oil (g)	Percentage of Oil Yield (%)
1	2	0.0919	0.1838
	4	0.0948	0.1896
	6	0.1062	0.2124
	8	0.1099	0.2198
	10	0.1118	0.2236
0.2	2	0.0969	0.1938
	4	0.1011	0.2022
	6	0.1045	0.2090
	8	0.1065	0.2130
	10	0.1086	0.2172
Powder	2	0.1243	0.2486
	4	0.1247	0.2494
	6	0.1248	0.2496
	8	0.1258	0.2516
	10	0.1290	0.2580

Based on Table 4.2, the mass of agarwood oil obtained and the percentage of oil yield was calculated from the pre-treatment of microwave for three different size of agarwood which is 1 cm, 0.2 cm and powder. The time of microwave as a pre-treatment on hydrodistillation process has been used is 2, 4, 6, 8 and 10 min. For the agarwood size of 1 cm, the highest of percentage of essential oil yield is 0.2236 % for the time of microwave is 10 min while the lowest of percentage of essential oil yield is 0.1838 % for the time of microwave is 2 min. Figure 4.2 showed that the data line obtained for agarwood size of 1 cm was slightly increasing.

For agarwood size of 0.2 cm, the highest of percentage of essential oil yield is 0.2172 % for the time of microwave is 10 min while the lowest of percentage of essential oil yield is 0.1938 % for the time of microwave is 2 min as shown in the Table 4.2. Thus, the highest mass of agarwood oil produced during the hydrodistillation process is 0.1086 gram by undergo the microwave pre-treatment that take about 10 min while the lowest mass of agarwood oil produced is 0.0969 gram for microwave pre-treatment time for 2 min. From Figure 4.2, the data line obtained based on the percentage of essential oil for agarwood size of 0.2 cm is nearly straight and slightly increasing when the time of microwave pre-treatment is increasing from 2 min to 10 min. It showed that when the pre-treatments time was increased, the essential oil extracted from the plant material was also increased (Michelon et al., 2017).

As stated in the Table 4.2, the highest percentage of oil yield for agarwood powder is 0.2580 % for the time of microwave is 10 min while the lowest of percentage of essential oil yield is 0.2486 % for the time of microwave is 2 min. Based on the data obtained, the highest mass of agarwood oil produced for the microwave pre-treatment is 0.1243 gram

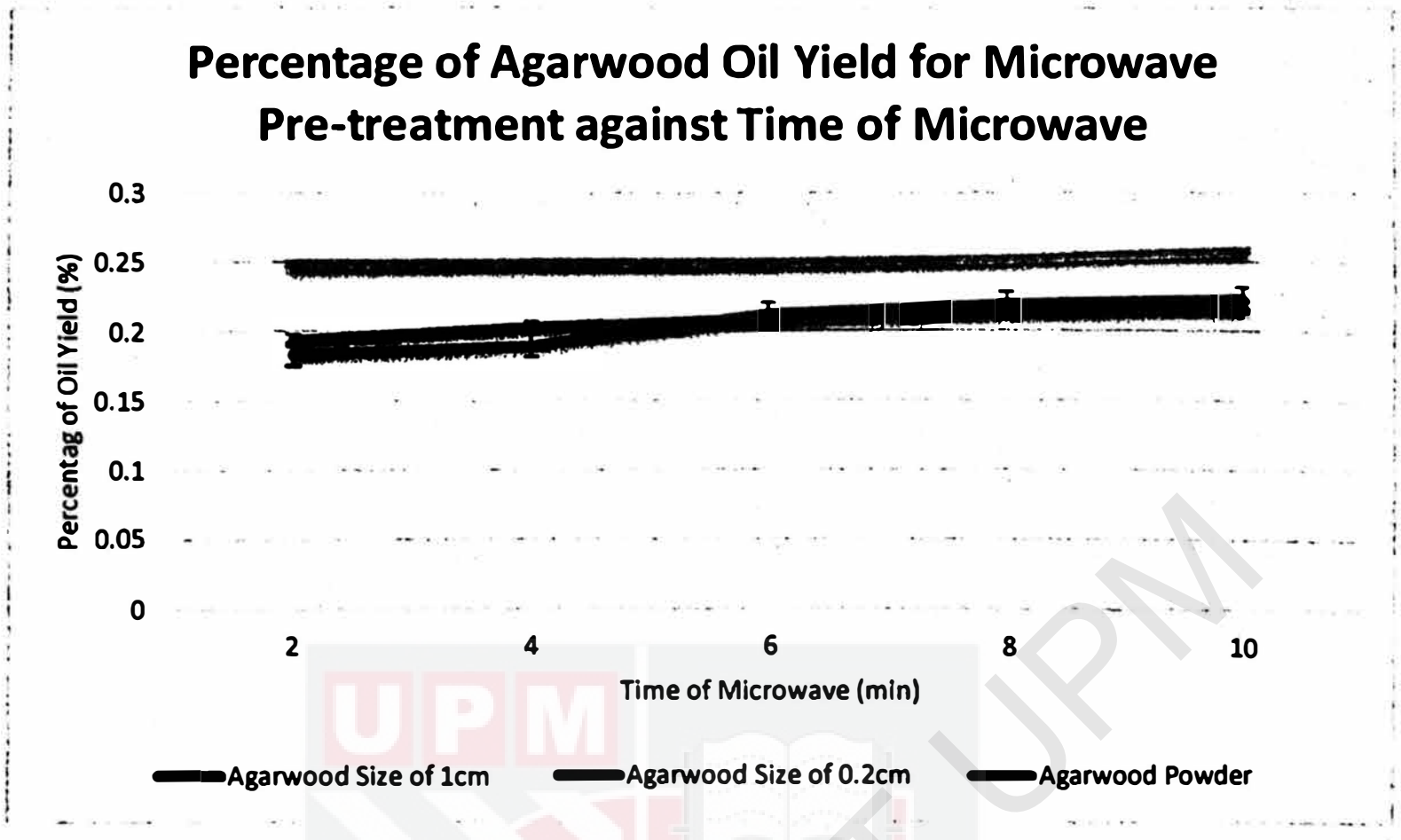

Figure 4.2: Graph of percentage of Agarwood oil yield against time of microwave for microwave pre-treatment

4.4 Chemical Composition of Different Parameters for Soaking Method

Referencing to study done by Siti Azura (2019), the chemical compounds of extracted oil from different parameters for soaking pre-treatment method were identified by GC-FID analysis. The six samples of extracted agarwood essential oil namely as sample A, sample B, sample C, sample D, sample E and sample F form different extraction, agarwood sizes, and soaking period were analyzed. Sample A and B shows the analysis of the sample by varying the extraction time, sample C and D shows the analysis of the sample from different size while sample E and F shows the analysis of the sample from different soaking of period. Table 4.3 shows the chemical composition of agarwood essential oil by three different parameters and Table 4.4 shows the percentage amount of hydrocarbon present in different sample.

Based on the analysis, there are more than 30 compounds were found in the sample A, B, C, D, E, and F. The compounds were classified into four groups, namely as other compound, sesquiterpene, sesquiterpenoid, and carboxylic acid. Sesquiterpenoid shows the highest total chemical compounds obtained in the extracted essential oil compared to other compound, sesquiterpene and carboxylic acid.

Based on the analysis, there are several major chemical compound present in the extracted oil namely 4-phenyl 2-butanone from other compound, 7-epi-γ-Eudesmol, agarospirol, β-Eudesmol, α-Eudesmol, Kusunol, , and Rotundone from sesquiterpenoid compounds. 7-epi-γ-Eudesmol shows the highest percentage relative peak area among all of the sample tested compared to another chemical compound.

Table 4.3 shows that sample A which is 8 hours of extraction has the highest percentage of hydrocarbon identified in the oil that consist of 67.54% compared to extraction time of 24 hours that consist of 66.48%. 35 number of compound present in agarwood oil extracted at 8 hours while only 34 number of chemical compound present in agarwood oil extracted at 24 hours. This indicates that, extraction time had an effect on compound present in essential oil.

For sample D which is agarwood size of 1.0 cm shows the highest percentage amount of hydrocarbon present in the agarwood oils extracted which is 68.94 % compared to the extracted oils from agarwood size 0.2 cm which 67.65 % as shown in the Table 4.3. However, same number of compounds were identified in both agarwood essential oil samples from 0.2cm and 1.0 cm which is 35 number of compounds.

Form Table 4.3, sample F for 9 days of soaking period shows the higher amount of hydrocarbon identified in the extracted agarwood oil which is 67.54 % compared to the sample E for 5 days of soaking period which only has 66.21 %. The number of compounds present in the both samples is also different which is essential oil for 9 days of soaking period give 35 number of compounds while the essential oil for 5 days of soaking period give 34 number of compounds

Table 4.3: Percentage amount of hydrocarbon present in different sample

Compounds	Sample					
	A	B	C	D	E	F
Total other compound	5.81	3.13	7.01	5.22	4.78	3.87
Total sesquiterpene	2.38	2.56	3.03	3.14	2.56	3.55
Total sesquiterpenoid	51.80	50.41	51.37	53.94	50.83	50.77
Total carboxylic acid	7.55	10.38	6.24	6.64	8.04	9.35
TOTAL (%)	**67.54**	**66.48**	**67.65**	**68.94**	**66.21**	**67.54**

Compounds	Molecular formula	Relative peak area (%) Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Ident.
Other compound								
Benzaldehyde	C_7H_6O	0.16	0.11	0.18	0.15	0.19	0.12	FID
4-phenyl-2-butanone	$C_{10}H_{12}O$	4.85	2.32	5.76	3.95	3.45	2.57	FID
Sinenofuranol	$C_{14}H_{24}O_2$	0.80	0.70	1.07	1.12	1.14	1.18	FID
Sesquiterpene								
α-Caryophyllene	$C_{15}H_{24}$	0.15	-	0.25	0.12	0.12	0.12	FID
allo-Aromadendrene	$C_{15}H_{24}$	0.49	0.86	0.94	1.05	0.86	1.11	FID
δ-Guaiene	$C_{15}H_{24}$	0.15	0.18	0.27	0.28	0.18	0.35	FID
cis-Calamenene	$C_{15}H_{22}$	0.14	0.16	0.11	0.17	0.14	0.14	FID
Dehydro-aromadendrene	$C_{15}H_{22}$	1.45	1.36	1.46	1.52	1.26	1.83	FID
Sesquiterpenoid								
Dihyro-β-Agarofuran	$C_{15}H_{26}O$	0.17	0.39	0.28	0.45	0.23	0.46	FID
Kessane	$C_{15}H_{26}O$	0.21	0.20	0.21	0.23	0.18	0.24	FID
α-Agarofuran	$C_{15}H_{26}O$	0.46	0.41	0.46	0.50	0.46	-	FID
nor-Ketoagarofuran	$C_{15}H_{24}O$	0.83	1.03	0.81	1.13	0.65	0.84	FID
Epoxybulnesene	$C_{15}H_{24}O$	1.32	1.14	1.28	1.18	1.02	1.38	FID
Caryophyllene oxide	$C_{15}H_{24}O$	0.34	0.29	0.34	0.33	0.42	0.45	FID
7-epi-γ-Eudesmol	$C_{15}H_{26}O$	6.80	7.51	7.06	7.45	6.38	7.34	FID
Agarospurol	$C_{15}H_{26}O$	3.10	3.37	3.22	3.13	2.88	3.55	FID
β-Eudesmol	$C_{15}H_{26}O$	6.82	7.04	6.48	6.83	6.27	6.61	FID
α-Eudesmol	$C_{15}H_{26}O$	6.54	6.29	6.14	6.30	6.15	6.40	FID
Kusunol	$C_{15}H_{26}O$	3.24	3.42	3.17	3.87	3.68	3.12	FID
Dehydrojunikob-cremol	$C_{15}H_{26}O$	1.22	1.18	1.31	1.11	1.20	1.46	FID
epi-α-Bisabolol	$C_{15}H_{26}O$	3.07	2.32	2.62	3.20	2.86	2.53	FID
α-Bisabolol	$C_{15}H_{26}O$	1.08	0.93	0.89	1.12	0.94	0.84	FID
Rotundone	$C_{15}H_{22}O$	3.07	2.33	3.36	3.40	2.87	2.64	FID
Selina-3,11-dien-9-ol	$C_{15}H_{24}O$	0.92	0.85	1.03	0.75	0.88	0.96	FID
Selina-3,11-dien-9-al	$C_{15}H_{22}O$	0.78	0.78	0.83	0.68	0.72	0.69	FID
9,11-Eremophiladien-8-one	$C_{15}H_{22}O$	1.14	1.21	1.13	1.13	1.19	1.17	FID
Guaia-1(10),11-dien-9-one	$C_{15}H_{22}O$	1.89	1.40	1.37	2.11	1.27	1.17	FID
Selina-4,11-dien-14-al	$C_{15}H_{22}O$	1.51	1.72	1.79	1.71	2.05	2.28	FID
Nootkatone	$C_{15}H_{22}O$	0.71	0.68	0.71	0.70	0.73	0.65	FID
Selina-3,11-dien-14-oic acid	$C_{15}H_{22}O_2$	0.92	1.09	1.04	1.07	1.17	0.84	FID
Dihydrokaranone	$C_{15}H_{24}O$	1.72	1.62	2.12	1.51	1.56	1.53	FID
Karanone	$C_{15}H_{22}O$	0.81	0.86	0.85	0.84	1.18	0.73	FID
Eudesmol	$C_{15}H_{26}O$	0.58	0.53	0.58	0.43	0.77	0.71	FID
2-hydroxyguaia-1(10),11-dien-15-oic acid	$C_{15}H_{22}O_3$	2.55	1.82	2.29	2.78	3.12	2.18	FID
Carboxylic acid								
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	7.55	10.38	6.24	6.64	8.04	9.35	FID
Total other compound		5.81	3.13	7.01	5.22	4.78	3.87	
Total sesquiterpene		2.38	2.56	3.03	3.14	2.56	3.55	
Total sesquiterpenoid		51.80	50.41	51.37	53.94	50.83	50.77	
Total carboxylic acid		7.55	10.38	6.24	6.64	8.04	9.35	

Note:
Sample A: 0.6cm, 7 days, 8 hours; Sample B: 0.6cm, 7 days, 24 hours; Sample C: 0.2cm, 7 days, 16 hours; Sample D: 1.0cm, 7 days, 16 hours; Sample E: 0.6cm, 5 days, 16 hours; Sample F: 0.6cm, 9 days, 16 hours

Figure 4.3: Chemical composition of Agarwood essential oil obtained by three different parameters (Extraction Time: A&B, Agarwood Size: C&D, Soaking Period: E&F)

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Essential oil are derived from various part of the plants. Agarwood is known as finest natural incense and has been used in many countries to fulfil cultural, religious, medical and pharmaceutical purposes. Extraction of essential oil form Agarwood can be carried out by various technique of extraction. Hydrodistillation is a popular extraction technique used for obtaining essential oils form plants. Hydrodistillation is known as a traditional and conventional technique that safe to operate and environmentally friendly. However, these traditional hydrodistillation method to extract an agarwood oil are time consuming process, low in process efficiency and higher operating cost. Pre-treatment techniques are one of the way to improve the extraction of agarwood oil.

The hydrodistillation extraction method assisted with the pre-treatment of ultrasound and microwave has been studied to determine the effects of ultrasound and microwave pre-treatment on yield of essential agarwood oil. Different parameters has been used which is agarwood size, time of sonication, and time of microwave to determine the percentage of essential oil yield in the end of this study.

As conclusion, hydrodistillation assisted with ultrasound and microwave pre-treatment has been proven can improve the efficiency and the production of Agarwood oil extraction. Ultrasound and microwave pre-treatments on oil yield optimization showed that the percentage of oil yield obtained is higher compared to the traditional hydrodistillation techniques.

From the study above, the recommended things to be done is pre-treatment via enzymatic hydrolysis which is treat the sample with enzyme to increase the oil productivity. More studies can be conducted to see more effect of soaking method on the production of oil yield and have other innovative methods to reduced chemical risk, extraction time and have a better quality of essential oils.

CHAPTER 6

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APPENDICES

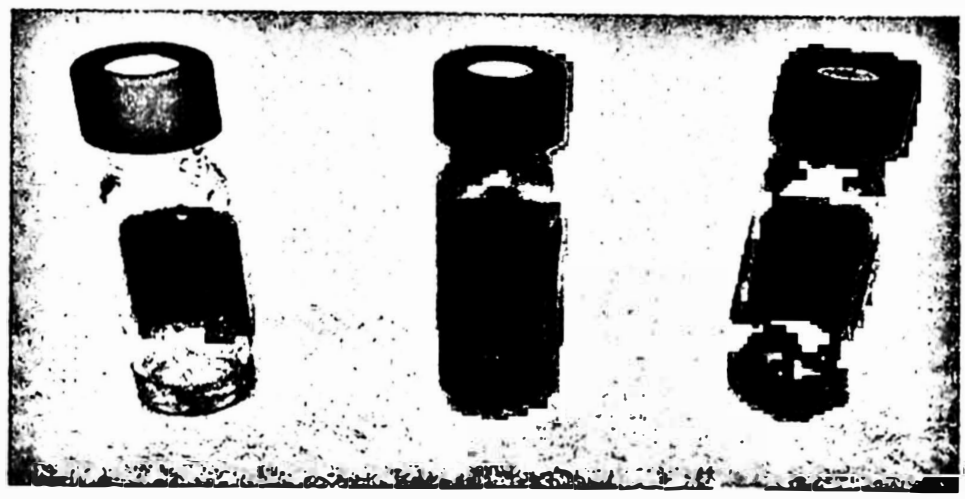

Figure A.1: Essential oil produced from Agarwood size of 1 cm for ultrasound pre-treatment

Figure A.2: Essential oil produced from Agarwood size of 0.2 cm for ultrasound pre-treatment

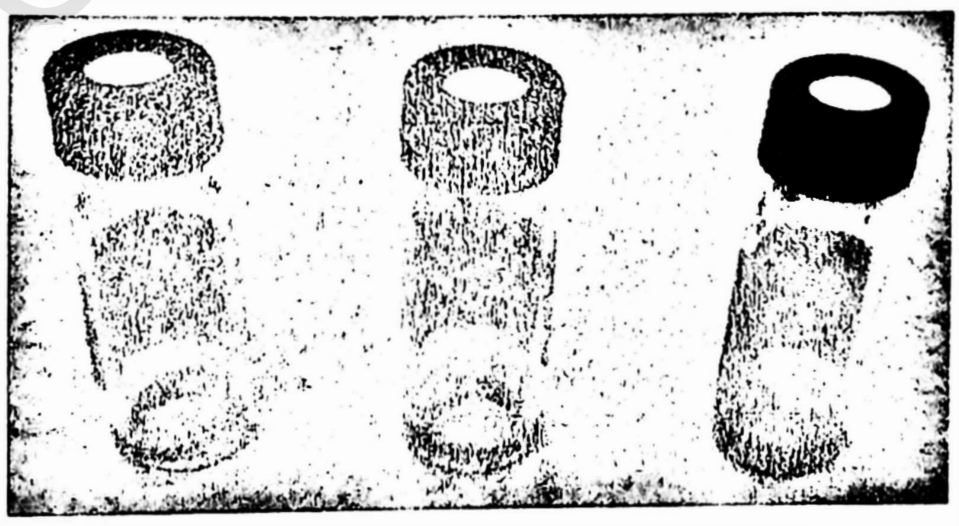

Figure A.3: Essential oil produced from Agarwood powder for ultrasound pre-treatment

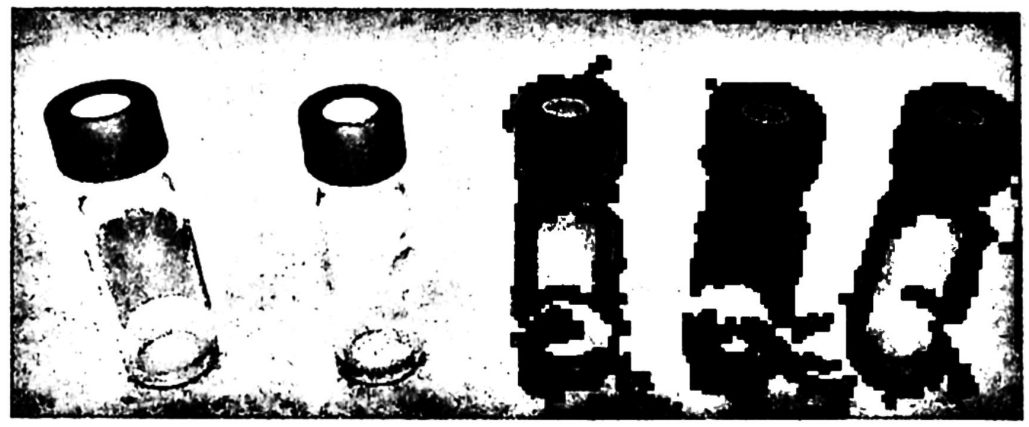

Figure A.4: Essential oil produced from Agarwood size of 1 cm for microwave pre-treatment

Figure A.5: Essential oil produced from Agarwood size of 0.2 cm for microwave pre-treatment

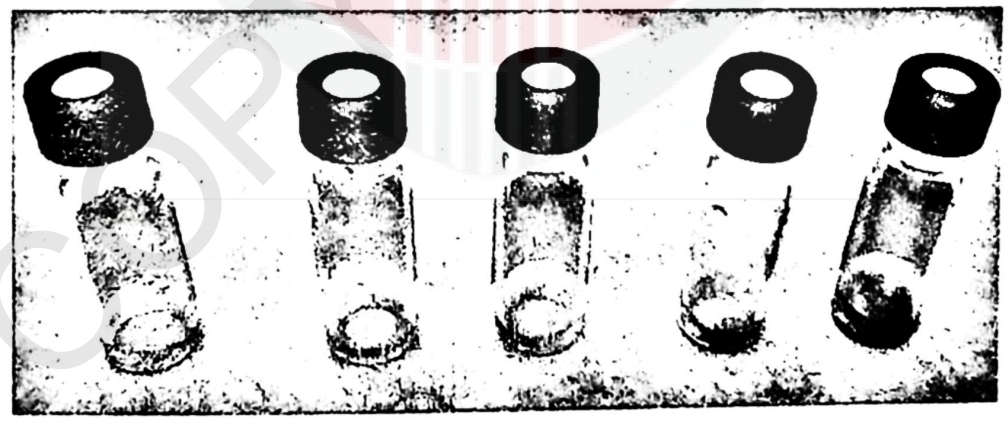

Figure A.6: Essential oil produced from Agarwood powder for microwave pre-treatment

Figure A.7: Grinder

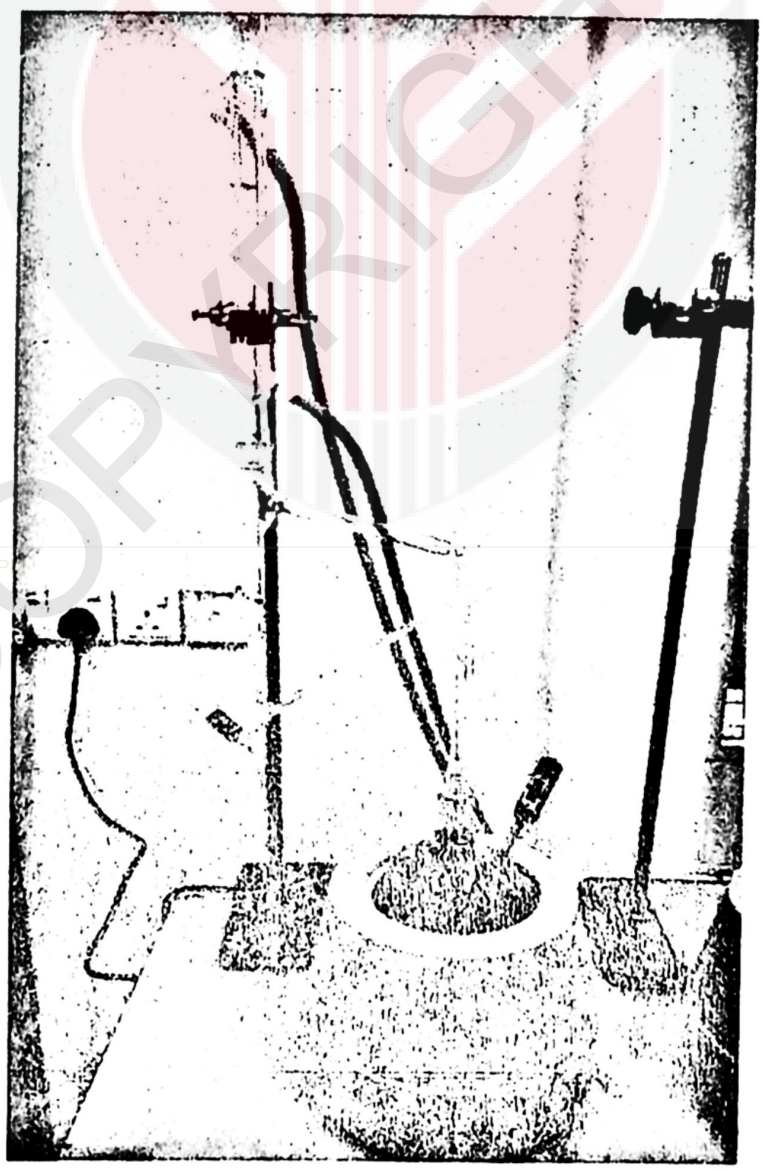

Figure A.8: Clevenger-type apparatus for hydrodistillation

Figure A.9: Microwave for microwave pre-treatment

Figure A.10: Ultrasound Horn for ultrasound pre-treatment