Microwave-Assisted Hydrodistillation of *Aquilaria Subintegra*

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Abstract— **Higher demanding of essential oil as pharmaceutical, aromatherapy aid and cosmetics ingredients give large opportunities for global marketing. The international market for essential oil growth rapidly. Thus, it is necessary to find the most suitable method to enhance the quality of essential oil. Conventional method that usually to obtain essential oil have several disadvantages such as longer extraction time, have low extraction yields, laborious and higher operational costing. Presently, alternative extraction method which is able to overcome disadvantages like mentioned above by using microwave-assisted hyrodistillation(MAHD) method. Hence, conventional hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) methods has been compared to evaluate their efficiency in terms of yield of extraction of *Aquilaria Sub-integra* grounded chips and for MAHD only, to investigate effect the power of microwave on the yield of essential oil. The MAHD offers several benefits compared to HD which are shorter extraction time, better yields, and provides a more valuable essential oil from agarwood grounded chips. The experimental results shows the operation time for MAHD was shorter than HD and higher yield results from higher extraction rates by microwave.**

Keywords— Aquilaria Sub-integra, essential oil, extraction, hydrodistillation, microwave-assisted hydrodistillation

I. INTRODUCTION

Extraction oils are also known as essential oils produced by the plant. Extraction oils can be get from the roots, stems, leaves and flowers of plant. Essential oils are commonly used in industry for cosmetics, perfumes, medicines and also for flavoring agent in food and drinks. (Heri Septya Kusuma, 2016).

One type of the essential oils is agarwood oil. Agarwood oil is a highly prized type of oil due to its unique aroma. The oil is extracted from the fragrant resin found in the agarwood tree trunk (Yumi Z.H.Y Hashim, 2014). *Aquilaria* species is an aromatic plant that is normally known as gaharu, jinkoh, eaglewood and aloeswood.

There are many grades of agarwood, and the highest quality wood is extremely expensive. In fact, the first-grade wood is one of the most expensive natural products in the world can be over thirty thousand US dollars per kilo. Prices range from a few dollars per kilo for the lowest quality according to The Rainforest Project, 2015. However the finest grade of agarwood is produced from naturally occurring fungal infection which happens slowly and very rarely.

Agarwood has been valued among people from Asia and currently, it is trendy commodity in many Asian areas. Because of its immense value and rarity, indiscriminate cutting of trees and over harvesting in hope of finding the treasured resin has lead to depletion of wild trees. The extraordinary esteem of agarwood has resulted in the overexploitation of this natural resource up to the most remote region of its development (Angela Barden, 2008). The levels of agarwood utilization are still increasing due to variety factors such the global migration of consumers, global trade facilities and business goals, professional marketing strategies, media, the conclusive constellation of increasing poorness in source countries and the growing affluence of consumer groups abroad (Jung, 2009). In addition, the global perfume industry has become an interested product, and agarwood has the interest of esoteric circles of non-Asian societies (Angela Barden, 2008). As a result, agarwood has become the most expensive perfumery raw material in the world, also exceeding the value of gold. The economic dimension of its global trade is inestimable, while wild resources have declined and are nearly extinct. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has listed *Aquilaria Malaccensis* as one of Red List of Endangered Species (Jung, 2009).

It is important to increase public awareness of threat of extinction of this natural resource to ensure agarwood's availability in the future. Furthermore, there must be a significant growth in the identification of agarwood species and refined products on sale for better understanding in their materiality and origin. Lastly, support should be supplied for the sale and investment of certified agarwood from audited wild resources and cultivation projects. The sustainable management of this natural resource can be achieved as chances of agarwood to be acknowledged for its cultural value and beneficial properties in global market especially Asian countries (Jung, 2009).

Essential oils from agarwood can be extracted by several methods which include hydrodistillation (typically using water or steam), solvent extraction, carbon dioxide extraction, cold pressing as well as florasol/phytol extraction. For example, (Lucchesi ME., 2004) had used solvent free microwave extraction technique from its aromatic herbs. Besides, the process of extracting gaharu oil steam distillation takes several hours with low yield, thus making the process less effectives (Mazni, 2007). The coupling of thermodynamic modeling with experimental work has offered an efficient low-time consuming tool to analyze the viability of supercritical extraction processes (Fonari, 2008). In contrast, these common methods can influence thermal degradation, hydrolysis and water solubility of some fragrance constituents. In addition, the oil obtained through solvent aided extraction contains residues that pollute the foods fragrances to which they are added. As a means to overcome this sort of drawbacks, an advance and improved method such as microwave-assisted extraction (Hong-Wu Wang, 2010) and separation and fractionation of Aquilaria Malaccensis oil using superctitical fluid extraction and the cytotoxic properties of the extracted oil by A. H. Ibrahim(2011).

Recently, microwave-assisted hydrodistillaton (MAHD) is one of the procedures for isolating essential oils that become attractive for use in laboratories and industry due to its effective heating, fast energy transfer and also an environmental friendly extraction technique. Its acceptance as prospective and powerful alternative for traditional extraction techniques has been verified through several researches according to (Jila, 2012a), (Abderrahmane, 2013), (Mohammad T. a., 2008). Moreover, the low content of essential oil in plant materials need an extraction technique with a high performance to achieve higher yields, thus parameter optimization of that particular technique is the most vital process to achieve this (Ranitha M, 2014)..

Microwave assisted technique have been described time saving, energy saving and highly efficient (P. Abroomand Azar, 2011). Thus, the objectives of this research was to use the microwave assisted hydrodistillation(MAHD) method for extraction of agarwood(*Aquilaria Subintegra* species). Another concern was to compared extraction time, extraction yield with conventional hydrodistillation(HD) method.

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II. METHODOLOGY

A. Material and chemicals

The sample of Agarwood(*Aquilaria Subintegra* species) grounded chips was obtained from Biobenua, Lojistik Sdn Bhd and stored at room temperature until required. Distilled water, anhydrous sodium sulphate and n-hexane used in the experiment work were all of analytical grade.

B. Microwave-assisted hydrodistillation

In applying MAHD, we used , a commercial microwave oven (MC456TBRCSR, Samsung, 45 L, 1100W, 2.45 GHz) with a cavity dimension of 418 x 414 x 262 (W x D x H). The microwave oven was modified by drilling a hole at the top. Around bottom flask with a capacity of 3000mL was placed inside the oven and was connected to the three-way adapter and liebig condenser through the hole. Then, the hole was closed with PTFE to prevent any loss of the heat inside.

200g of Agarwood grounded chips and 2000mL of distilled water (samples-to-water-ratio of 1:10) were placed in the flask and heated by microwave for 6 hours with different irradiation power at 300W, 400W, 600W and 800W. For this experiment, the time was observed within a 10 minutes interval until no more oil is produced. The oil extracted was collected, dried with anhydrous sodium sulfate and stored at 4°C. The dried extract was dissolved with n-hexane and then it was analyzed using GC-MS

C. Hydrodistillation

Hydrodistillation was conducted approximately same like MAHD but a heating mantle was used as the source of heat. 200g of Agarwood grounded chips and 2000mL of distilled water (weight samples-to-volume water-ratio of 1:10) were placed in the flask and heated by heating mantle for 6 hours. For this experiment, the time was observed within 10 minutes interval until no more oil is produced. The oil extracted was collected, dried with anhydrous sodium sulfate and stored at 4°C. The dried extract was dissolved with n-hexane and then it was analyzed using GC-MS.

D. Gas Chromotography-Mass Spectrometry(GC-MS) analysis

Essential oils composition was performed by a GC-MS Model; QP 2010 series which is equipped with a VF-5ms fused silica capillary column of 80m length, 0.25mm diameter and 0.25μm film thickness. For the GC-MS detection, an electron ionization system, ionization energy of 70ev was used. Helium gas (99.99%) was used as a carrier gas at constant flow rate of 1.51 ml/min, injector and mass transfer line temperature were set at 200 and 240°C respectively.

III. RESULTS AND DISCUSSION

A. The effects of MAHD and HD on extraction yield

The extractions obtained from the standard hydrodistillation(HD) and microwave-assisted hydrodistillation(MAHD) were tabulated in table 4.1. The volume of essential oil been extracted for HD and MAHD shows a different values meanwhile for different power of microwave power used it shows a quite similar amount of oil been extracted. The yield of oil obtained was difficult to be determined accurately but normally less than 0.1%.

Table 1: Tabulated data for Oil Been Extracted from HD and MAHD

Type of Sample	**Mass Before (g)**	**Mass After (g)**	**Mass oil extracted (g)**	**Yield of extraction (%)**
Sample 1 (HD)	198.36	198.51	0.15	0.0068
Sample 2 (MAHD= 300W)	198.51	198.75	0.24	0.011
Sample 3 (MAHD= 400W)	198.62	198.85	0.23	0.010
Sample 4 (MAHD= 600W)	198.66	198.91	0.25	0.011
Sample 5 (MAHD= 800W)	198.72	198.94	0.22	0.010

From table 1, Sample 1 is the lowest oil been extracted based on weight comparison which 0.15g whereas for MAHD process, the amount of extracts when oil fully produces are approximately same. As stated before, MAHD had more higher amount of extracts compare to HD, thus, the yield also obeyed have the same patterns too. Thus we can say that the MAHD produced more amount extraction oil compared to HD which is have been proven by Hong-Wu Wang, (2010) and Heri Septya Kusuma (2015) that mentioned MAHD methods offers better advantages over HD such as shorter extraction time and better yields. This could be due to the synergy of two transfer phenomena: mass and heat acing in the same time. Hence from the table 1, it can be concluded that even different power of microwave had been used, the extraction yield approximately equal at amount of 0.010-0.011%.

B. TemperatureProfile

Figure 1 shows the temperature profile during extractions by MAHD from agarwood grounded chips. In all extraction methods, the initial temperature was around 25°C. The extraction temperature was equal to the boiling point of water(100°C). It can said that variation power of microwave power from 300W to 800W had no significant effect on the extraction yield as the they were heated long enough more than 3 hours, the yields under different power were almost similar.

When the sample starts to boil, it will condense the vapor that will become the oil. The graph shows that the time required for boiling samples which are including standard hydrodistillation(HD) and microwave-assisted hydrodistillation(MAHD) at power of 300W and 400W are approximately equal. The time required for these three methods to boil are around 40-45 minutes for each of them. Meanwhile, for microwave power of 600W and 800W, the time required for both methods are around 20-25 minutes.

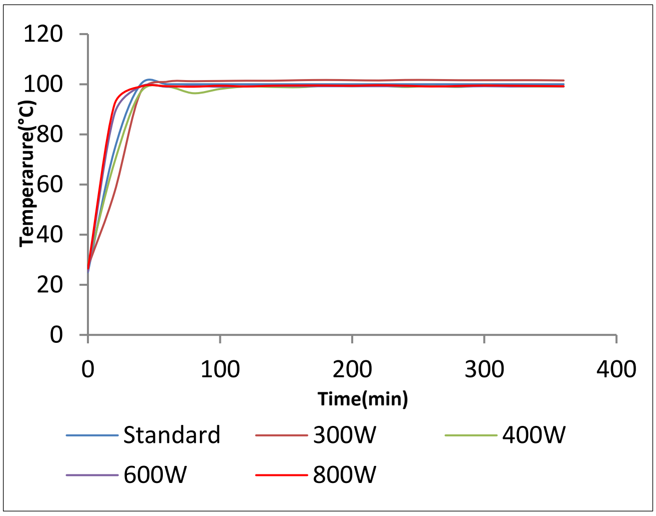
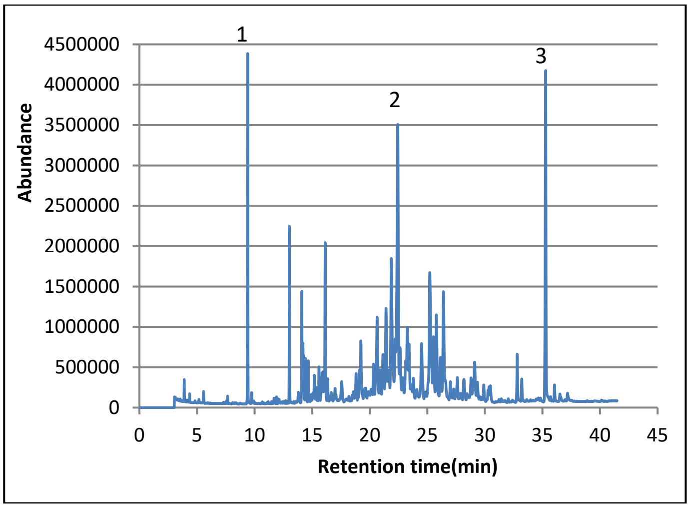


Figure 1: Comparison of time of extraction between HD and MAHD

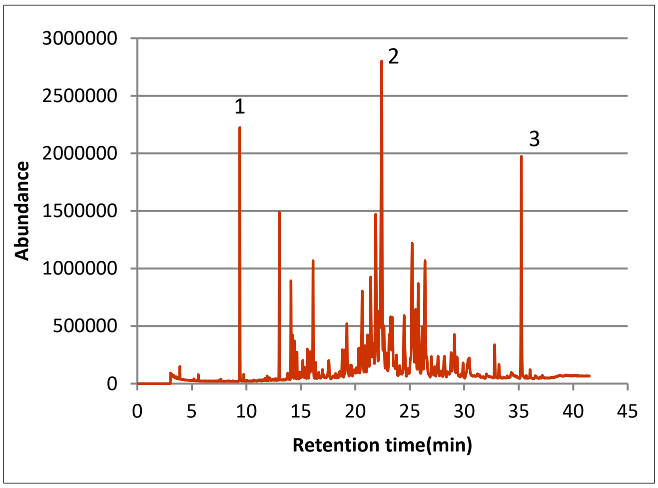
From the observation during each of experiment, it shows that standard hydrodistillation and microwave-assisted hydrodistillation at power of 300W and 400W are having approximately same initial time which are 25-30 minutes for oil to start producing after the sample is boiled. In the other hand, for the microwave power of 600W, the time required for oil to begin produce is around 15-20 minutes after boiling. Lastly, for the microwave power of 800W, the time needed for oil to form is 10-15 minutes. Thus, from the five method, it clearly state that when the power of microwave is high, the time for sample to producing the oil will become shorter. Hence, microwave power of 800W has the less time to extract oil.

C. *Composition of essential oil*

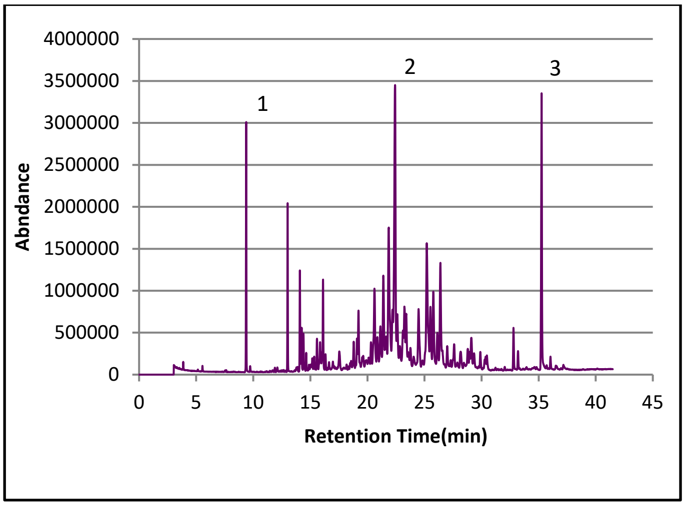
The essential oil from the Aquilaria Sub-integra grounded chips was extracted by the standard hydroditillation(HD) and microwave-assisted hydrodistillation(MAHD) and analysed by gas chromatography-mass spectrometry(GC-MS). For each method of extraction given different total compounds that were identified from the analysis. Examination of five samples of agarwood oils from different method of extraction showed some variations in GC profile an chemical components. The variations of GC profiles are shown in Figure 4.2(a-e).



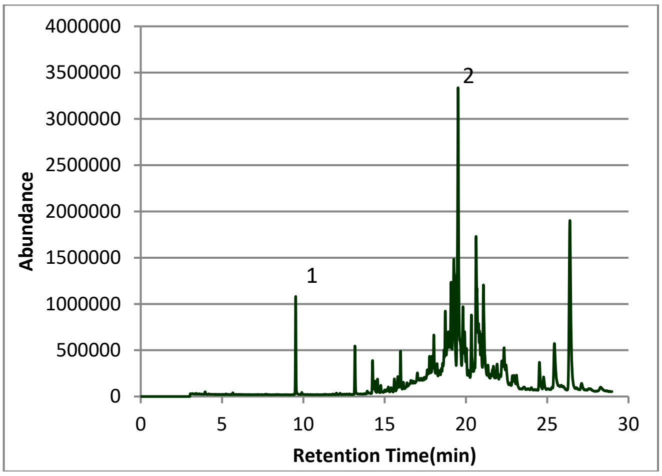
(a)



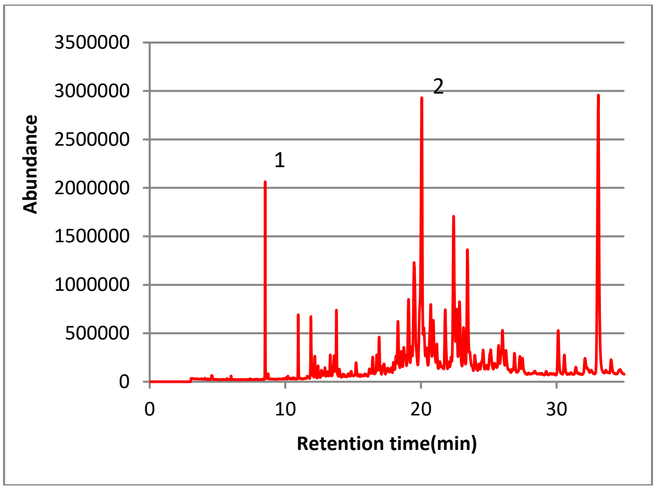
(b)



(c)



(d)



(e)

Figure 4.2: Total ion chromatogram for agarwood essential from different extraction samples a)Sample A(HD), b)Sample B(MAHD=300W), c) Sample C(400W), d)Sample D(MAHD=600W) and e)Sample E(MAHD=800W). Labeled peaks are 6-Octen-1-ol, 3,7- dimethyl-propanoate(1), (gurjunene)Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7 (2) and Verrucarol(3)

A total of 241 compounds were identified from the five samples of agarwood oils.48 components were identified in sample A which are the standard hydrodistillation method. In sample B, sample C, sample D and sample D, there were 48,47, 49 and 49 compounds identified respectively. About 15% of the compounds identified were aromatic, and terpene(monoterpene and sesquiterpenes) have been disclosed to the main active compounds of agarwood oils (Abbas, 2014). These main active components play important roles in giving the aroma and pleasant odor of agarwood (Chen, 2011).

Two compounds were commonly identified in all five samples which are 6-Octen-1-ol, 3,7-dimethyl-,propanoate (Compound 1 in graph of GC-MS analysis) and (gurjunene)Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-**(**1-methylethenyl) (Compound 2). Citronellyl propionate or 6-Octen-1-ol,3,7-dimethyl-,propanoate is colorless liquid that can be categorized as one of flavor and fragrance agents. Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-**(**1-methylethenyl) or normally known as gurjunene are one of the highest composition of compounds in each samples. From the Figure 4.2(a-c), Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene), 6-Octen-1-ol, 3,7-dimethyl-propanoate and verrucarol are the three component that have higher percentage in Sample A, Sample B and Sample C.

Verrucarol has same fundamental structure as the antibiotics trichothecin (Tadeusz Korzybski). Verrucarol only appears in Sample 1, Sample 2 and Sample 3. It shows that this compound are absence or ruptured if power of microwave is too high which are 600W and 800W. antibiotics is the component only exist and functional when it is available at its optimum condition such as suitable temperature and so on.

Most of the compounds found in the samples are sesquiterpenes such as gurjunene, azulene, ferrocene and spathulenol meanwhile 3-carene are example of monoterpenes. All of this component that mentioned before this were found in all five samples. Table 2 shows the total compound that were found in Sample A, Sample B, Sample C, Sample D and Sample E. There were total 34 components that were found in all five samples such as Caryophyllene, (aromadendrene)1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl, α-Vatirenene, Azulene-1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyle), à-Cubebene and 1,9-Dihydropyrene.

Thus, it can concluded that when the microwave power used id different, it will affect the composition of oil that will present in essential oil. It can be increased or decreased the total number of components exist in extraction oil. The insignificant amount of compounds may also be affected due to the sample preparation or extraction time and microwave power that had been used.

Table 2: Comparison of HD and MAHD in terms of GC-MS analysis

	Standard Hydrodistillation (Sample A)	Microwave-assisted Hydrodistillation			
		300W (Sample B)	400W (Sample C)	600W (Sample D	800W (Sample E)
Total compounds found	48	48	50	49	49
10 compounds that has higher composition	1)6-Octen-1-ol, 3,7-dimethyl-, propanoate 2) Verrucarol 3)Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene) 4) α-Pinene 5) Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyle) 6) Limonene oxide, cis- 7)3-Carene 8) Caryophyllene 9) Squalene 10) Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7(valencene)	1)Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene) 2) 6-Octen-1-ol, 3,7-dimethyl-, propanoate 3) Verrucarol 4)3-Carene 5) (-)-Spathulenol 6) ç-Gurjunenepoxide- 7) Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyle 8) Phenanthrene, 2-nitro- 9)Humulane-1,6-dien-3-olthrene, 10) Caryophyllene	1)Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene) 2) Verrucarol 3) 6-Octen-1-ol, 3,7-dimethyl-, propanoate 4) Ecgonine 5)Spathulenol 6) 3-Carene 7) á-Vatirenene 8) 6-Octenal, 3,7-dimethyl- 9) Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyle 10) Ferrocene	1)Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene) 2) Ferrocene 3) 3-Carene 4) Aristolene 5)Naphthalene, 1,6-dimethyl-4-(1-methylethyl) 6) 1,9-Dihydropyrene 7) 2-Butanone, 4-phenyl- 8) 1-Penten-3-one, 1,5-diphenyl- 9) á-Panasinsene 10) Humulane-1,6-dien-3-ol	1)Piperidine, 1-methyl- 2) Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7(gurjunene) 3) 2-Butanone, 4-phenyl- 4) ç-Himachalene 5) 3-Carene 6) à-Cubebene 7) Limonene oxide, cis 8) Caryophyllene 9) Mequinol 10)Aristolene
Number of similar compound that found in all five samples	34 compounds 1) Ecgonine 2) c-Elemene 3) 6-Octen-1ol,37dimethyl-propanoate 4)Humulane-1,6-diene-3-ol 5)Caryophyllene (ID#:160525~3-N1001) 6)cis-á-Terpineol 7)a-pinene 8)Caryophyllene (ID#:250516~1-N1001) 9)(aromadendrene) 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl- 10)á-Vatirenene 11)Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyle 12)à-Cubebene 13)1,9-Dihydropyrene 14)à-Farnesene 15)á-Panasinsene 16)Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyle 17)(valencene) Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7 18)Limonene oxide, cis- (ID#:16DA1E~1-N1003) 19)6-Octenal, 3,7-dimethyl- (ID#:160616~1-N1002) 20)(gurjunene)Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1- 21)Limonene oxide, cis- (ID#:160616~1-N1003) 22)Mequinol 23)Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethen 24)3-Carene 25)Camphor 26)Aristolene 27)Alloaromadendrene oxide-(1) 28)6-Octenal, 3,7-dimethyl 29)Phenantherene,2-nitro 30)Naphthalene,16-dimethyl-4-(1-methylethyl) 31)Ferrocene 32)(guaiene) Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7- 33)Spathulenol 34)1H-Imidazole, 2-propyl				

IV. Conclusion

As conclusion, this experiment has managed to compare between HD and MAHD in terms extraction time and yield of extraction oil and also effect of power microwave on the yield essential oil. From the results obtained, MAHD offers better benefit over HD which are shorter extraction time and better yields. From the MAHD method, microwave power of 800W has the less time to extract oil. Thus, oils extracted using both methods must contain a large amount of high vitality compounds and their composition should be same. Microwave irradiation may improve or decrease release of the volatile compounds. MAHD had offered important advantages over HD such as shorter extraction times and better yields.

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