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Characterization of bioactive compounds extracted from patchouli: Comparing microwave-assisted hydrodistillation and hydrodistillation methods

N H Abdurahman and S Sundarajan*

Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia

*E-mail: ssgeetha18rajan@gmail.com

Abstract. Microwave-assisted hydrodistillation (MAHD) is a promising and advanced technology that uses microwave heating to extract essential oils from dry leaves. Currently available conventional methods are time consuming, thermally unsafe and require costly solvents. In the current study, MAHD and traditional hydrodistillation (HD) techniques were compared and their effectiveness in the separation of the essential oil from dry patchouli leaves have been studied. The patchouli oil that obtained from MAHD and HD extraction methods were characterized using gas chromatography-mass spectrometry (GC-MS) analysis and the impact of the two extraction methods on the plant morphology were observed using scanning electron microscopy (SEM) analysis. The results indicated that MAHD method extracted 5 % of essential oil at solid to solvent ratio of 1:12 g/ml from patchouli leaves as compared to 4 % obtained using HD method at 45 minutes of extraction time. In addition, MAHD method can retain 16 compounds while only 11 chemical compounds were extracted using HD. Thus, MAHD showed a greater potential for better production of the essential oil from patchouli leaves as compared to HD method.

1. Introduction

Essential oils are fragrant, slick and unstable concentrates from different parts of plants, for example, leaves, seeds, barks, and roots. Over 150 sorts of essential oils currently being exchanged in international markets [1]. Among these essential oils, patchouli oil has higher demand due to its various applications. Patchouli (*Pogostemon cablin*) oil can be extracted from the leaves of patchouli plants which are commonly cultivated in tropical areas especially Indonesia, Philippines [2] and Malaysia [3]. Patchouli oil has many applications in various industries. The oil plays an important role in Chinese traditional medicine [4]. It acts as antidepressant, antiseptic, antiphlogistic, and astringent [5-7]. These pharmacological properties might be due to several chemical compositions of the oil [8]. Moreover, patchouli oil can be used in the cosmetic [2] and fragrance industry to provide the base and lasting character to the fragrance because of its fixative properties [9]. Furthermore, the oil can be used as a fragrance in soaps, detergents and deodorants [10]. Currently the demand for the patchouli oil is about 587 tons per annum [4]. Since there is no synthetic replacement for the patchouli oil and new applications of the oil continuously being introduced, the interest for patchouli oil will be increasing annually and the price will remain high in future. Hence, to meet international demands and contribute to country's economic growth, an efficient extraction technology that is rapid, energy-efficient, thermally safe, and capable of recovering higher yield of oil is inevitable for the separation of patchouli essential oils.

Steam distillation, hydrodistillation and Soxhlet extraction are the conventional methods that are currently used in the separation of patchouli oil. In hydrodistillation, the plant sample to be extracted is soaked in solvent and then boiled using a heating mantle [11] while in steam distillation, steam passes through the bed of plant material to be extracted [9]. For Soxhlet extraction, the plant sample is set in a thimble while condensed solvent topped off the thimble and siphoned down into the container of solvent continuously until the oil has been extracted from solid in the thimble into the extracting solvent [12]. Yield of patchouli essential oil obtained from hydrodistillation was 2.61 % for 6 hours [4] and 3.4% for 4 hours of extraction [2] while 1.5 % of patchouli oil was obtained when using steam distillation for 2 hours [9]. When using Soxhlet extraction for one hour, 0.84 % of patchouli oil was obtained [13]. However, the traditional methods possess several drawbacks such as long extraction time, cause thermal degradation, consumption of high energy, and give a low yield of patchouli oil [12, 14]. Most of the conventional techniques employed in the extraction of oil are being conducted at high temperatures, hence the chemical compounds might have been degraded which can reduce the quality of oil. Since conventional techniques require longer time of extraction, the amount of heat absorbed by the solvent will be higher which can lead to high temperature [15]. Thus, an advanced extraction technique that can extract higher amount of essential oil at shorter time need to be studied.

In this study, MAHD was studied for its effectiveness in extracting oil from patchouli leaves. Fast heating is one of the advantages of microwave extraction because it can heat up the plant materials simultaneously at higher rate [11]. MAHD had been reported to recover higher yield of essential oils from Mentha longifolia L., rosemary and damask rose [16-18]. According to [4], the obtained yield of patchouli essential oil was 2.72 % when using microwave extraction for two hours while 2.61% of patchouli oil was obtained after six hours of hydrodistillation. This shows that higher amount of patchouli oil can be obtained at shorter time when using microwave extraction. The results may vary from this project because patchouli leaves were obtained from Sabah, Malaysia for this current experiment. Cultivation region can change the qualitative and quantitative properties of patchouli leaves [9]. In addition, [19] reported that it took 15 minutes for the full recovery of essential oil from orange peels when using MAHD method while [20] obtained 5% of essential oil from Tongkat Ali when using 20 min of MAHD. Therefore, in this research, 60 minutes was used for MAHD to study the effect of longer extraction time on the yield of patchouli oil. Although methanol [21-22], CaO catalyst [22] and ethanol [21] can accelerate the extraction process, only distilled water was used as a solvent for this study to promote an environmentally-friendly extraction method. Thus, this study investigated the potential of MAHD as the alternative extraction method compared with HD method in the separation of oil from patchouli leaves. The effects of MAHD and HD parameters (extraction time and solvent to feed ratio) on the recovery of oil were studied. Moreover, the chemical compositions of the extracted oil at the highest extraction parameters for both methods were examined using GC-MS and the influence of the extraction techniques on the morphological nature of patchouli leaves before and after extraction were as well investigated using SEM analysis.

2. Materials and Methods

2.1. Raw Material
Dried patchouli leaves were obtained from Gaya Naturals Company, Tawau, Sabah. Before starting the experiment, the leaves isolated from stems and dried using oven for four days. Then, the leaves were blended using a grinder into fine powder.

2.2. Moisture Content of Patchouli Leaves
The moisture content was determined by weighing 5g of dry patchouli leaves, the plant sample was further dried for 4 days at room temperature until stable weight was attained. After 4 days of drying, the dry patchouli leaves were weighed again and the moisture content was calculated using equation (1).

$$Moisture\ content\ (\%) = \frac{Weight\ before\ drying - Weight\ after\ drying}{Weight\ before\ drying} \times 100 \quad (1)$$

2.3. Operating Procedure of Essential Oil Extraction Using MAHD and HD

A modified domestic microwave extractor comprising of power and time controller was used for this experiment. Figure 1 (a) shows the set up for the MAHD study. 30 g of patchouli powder was added to 180 ml of distilled water in a flat-bottomed glass flask. According to a previous study by [20], 250W was used for MAHD and the highest yield of essential oil obtained was 5% after 20 minutes of extraction. Thus, to study the effect of higher microwave power on the yield, the microwave power and time of extraction were kept constant at 400W and 60 minutes respectively. After one hour, the hydrosol (water + essential oil) was gathered from the Clevenger. The hydrosol was placed in a separating funnel to separate essential oil from water. The oil was filled in a vial and stored at -4 °C for further analysis. Essential oils need to be stored at below -4 °C to prevent oxidation. The experiment was repeated for 240, 300, 360, and 420 ml of distilled water.

The experiment for HD [figure 1 (b)] was carried out in the same manner as MAHD, however instead of modified microwave, heating mantle was used. 30 g of patchouli powder was added to 180 ml of distilled water in a flat-bottomed glass flask. The experiment was conducted for three hours and was repeated using 240, 300, 360, and 420 ml of distilled water. The extracted oil was filled in a vial and stored at -4 ° C until further analysis. According to [27] research, highest yield (1.3%) was obtained at 150 minutes using HD, thus 3 hours of extraction was used for this project to study the effect of longer extraction time on the yield of patchouli oil.

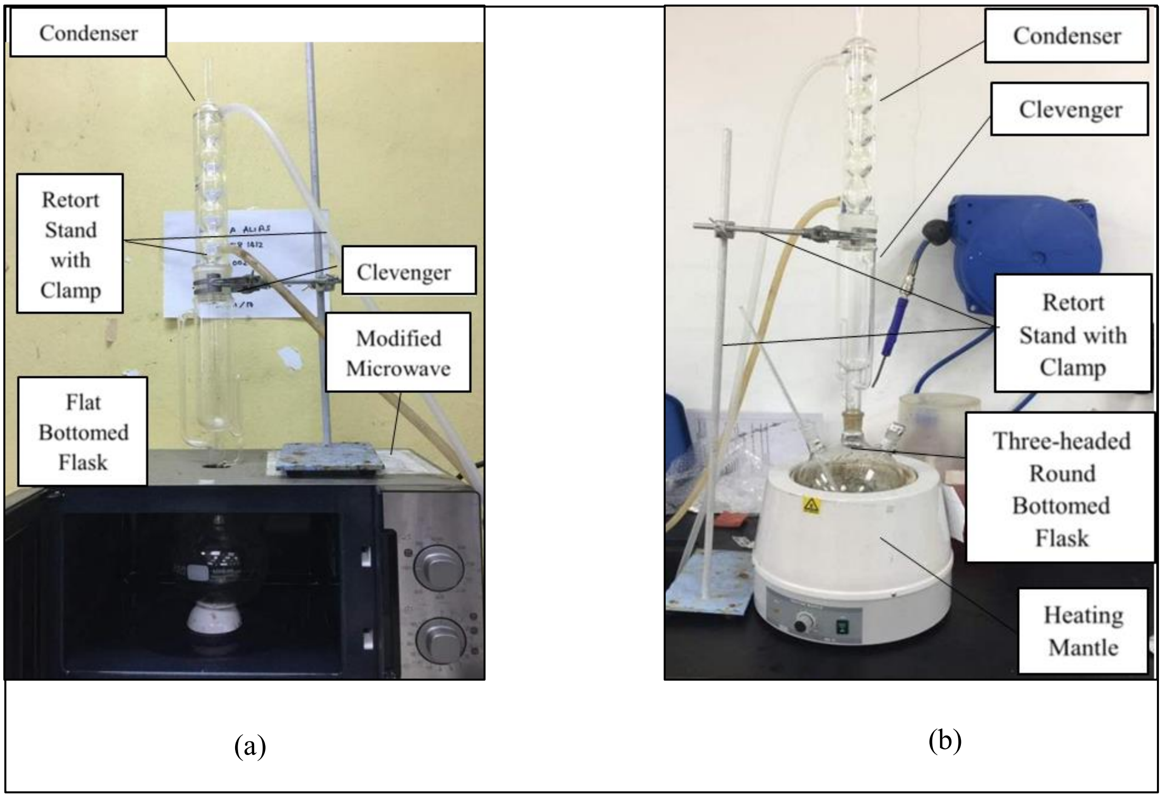

Figure 1. (a) MAHD set up and (b) HD set up.

2.4. Yield of Patchouli Essential Oil

The yield of patchouli essential oil was calculated as shown below:

$$Mass\ of\ patchouli\ essential\ oil\ (g) = Density\ (\rho) \times volume\ (ml) \quad (2)$$
$$Density\ of\ patchouli\ oil = 0.963\ g/ml$$
$$Yield = (Weight\ of\ essential\ oil\ (g)) / (Weight\ of\ plant\ sample\ (g)) \times 100\ \% \quad (3)$$

2.5. GC-MS Analysis
GC-MS analysis of the extracted oil was carried out using Hewlett–Packard 6890 gas chromatograph coupled to a 5973A mass spectrometer, comprised of two fused-silica-capillary columns with different stationary phases. HP5MS™ was used as a non-polar column (30 m length, 0.25 mm diameter and 0.25μm film thickness) and Stabilwax ™ as polar column consisting of Carbowax™-PEG (60 m length, 0.25 mm diameter and 0.25μm film thickness). The following conditions were used to obtained GC-MS spectra: Helium as the carrier gas; flow rate of 1.0 ml/min; split ratio of 1:50; injection volume of 1.0μL; injection temperature of 300 ºC; oven temperature progress from 100 to 250 ºC at 10 ºC/min; the ionization mode used was electronic impacted at 70 eV. The identification of chemical compounds in the patchouli oil was performed by comparing the mass spectral fragmentation patterns with those stored in MS database (National Institute of Standards and Technology and Wiley libraries).

2.6. SEM Analysis
SEM analysis of raw dried patchouli leaves, residue of patchouli sample where highest yield was obtained for MAHD and HD method. The samples were fixed on the specimen holder and then sputtered with gold in a sputter coater (BAL-TEC SCD 005, Balzers, Switzerland). All the specimens were examined with a Philips XL 30 scanning electron microscope under high vacuum condition and at an accelerating voltage of 20.0 keV and at a working distance of 8–9 mm.

2.7. Statistical Analysis
Each experimental run was performed in triplicate, and average values were recorded as mean ± standard deviation.

3. Results and Discussion

3.1. Effect of Solvent Volume on the Patchouli Oil Yield
Figure 2 shows the results obtained for the influence of varying solvent volume (180-420 ml) on patchouli oil yield. The microwave power and extraction time were fixed at 400 W and one hour for MAHD, respectively. However, the extraction time was fixed at three hours for HD method. From Figure 2, the highest yield of patchouli oil was acquired when 360 ml of solvent (distilled water) was used. At ratio of 12:1 ml/g, the highest yield of oil was extracted for both MAHD and HD methods. However, when using MAHD, the yield of patchouli oil obtained was higher (5 %) compared to HD (4 %). Higher volume of solvent caused more heat absorption that transferred to plant samples; hence, higher yield can be extracted from the oil gland [23]. In addition, solvent act as a pumping agent that can extract the essential oil from oil glands of the plant sample. [24] had reported 1.9437 % of the yield of essential oil obtained at the solid to solvent ratio of 1:10 g/ml using microwave extraction. The oil yield obtained in this current study (5 % at ratio of 12:1 ml/g) was higher compared to the previous study. The yield of patchouli oil increased when solvent volume increased. Although the volume of extracted essential oil from both extraction methods were different, the composition percentage of oils were similar (Table 1). The number of compounds identified when using both extraction methods depend on the ability of the extraction technique to extract the bioactive compounds from the oil glands. However, beyond 360 ml of solvent, the yield of patchouli oil declined because once the solvent hit the maximum volume use, each extraction technique could reach maximum oil yield [25].

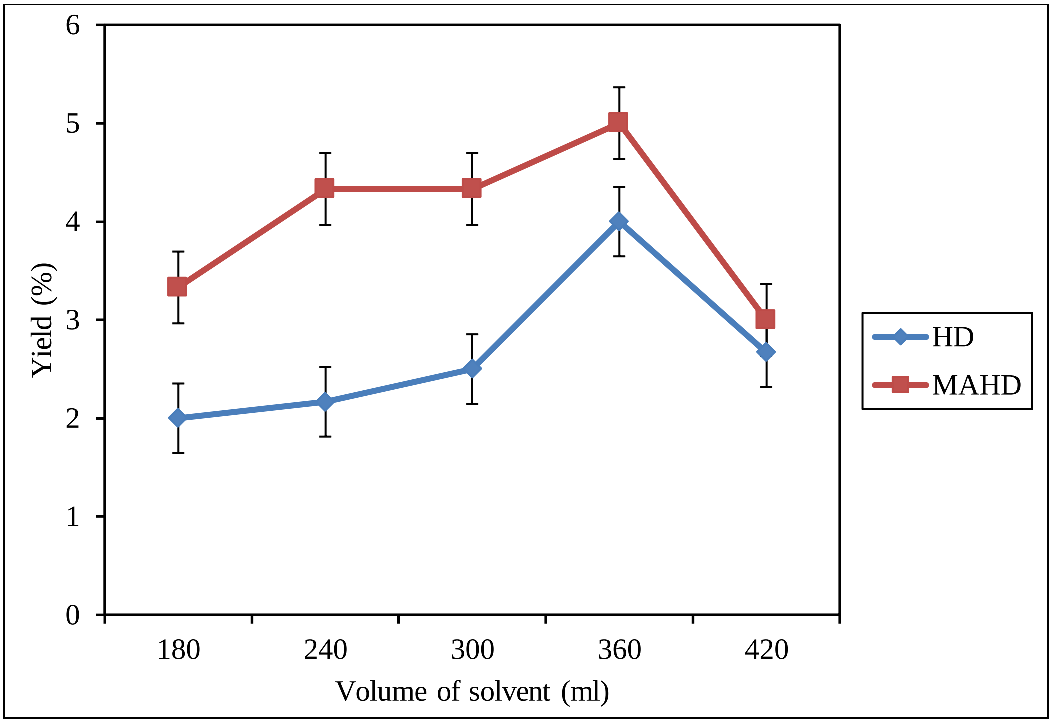

Figure 2. Yield of patchouli oil against volume of solvent (ml).

3.2. Effect of Extraction Time on the Patchouli Oil Yield

Figure 3 shows the effect of extraction time between 15-60 min on the yield of patchouli leaves at fixed microwave power of 400 W and solvent to solid ratio of 12:1 ml/g for MAHD and extraction time of three hours at fixed solvent to sample ratio of 12:1 ml/g for HD. According to [11], 4 hours and 2 hours of extraction time used for HD and MAHD methods respectively to extract essential oil from *Thymus vulgaris* L. Although 2 hours of extraction time was used for MAHD by [11], in this study, 1 hour of extraction time was used because all the water in the flask was evaporated and the extraction was stopped after 60 minutes of extraction. On the other hand, after 3 hours of extraction of patchouli oil using HD method, there is no essential oil extracted as shown in Figure 3. These are due to the difference of dielectric constants of the solvent and the sample [15]. The highest yield of patchouli oil was obtained at 45 minutes for both MAHD and HD. It can be obviously observed that the yield acquired utilizing MAHD was higher (2.33 %) compared to HD (0.9 %). This might be because of the different heat and mass gradients in the extraction techniques [15]. In MAHD, the heat and mass gradients work in the similar direction, whereas in HD, the gradients are working in opposite directions. The same direction of heat and mass gradients can heat up the samples faster and simultaneously, hence the amount of patchouli oil extracted by MAHD is greater than conventional hydrodistillation [15]. Yield of patchouli oil decreases after 45 minutes where the yield of oil from MAHD decreases from 2.33% to 1% and oil from HD goes from 0.9% to 0.43% at 60 minutes of extraction. Longer extraction time can degrade heat sensitive bioactive compounds, hence decreases the yield obtained. Exposure to high temperature for a longer time also can vaporize the solvent and eventually lower the yield of patchouli oil [26].

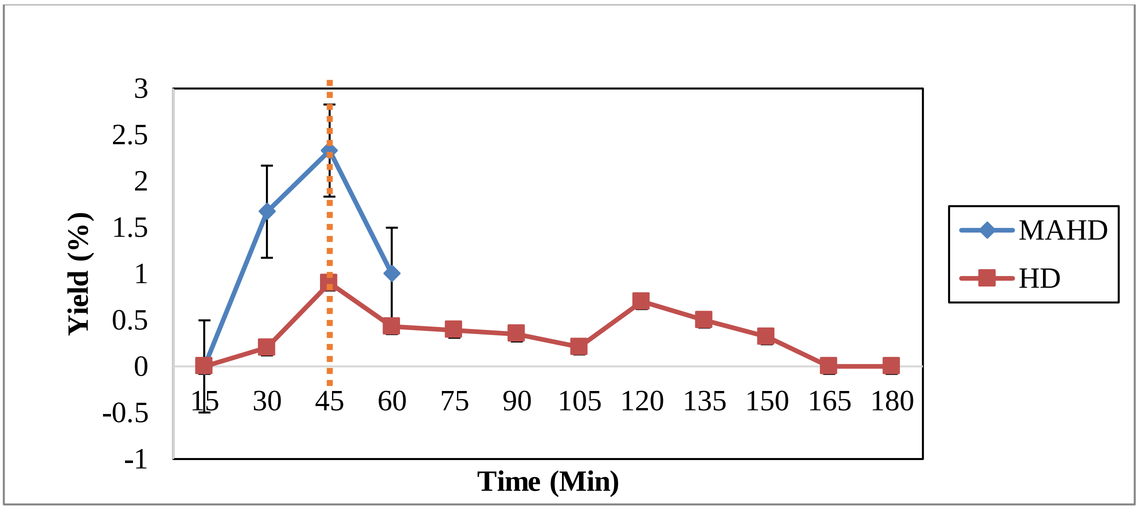

Figure 3. Yield of patchouli oil versus Time (min).

3.3. Composition of Oil from Patchouli Leaves

Patchouli essential oil at the optimal conditions was analyzed using GC-MS to identify the bioactive compounds in the oil. Table 1 illustrates the results obtained for both extraction methods. Sixteen chemical compounds were identified using MAHD while eleven chemical compounds were identified using HD. Oil obtained through HD contained higher contents of sesquiterpenes while oil obtained through MAHD packed with oxygenated compounds. This is because the heat transfer in MAHD is from the oil gland to the surrounding solvent while the heat transfer in HD is vice versa [15]. Hence, maximum quantity of essential oil can be extracted from patchouli oil gland using MAHD. Since the higher amount of essential oil can be extracted utilizing MAHD technique, higher number of oxygenated compounds were recognized in the oil. Patchoulol is the main oxygenated compound extracted from patchouli leaves. Moreover, the higher the number of oxygenated compounds, the higher the quality of essential oil. MAHD method shows a greater probability in the production of natural fragrance of the patchouli oil. Thus, MAHD method could be a better alternative extraction technique for the extraction of essential oil.

Table 1. Chemical compositions of patchouli oil extracted from patchouli leaves through HD and MAHD using GC-MS analysis

No.	Compounds	Molecular Formula	Area (%)	
			HD	MAHD
Sesquiterpenes				
1	δ-Elemene	$C_{15}H_{24}$	nd	0.2
2	β-caryophellene	$C_{15}H_{24}$	4.94	4.64
3	α-Gurjunene	$C_{15}H_{24}$	1.45	11.01
4	Aromandendrene	$C_{15}H_{24}$	nd	0.25
5	Valencene	$C_{15}H_{24}$	nd	1.91
6	β-Patchoulene	$C_{15}H_{24}$	2.4	2.6
7	β-Elemene	$C_{15}H_{24}$	1.17	nd
8	α-Patchoulene	$C_{15}H_{24}$	5.4	nd
9	Selina-3,7(11)-diene	$C_{15}H_{24}$	nd	0.7
10	α-Guaiene	$C_{15}H_{24}$	15.94	12.2
11	β-Maaliene	$C_{15}H_{24}$	nd	0.74
12	δ-Guaiene	$C_{15}H_{24}$	25.06	17.5
13	Seychellene	$C_{15}H_{24}$	7.1	8.3
14	β-Chamigrene	$C_{15}H_{24}$	3.72	nd

No.	Compounds	Molecular Formula	Area (%)	
			HD	MAHD
Oxygenated terpenes				
15	Iso-. alpha. -cedren-15-al	$C_{15}H_{22}O$	nd	0.4
16	Patchoulol	$C_{15}H_{26}O$	27.1	26.8
17	Hexahydrothunbergol	$C_{20}H_{40}O$	nd	0.05
18	Aromandendrene oxide	$C_{15}H_{24}O$	nd	2.78
19	2,6,6-Trimethyl-cyclohex-2-en-1-yl	$C_{14}H_{24}O$	0.3	nd
20	Viridiflorol	$C_{15}H_{26}O$	nd	6.1
Total non-oxygenated compounds (%)			67.18	60.05
Total oxygenated compounds (%)			27.4	36.13
Total identified (%)			94.58	96.18

3.4. Structural Changes Before and After Extraction Processes

The morphological images of the powdered patchouli leaf before and after extraction using SEM analysis are shown in Figure 4. In Figure 4, the oil glands were identified and circled. The vegetal cell of patchouli leaf was closely packed before the extraction as shown in Figure 4(a). The morphological variations in the cell walls after MAHD and HD are illustrated in Figures 4(b) and 4(c), respectively. It can be observed that the oil gland had undergone complete rupture at 60 min of MAHD, whereas, a partial rupture was observed using HD at 180 min of extraction time. These reflected that higher quantity of essential oil can be extracted in shorter time using MAHD method. During the MAHD extraction, the microwave energy is transferred into oil gland and converted into heat energy. The heat energy then transferred to plant samples to heat up the plant matrix to expel oil from the gland. Furthermore, heat is being transferred through three ways: conduction, convection and radiation. Hence, the oil glands get heated faster and essential oil can be extracted completely [4]. However, the heat energy will first reach the surface of the solvent before being transferred to plant samples in a conventional HD. In HD, the heat is transferred through conduction and convection. So, it will take longer time for the oil gland to be heated and quantity of essential oil extracted will be less as compared to MAHD method.

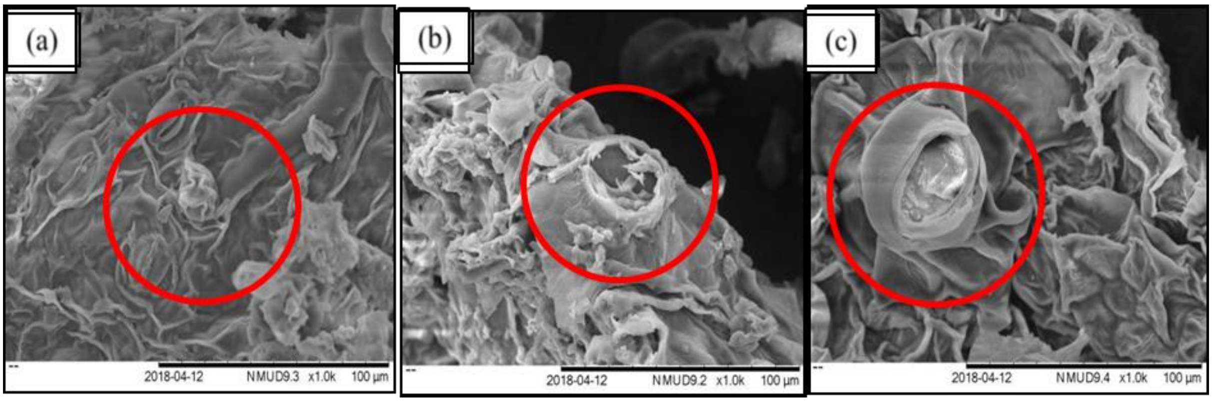

Figure 4. Scanning electron micrographs of patchouli samples; (a) raw, (b) after MAHD for 60 min and (c) after HD for 180 min.

4. Conclusion

This study investigated the potential of MAHD as compared to conventional HD method in extracting essential oil from patchouli leaves. The results showed that higher oil yield of 5 % was obtained from patchouli leaves in 60 min of extraction time as compared to HD (4 % of oil) in 180 min using a solid to solvent ratio of 1:12 (Figure 2). Moreover, higher number of chemical compounds were observed in the oil through MAHD as compared to HD method. 16 bioactive compounds were extracted using MAHD and 11 compounds were obtained using HD, still the percentage of identified compounds were not too distinct between both methods. The yield of patchoulol when using MAHD and HD extraction techniques were 26.8% and 27.1% respectively. In addition, SEM results confirmed that higher recovery of oil through MAHD as compared to HD method was due to complete rupture of oil glands in the plant matrix. Thus, MAHD showed a greater potential for better production of the essential oil from patchouli leaves as compared to HD method. In addition, MAHD can be proposed industrially as an alternative technique to separate essential oil in a larger scale.

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Appendix
Microwave-assisted hydrodistillation

a) Effect of volume of solvent

Volume of solvent (ml)	Yield of Patchouli oil (ml)	Yield of Patchouli Oil (g)	Yield of Patchouli oil (%)
180	1.04	1.0	3.33
240	1.35	1.3	4.33
300	1.35	1.3	4.33
360	1.56	1.5	5
420	0.93	0.9	3

b) Effect of time of extraction

Time of extraction (min)	Yield of Patchouli oil (ml)	Yield of Patchouli Oil (g)	Yield of Patchouli oil (%)
15	0	0	0
30	0.52	0.5	1.67
45	0.73	0.7	2.33
60	0.31	0.3	1

Hydrodistillation

a) Effect of volume of solvent

Volume of solvent (ml)	Yield of Patchouli oil (ml)	Yield of Patchouli Oil (g)	Yield of Patchouli oil (%)
180	0.62	0.6	2
240	0.68	0.65	2.167
300	0.78	0.75	2.5
360	1.25	1.2	4
420	0.83	0.8	2.67

b) Effect of time of extraction

Time of extraction (min)	Yield of Patchouli oil (ml)	Yield of Patchouli Oil (g)	Yield of Patchouli oil (%)
15	0	0	0
30	0.062	0.06	0.2
45	0.28	0.27	0.9
60	0.135	0.13	0.43
75	0.121	0.12	0.39
90	0.11	0.11	0.35
105	0.065	0.063	0.21
120	0.22	0.21	0.7
135	0.156	0.15	0.5
150	0.1	0.096	0.32
165	0	0	0
180	0	0	0