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Chemical Compound Characterizations of Patchouli Leaf Extract via GC-MS, LC-QTOF-MS, FTIR, and 1H NMR

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***Abstract*— The demand for patchouli (*Pogostemon cablin)* essential oil is increasing globally due to its diverse importance in the nutraceutical and pharmaceutical industries. The oil from this plant is an embodiment of different varieties of chemical compounds. It is then important to identify the bioactive compounds in the extracted oil to identify the embedded potential uses of the oil. Hence, this study focused on the characterization of extracted patchouli oil through microwave-assisted hydrodistillation (MAHD) technique using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry quadrupole time of flight (LC-Q-TOF-MS), nuclear magnetic resonance (NMR), and Fourier transform infrared transmission (FTIR). The obtained results reflected that patchouli leaf oil is endowed with appreciable quantities of non-oxygenated and oxygenated compounds (from GC-MS analysis); 18 tentatively identified phenolic compounds from LC-Q-TOF-MS analysis; several peaks showing the presence of O-H stretching in alcohol, C=O stretching vibration of carbonyl aldehyde, C-H bending stretching of alkane, and C=C stretching from aromatic rings were identified from the FTIR analysis. Additionally, the NMR results reflected the abundance of patchouli alcohol in the oil which can contribute to its aroma. Thus, patchouli leaf oil is an important plant endowed with different bioactive compounds.**

Keywords*— *Bioactive compounds; Characterization; Microwave-assisted hydrodistillation; Patchouli; Gas-chromatography mass spectrometry; Nuclear magnetic resonance

I. INTRODUCTION

Patchouli leaves (*Pogostemon cablin*), a plant belonging to the Lamiaceae family is endowed with essential oil [1]. This plant is usually cultivated in a tropical area particularly southeast Asia [2]. It is an aromatic plant that possesses higher content of essential oil that emanates from its young twigs and leaves. Patchouli oil is a dark orange or brownish colored liquid with a woody, earthy and camphoraceous odour [3]; [1]; [2]. The oil plays an important role in the production of perfumery products such as soaps, cosmetics products and detergents because it possesses a long-lasting odour with fixative properties [4]. Moreover, the patchouli plant is used in traditional Asian medicine as an anti-stress, antiseptic, relieve headaches and fever [5]. Several therapeutic activities of patchouli oil had previously been reported, including antifungal, anti-depressive, anti-bacterial, anti- inflammatory, sedative, febrifuge, and diuretic [4]; [1].

The composition of patchouli essential oil is complex and unique in relation to other essential oils [4]; however, it is distinct due to the presence of sesquiterpenes. Patchoulol is one of the main constituents of this oil and a primary compound accountable for its fragrance [6]. Patchoulol acts as fragrance binder to give long-lasting characteristic for the fragrance as compared to patchoulol, α-himachalene, α-/β-/γ-patchoulenes, seychellene, and α-guaiene [4]; [5]. Moreover, a report had illustrated that patchoulol and α-patchoulene are responsible for the oil aroma [4]. Hence, this can increase the trading values of this oil if patchoulol and α-patchoulene are present in larger amounts. Conventionally, the essential oil is obtained from a plant matrix through hydrodistillation technique. However, this technique had been reported to take a longer period of extraction, consumed a larger amount of energy with a potential of degenerating the bioactive compounds in the oil. Thus, employing a modern technique of extraction is important to fill up the shortcomings of hydrodistillation technique. The microwave-assisted hydrodistillation extraction technique is being employed due to its fast start-up, efficient heating and faster energy transfer [7]. In addition, characterization of plant extract/oil is essential to tentatively identify the embedded bioactive compounds. Several characterization techniques are being employed to tentatively identify the chemical compounds, these include gas chromatography-mass spectrometry (GC-MS), gas chromatography (GC), liquid chromatography-mass spectrometry quadrupole time of flight (LC-Q-TOF-MS), Fourier transform infrared transmission (FTIR), nuclear magnetic resonance (NMR), and among others.

Although, the bioactive compounds in the patchouli oil had previously been reported [5]; [8], however, comprehensive tentative characterization using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry quadrupole time of flight (LC-Q-TOF-MS), nuclear magnetic resonance (NMR), and Fourier transform infrared transmission (FTIR) is yet to be reported. Thus, this study focuses on the characterization of oil from patchouli leaves oil to disclose the embedded bioactive compounds.

II. MATERIALS AND METHODS

A. Raw material and chemicals

Dried samples (patchouli leaves) were obtained from Gaya Naturals company, Tawau, Sabah. The dry leaves were separated from stems and further dried at room temperature for several days. Then, the patchouli leaves were blended into powder form. Anhydrous sodium sulphate and

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dichloromethane utilized in this study were obtained Faculty of Chemical and Natural Resources Engineering laboratory.

B. The procedure of essential oil extraction using MAHD

A modified domestic microwave extractor comprising power and time control was used to extract oil from patchouli leaves. Fig. 1 shows the set up for the MAHD extractor for this study. A 30 g of patchouli powder was mixed with 180 mL of distilled water in a round-bottomed glass chamber. The microwave was operated at 400 W for 1 h. After 1 h, the hydrosol (water + essential oil) was collected from the Clevenger. Then, the hydrosol was placed in a separating funnel by adding few drops of dichloromethane to separate the essential oil from water. Thereafter, anhydrous sodium sulphate was added to eliminate any trace of water in the extracted oil. The obtained oil was then stored in a vial at -4º C for further analysis. The experiment was repeated for 240, 300, 360, and 420 mL of distilled water.

C. Liquid chromatography-mass spectrometry (LC-Q-TOF-MS) analysis

The chemical constituents in the patchouli essential oil were identified using LC-Q-TOF- MS (Waters, USA). LC-Q-TOF-MS has higher sensitivity and selectivity in characterizing and identifying the chemical compounds in the extracted oil of patchouli leaf when compared with other characterization methods. The patchouli oil was diluted using analytical grade ethanol to prepare a concentration of 100 mg/mL. Then, the concentration of the oil is further re-diluted to obtain 20 ppm prior to injection into the mass spectrometer. The QTOF-MS instrument was operated under the following conditions viz; desolvation flow rate (800 L/h), desolvation temperature (550 °C), operation mode (+ve and – ve mode), scan time (0.200 s to 4.00 min), ms mode (high definition), collision energy interval (4.00-45.00 eV), and scanning range (100-1000 m/z).

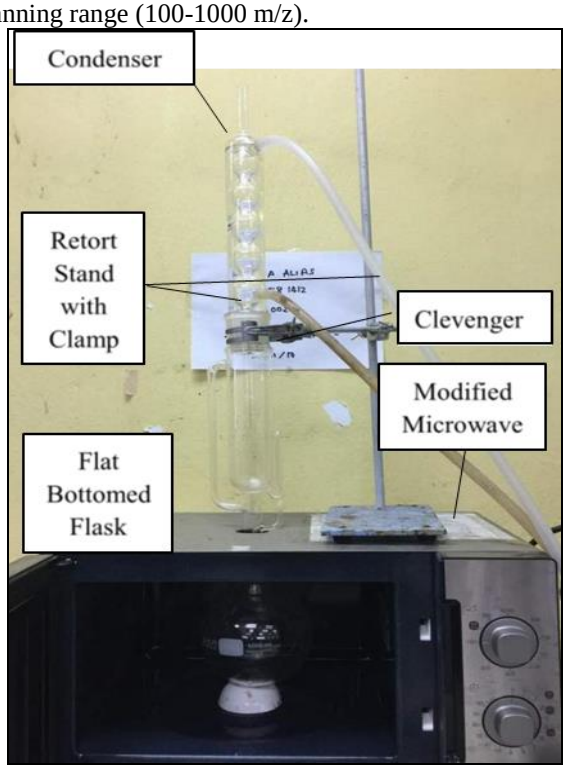

Fig. 1. MAHD set-up

D. Fourier transform infrared transmission (FTIR) analysis

Fourier transform infrared transmission was utilized to recognize functional groups in the patchouli leaf oil. This analysis was carried out to determine the bonding structures present in the essential oil by studying the position of peaks in the IR spectra. The IR spectra were acquired by utilizing an FTIR spectrometer (Nicolet iS5 iD7 ATR; Thermo Scientific, Germany) equipped with OMNIC software. The wavenumber ranging from 4000-500 cm-1 was used to analyze the oil.

E. Nuclear magnetic resonance (NMR)

1H NMR spectra were recorded on a Bruker AMX500 spectrometer operating at 500 MHz for the proton nucleus at room temperature. The patchouli oil samples were used to obtain 1H NMR spectra with the following acquisition parameters: Acquisition time 3.75 s, 16 scans, 10 s D1, spectral width 4370.63 Hz, and FID resolution 0.133Hz. Phase correction and baseline correction were manually performed.

III. RESULTS AND DISCUSSIONS

A. Identified chemical compounds through GC-MS analysis

Patchouli essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS) to identify the chemical compounds in the oil (Table 1). A total number of twenty-nine chemical compounds were identified. The oil reflected about 60.25% non-oxygenated and 39.49%

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Table 1 List of chemical compounds in patchouli essential oil using GC-MS analysis

Number	Compounds	Molecular Formula	Mass percentage of chemical compounds in crude extracted (%)
Oxygenated Terpenes			
1	Patchouli alcohol	$C_{15}H_{26}O$	26.25
2	3-Ethylphenol	$C_8H_{10}O$	6.69
3	Aristol-9-en-8-one	$C_{15}H_{22}O$	1.61
4	Cashmeran	$C_{14}H_{22}O$	1.49
5	Ethyl chrysanthemate	$C_{12}H_{20}O_2$	0.83
6	β-Pinone	$C_9H_{14}O$	0.6
7	Turmerone	$C_{15}H_{20}O$	0.48
8	5-Isopropenyl-1,2-dimethylcyclohex-2-enol	$C_{11}H_{18}O$	0.44
9	2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	0.42
10	2-(2-Methylpropylidene)-1H-indene-1,3(2H)-dione	$C_{13}H_{12}O_2$	0.29
11	Corymbolone	$C_{15}H_{24}O_2$	0.28
12	2-(2-Furyl)-1,3-thiazolidine	C_7H_9NOS	0.11
13	2-Ethylphenol	$C_8H_{10}O$	nd
14	3,4-Dimethyl-3-cyclohexene-1-carboxaldehyde	$C_9H_{14}O$	nd
15	4-Fluoro-3-nitrotoluene	$C_7H_6FNO_2$	nd
16	2-Propenoic acid, 6-methylheptyl ester	$C_{11}H_{20}O_2$	nd
Sesquiterpenes			
17	Valencene	$C_{15}H_{24}$	13.29
18	Aromadendr-1-ene	$C_{15}H_{24}$	11.86
19	Azulene	$C_{15}H_{24}$	11.76
20	β-Patchoulene	$C_{15}H_{24}$	9.57
21	Aromandendrene	$C_{15}H_{24}$	3.61
22	Patchoulene	$C_{15}H_{24}$	2.96
23	Dehydroaromadendrene	$C_{15}H_{22}$	1.65
24	Caryophyllene	$C_{15}H_{24}$	1.43
25	Longifolene	$C_{15}H_{24}$	1.23
26	alpha-guaiene	$C_{15}H_{24}$	0.61
27	Guaiazulene	$C_{15}H_{18}$	0.47
28	o-Xylene	C_8H_{10}	0.39
29	m-Xylene	C_8H_{10}	0.36
30	Cyclopentane-3'-spirotricyclo [3.1.0.0(2,4)] hexane-6'-spirocyclopentane	$C_{14}H_{20}$	0.33
31	3,4-Dimethylstyrene	$C_{10}H_{12}$	0.16
32	Naphthalene, 6-butyl-1,2,3,4-tetrahydro-	$C_{14}H_{20}$	0.05
33	γ-Gurjunene	$C_{15}H_{24}$	nd
34	Guaia-3,9-diene	$C_{15}H_{24}$	nd
35	Cyclohexane	$C_{15}H_{24}$	nd
36	Benzene, (2-methyl-1-propenyl)-	$C_{10}H_{12}$	nd
37	5-(3-Fluorophenyl)-2H-tetrazole	$C_7H_5FN_4$	nd
38	(-)-Tricyclo [6.2.1.0(4,11)] undec -5-ene,1,5,9,9 tetramethyl – (isocaryophyllene-I1)	$C_{15}H_{26}$	nd
39	Bicyclo [4.2.0] oct-1-ene, exo-7-(1-cyclohexen-1-yl)-	$C_{14}H_{20}$	nd
40	Clovene	$C_{15}H_{24}$	nd
41	γ-Selinene	$C_{15}H_{24}$	nd
42	alpha-Himachalene	$C_{15}H_{24}$	nd
43	1,4-Dimethyladamantane	$C_{12}H_{20}$	0.52
Total non-oxygenated compounds (%)			60.25
Total oxygenated compounds (%)			39.49
Total identified (%)			99.74

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Table 2 Phenolic compounds in patchouli leaf oil

No.	Observed RT (min)	Component name	Chemical formula	Observed (m/z)	Response	Adducts	Total fragment found
1	5.42	Tellimagrandin II	C41H30O26	983.1007	4645	+HCOO	23
2	5.53	Pedunculagin	C34H24O22	783.0700	3582	-H	41
3	7.29	Furosin	C27H22O19	695.0756	4773	+HCOO	71
4	5.82	Kukoamine A	C28H42N4O6	529.3018	4237	-H	11
5	6.02	1,2,3,4,6-Penta-O'Galloyl-β-D'Glucopyranoside	C41H32O26	985.1175	7074	+HCOO	21
6	6.57	Geraniin	C41H28O27	951.0737	5234	-H	55
7	6.64	Casuarinin	C41H28O26	981.0840	4895	+HCOO	28
8	6.84	Terchebin	C41H30O27	953.0916	4723	-H	64
9	7.69	Mallotinic acid	C34H26O22	831.0899	3684	+HCOO	46
10	0.51	Schizonepetoside E	C16H28O8	349.1838	428455	+H	7
11	1.45	Xanthumin	C17H22O5	307.1520	5614	+H	0
12	3.89	Sonchuside A	C21H32O8	435.1989	176863	+Na, +K	34
13	4.41	Hookeroside C	C38H62O15	781.3982	110716	+Na	16
14	5.45	2-Hydroxyesculentic acid	C30H46O7	541.3127	290124	+Na	48
15	6.13	11-Oxo-kansenonol	C30H46O4	493.3288	813628	+Na	30
16	7.02	Akebonoic acid	C29H44O3	463.3179	420054	+Na	30
17	7.51	3α,30-Dihydroxylup-20 (29)-en-27-oic acid	C30H48O4	495.3443	73459	+Na	19
18	3.28	2β-Hydroxyilicic acid	C15H24O4	291.1569	77053	+Na	5

oxygenated compounds. This is because the heat transfer in MAHD is generated from the oil gland to the surrounding solvent [9]. Hence, the maximum quantity of essential oil can be extracted from patchouli oil gland using MAHD. 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 higher probability in the production of natural aroma of the patchouli oil.

B. Identified chemical compounds through LC-Q-TOF-MS analysis

The phenolic compounds in the patchouli leaf oil were analyzed using LC-Q-TOF-MS. A total of 18 phenolic compounds were identified as shown in Table 2. Tannins, phenolic alkaloids, flavonoids, sesquiterpenoids lactones, and triterpenoids are the phenolic compounds found in the patchouli oil. From the identified compounds, eight chemical compounds are tannins. Tannins are the complex mixture of polyphenol which can act as an antioxidant [10], anti-inflammatory [11] and anti-microbial [12]. However, 1,2,3,4,6-Penta-O' galloyl-β-D'glucopyranoside is a compound reported to possess anti-cancer [13] and anti-tumor effects [14]. Geraniin has good radical scavenging activity [15] while Casuarinin possesses anti-oxidant properties that could inhibit the growth of T24 bladder cancer cells [15]. Moreover, Kukoamine A is a phenolic alkaloid reported to possesses natural anti-oxidant properties with mechanisms involving free radical scavenging [15]. Other pharmaceutical effects such as anti-hypertension, anti-analgesic, anti-inflammatory, antisepsis, and enhancing autoimmune still abound [16].

C. Identified chemical compounds through FTIR analysis

Fig. 2 illustrated the FTIR spectra for patchouli oil obtained from MAHD method. The infrared spectroscopy (IR) characteristics fingerprint peaks for patchouli oil falls within the range of 3400-800 cm-1. These spectra show that there is an overlap of the absorption spectrum of different components in the oil because patchouli essential oil is a complex mixture of volatile oils [17]. There are some observable peaks from MAHD spectrum. The peak at 3313 cm-1 represents O-H stretching in alcohol [18] which shows the abundance of patchoulol, an important material in perfumery. Another observable peak at 1635 cm-1 corresponds to C=O stretching vibration of carbonyl aldehyde [17]. It proves that patchouli oil contains higher amounts of aldehyde compounds. The peak at 1445 cm-1 can be attributed to C-H bending stretching in alkane [19] and C=C stretching from aromatic rings while the peak at 1373 cm-1 is a characteristic of O-H bending in a carboxylic acid [18]. The peak at 886 cm-1 is a representation of C-H bending vibration [17]. Summary of major peaks and their representation are listed in Table 3. From Table 3, five functional groups were identified from the MAHD spectrum.

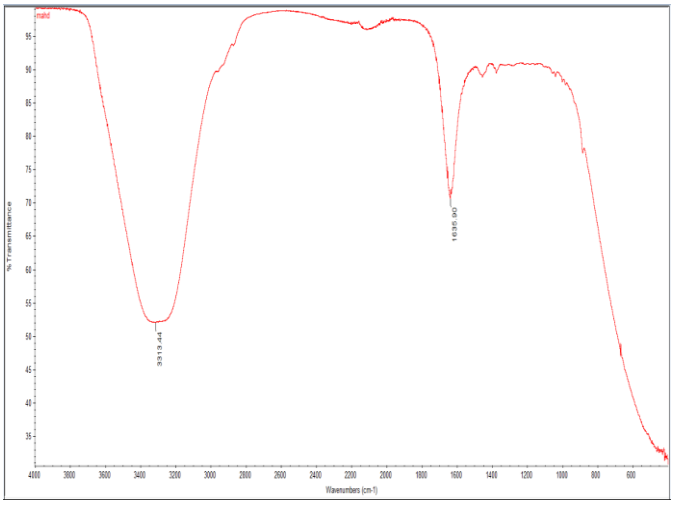

Fig. 2. FTIR spectrum

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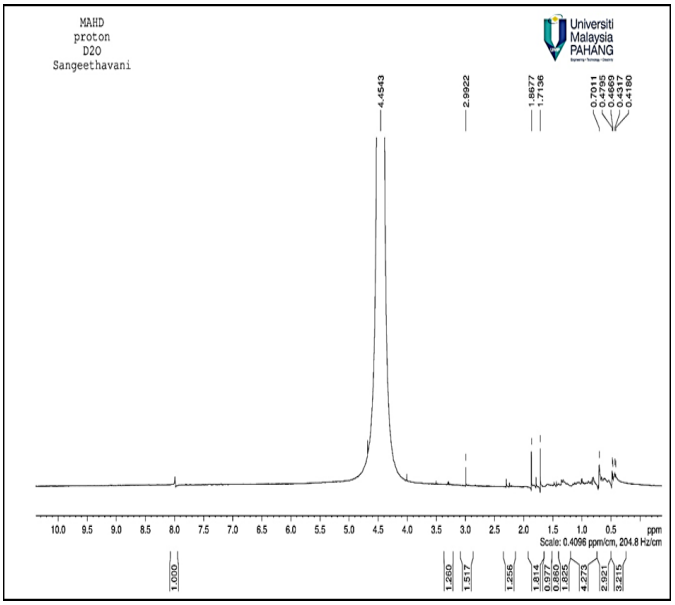
Table 3 Summary of important peaks and their representation

Functional group representation	Position of bands (cm^{-1})
C-H bending vibration	886
O-H bending	1373
C-H bending stretching alkane and C=C stretching aromatic	1445
C=O stretching vibration and N-H bending	1635
O-H stretching	3313

D. Identified chemical compounds through NMR analysis

The representative one-dimensional 1H NMR spectra of patchouli leaf oil are shown in Fig. 3. The vertical scale B was magnified for better visibility. Table 4 shows the chemical shifts from both NMR spectrum A and B with their protons. The chemical shift at 0 ppm is the reference point. The highest peak in the NMR spectrum A which is at 4.4543 ppm represents the alcohol group [20]. This proves the abundance of patchouli alcohol in patchouli leaves extract which contributes to the aroma of patchouli essential oil [21]. The peak of 8.0 ppm is characterized by the signals of aryl group (patchoulene, azulene and valencene) which play an important role in aroma [20]; [22] and act as anti-inflammatory [23], anti-ulcer [23]; [24], and anti-diabetic [24].



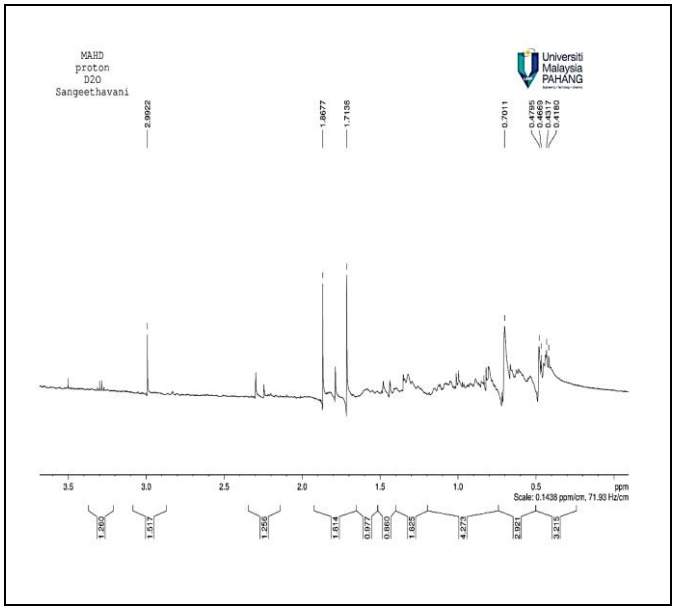


Fig. 3. 1H-NMR spectrum of ethanol–water extract of Patchouli essential oil. Vertical scale in B is magnified with respect to A.

Table 4 The chemical shifts with their protons

Chemical Shift, δ (ppm)	Protons
0.7011	$R\text{-}CH_3$
1.7136	R3C-CH
1.8677	=C-CH
2.9922	-C≡C-H
4.4543	HC–OH
8.0	Ar–H

IV. CONCLUSION

This study has successfully characterized the oil obtained from patchouli leaves through MAHD using GC-MS, LC-QTOF-MS, FTIR, and NMR. The obtained results have clearly indicated that the oil possessed about 60.25% non-oxygenated and 39.49% oxygenated compounds through GC-MS analysis; the LC-Q-TOF-MS results showed that the oil comprised 18 phenolic compounds which are majorly tannins, phenolic alkaloids, flavonoids, sesquiterpenoids lactones, and triterpenoids. Additionally, there was the presence of O-H stretching in alcohol indicating the abundance of patchoulol, C=O stretching vibration of carbonyl aldehyde showing that the patchouli oil contains higher amounts of aldehyde compounds, C-H bending stretching of alkane, and C=C stretching from aromatic rings. The NMR results indicated that the abundance of patchouli alcohol in the oil which can be contributed to the aroma. Thus, patchouli leaf oil is an important plant that is endowed with varieties of chemical compounds.

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