



EXTRACTION OF ESSENTIAL OIL *Citrus hystrix* BY USING ULTRASOUND-ASSISTED EXTRACTION

(Pengestrakan Pati Minyak *Citrus hystrix* Menggunakan Pengekstrakan Bantuan Ultrasonik)

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Abstract

Citrus hystrix is a famous aromatic plant that comprises biological properties such as antioxidant, antibacterial, antileukemic, and antitussive properties. Several conventional extraction methods like hydro-distillation, steam distillation and Soxhlet distillation have been used to extract essential oils (EOs), but they are time-consuming, expensive and necessitate the use of numerous solvents. Therefore, this study focused on the use of ultrasound-assisted extraction (UAE) which is a novel extraction method that has lower operating costs and shorter extraction time. UAE was performed to extract the EOs from *C. hystrix* peels by using methanol and petroleum benzene. The yield of extracted EOs were 9.43% and 2.36%, respectively. After extraction, a qualitative phytochemical analysis was carried out to detect chemical constituents in the EOs. Both EOs showed positive results in the tests of alkaloids and terpenoids. Besides, the surface morphology of fresh *C. hystrix* powder before extraction and the residual powder after extraction were compared by using a scanning electron microscope (SEM). SEM photographs of both residual powders revealed pores that appeared because of peel surfaces implosion which occurred during extraction. Functional groups at the configuration peaks were characterised by using Fourier transform infrared (FTIR) spectroscopy and chemical constituents in the EOs were analysed by using gas chromatography-mass spectrometry (GCMS). Results for both extracted EOs demonstrated the presence of OH stretching at $3625\text{--}3025\text{ cm}^{-1}$, CH_3 stretching at $2939\text{--}2834\text{ cm}^{-1}$, C=O stretching at $1721\text{--}1719\text{ cm}^{-1}$ and C=C stretching at $1624\text{--}1622\text{ cm}^{-1}$. The GCMS result indicated that the main chemical compounds of both EOs were d-limonene, citronellal, and terpinen-4-ol. According to the findings, UAE extraction method was recommended due to its environmental friendliness and short extraction time.

Keywords: *C. hystrix* peel, ultrasound-assisted extraction, essential oils, phytochemical screening

Abstrak

C. hystrix merupakan tumbuhan aromatik terkenal yang terdiri daripada sifat-sifat biologi seperti sifat antioksidan, antibakteria, antileukemik dan antitusif. Beberapa kaedah pengekstrakan konvensional seperti penyulingan hidro, penyulingan wap, dan penyulingan soxhlet telah digunakan untuk mengekstrak minyak pati (EOs) namun memerlukan penggunaan banyak pelarut, memakan masa, dan mahal. Oleh itu, kajian ini tertumpu kepada penggunaan pengekstrakan bantuan ultrasonik (UAE) yang merupakan kaedah pengekstrakan novel yang mempunyai kos operasi yang lebih rendah dan masa pengekstrakan yang lebih singkat. UAE telah digunakan untuk mengekstrak EOs daripada kulit *C. hystrix* menggunakan metanol, dan petroleum benzene. Hasil kedua-dua EO yang diekstrak adalah 9.43% dan 2.36%. Selepas pengekstrakan, analisis fitokimia kualitatif telah dilakukan

untuk mengesan konstituen kimia dalam EOs. Kedua-dua EOs menunjukkan keputusan positif dalam ujian alkaloid dan terpenoid. Selain itu, morfologi permukaan serbuk *C. hystrix* segar sebelum pengekstrakan dan serbuk sisa selepas pengekstrakan telah dibandingkan dengan menggunakan pengimbas mikroskopi elektron (SEM). Gambar-gambar SEM bagi kedua-dua serbuk sisa menunjukkan liang yang hadir disebabkan oleh letupan permukaan kulit yang berlaku semasa pengekstrakan. Kumpulan-kumpulan berfungsi pada puncak konfigurasi telah dicirikan menggunakan spektroskopi inframerah transformasi Fourier (FTIR), dan jujuk-jujuk kimia dalam EOs dianalisis menggunakan kromatografi gas-spektrometri jisim (GCMS). Hasil kajian menunjukkan kehadiran regangan OH pada $3625\text{--}3025\text{ cm}^{-1}$, regangan CH_3 pada $2939\text{--}2834\text{ cm}^{-1}$, regangan C=O pada $1721\text{--}1719\text{ cm}^{-1}$, and regangan C=C pada $1624\text{--}1622\text{ cm}^{-1}$ untuk kedua-dua EO yang diekstrak. Data GCMS menunjukkan komposisi kimia utama bagi kedua-dua EOs adalah D-limonene, sitronelal, dan terpinen-4-ol. Berdasarkan penemuan, kaedah pengekstrakan UAE disyorkan kerana mesra alam dan masa pengekstrakan yang singkat.

Kata kunci: kulit *C. hystrix*, pengekstrakan bantuan ultrasonik, minyak pati, penyaringan fitokimia

Introduction

In Malaysia, 17,000 tonnes of food wastes are reported per day [1]. Fruit peels are one of the wastes produced. Nowadays, fruit peels are collected and processed to extract EOs, which are applied in food and beverage industries, cosmetic industries and pharmaceutical industries. *C. hystrix*, or known as *kaffir lime*, is an aromatic plant part of the Rutaceae family with a green and bumpy peel. EOs of *C. hystrix* have bioactivities such as antioxidant, antibacterial, antileukemic and antitussive, which are functions in aromatherapy, insect repellent and additive in cosmetic products [2, 3].

Extraction methods such as hydro-distillation, steam distillation, and Soxhlet distillation [4] have been used to extract EOs from *C. hystrix* with a high yield production of EOs. However, the extraction period is quite long, and it requires a high operating cost [4]. The UAE extraction method extracts compounds from plants in a solvent by using soundwave at 20 kHz to 100 MHz frequency. Mass transfer occurs due to the ultrasound, which allows the solvent to access plant material cell components faster. Ultrasound also allows the process of erosion to occur, in which the cavitation bubbles produced by ultrasound will make the surface of plant tissues undergo implosion [5]. UAE is a beneficial method that has a short extraction time and high production yield [5]. It can provide a large yield of oil in a short extraction time by imploding cavitation bubbles caused by the ultrasound and triggering fissions at plant cell walls, whereby the percentage yield of oil obtained in 120 min of kaffir lime leaves was 1.75% and the percentage yield of oil obtained in 30 min of rapeseed oil was 21.36% [6, 7]. In this study, the peels of *kaffir lime* were employed to extract EOs with a high yield production in a short period by using UAE.

Solvent used in the extraction process is determined by the type and part of plant, bioactive chemicals of plant, compounds to be extracted from the plant, and

availability of solvent [8]. Different types of solvents have different polarities. The bonds between atoms in polar solvents contain very different electronegativities while the bonds between atoms in non-polar solvents contain similar electronegativities. Quantitative and qualitative extraction of plant compounds is affected by the polarity of solvent [9]. Therefore, the yield of EOs extracted from plants by using solvents of varying polarities depends on solubility of plant compounds with the solvent. Polar solvents such as methanol and ethanol are frequently used to extract polyphenols like antioxidant compounds from a plant [7]. Non-polar solvents such as *n*-hexane and petroleum ether are used to extract alkaloids, terpenoids, coumarins and fatty acids from a plant [8]. Purity and quality of the extracted compounds might be impacted because the polarity of a polar solvent like methanol could lead to an unintended chemical reaction, decomposition or degradation of the extracted compounds [8].

Phytochemicals are compounds present in plants which give colours, aromas and flavours, such as phenolics, flavonoids, terpenoids, alkaloids, steroids, proteins and tannins. The main phytochemical contained in *C. hystrix* peel is terpenoids such as β -pinene, limonene, citronellal and sabinene [10]. Terpenoids are the largest group found in natural products, which are known as oxygen-containing terpenes. A study by Soib et al. [11] showed that the UAE method could extract the same secondary metabolites from *Carica papaya* Linn leaves like reflux and agitation methods in terms of saponins, flavonoids, glycosides, alkaloids and phenolics. According to Pandhi and Poonia [12], several phytochemical compounds, including alkaloids, phenol, tannins and flavonoids were detected in Jamun seed extracts by using the UAE method, except saponins, phytosterols, carbohydrates, glycosides and protein. Another study by Mutalib [13] discovered that the UAE approach produced the same favourable findings in photochemical screening tests of *Rosmarinus officinalis*

leaf extracts as by using the conventional methods for extraction, such as maceration, decoction, infusion, regular reflux, and Soxhlet distillation. It can be concluded that the novel UAE extraction method has great possibility of extracting secondary metabolites from plant material with its unique advantages.

In this study, the UAE technique was applied to produce a high yield in a brief amount of time with a small peel-to-solvent ratio. The findings by Kasuan et al. [3] showed that a mere 1.87% of *kaffir lime* oil was extracted from 350g of peels in 10l solvent by using an automated steam distillation process at temperature of 80 °C in 75 min. Comparatively, in the present study a better yield of *kaffir lime* EOs was produced by using UAE method despite only 50 min of extraction time and a lower raw material-to-solvent ratio of 1:30 g/mL. The different polarity of solvents was utilised to investigate the effectiveness of different solvents to extract the compounds. Methanol (polar solvent) and petroleum benzine (non-polar solvent) were used in the extraction of EOs from *C. hystrix* with a ratio sample (g) to solvent (ml), was 1:30. Methanol was chosen to extract EOs from *C. hystrix* peels due to its high production yield and effectiveness in extracting phenolic, flavonoid, alkaloid and terpenoids. Petroleum benzine was used to extract EOs from *C. hystrix* peels due to its low boiling point and efficacy in removing alkaloids and terpenoids [8]. Continuously, the presence of alkaloids, protein and amino acids, phenolic compounds, saponins, tannins, flavonoids and terpenoids in the EOs was demonstrated by using phytochemical screening. The morphological properties of *C. hystrix* peels before and after extraction were examined by using SEM. The presence of functional groups in the EOs was confirmed by using FTIR. The analysis of the compounds in EOs extracted was conducted by using Gas chromatography-mass spectrometry (GC-MS).

Materials and Methods

Materials

Methanol (99.9 wt.%) (LabServ) and petroleum benzine (Supelco/Germany) were used as solvents. Sodium hydroxide (98%) (Emory), copper (II) sulfate pentahydrate AR ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (Emory), iron(III) chloride hexahydrate (Supelco), potassium sodium tartrate ($\text{C}_4\text{H}_4\text{KNaO}_6 \cdot 4\text{H}_2\text{O}$) Grade AR (QreC), potassium iodide (99.9%) (R&M Chemicals), iodine (PC Laboratory Reagent), sulfuric acid, chloroform, and hydrochloric acid were chemicals used in the phytochemicals screening testing.

Kaffir lime sample preparation

Fresh *kaffir lime* (*C. hystrix* DC) peels were purchased from a local supermarket located in Pagoh, Johor, Malaysia. The collected peels were washed several times with tap water to remove the remaining dust particles and impurities before the flesh were removed [10]. Following that, the washed peels were oven dried at 60 °C for 1 h to remove excess water [14]. The dried peels were blended until smooth and sieved by using an electric grinder to obtain uniform powders (Figure 1c) [15]. Then, the powders were weighed and sealed in an airtight container and stored at room temperature until ready to be used for extraction [16].

Extraction method

The extraction process was performed by using UAE method. Methanol was used as the polar solvent and petroleum benzine was used as the non-polar solvent for the extraction of *kaffir Lime* EOs. Then, 5g of powders were weighed and added into a conical flask which contained the solvents. The ratio of powder to solvent and extraction time was fixed at 1:30 g/mL and 50 mins [14, 17]. According to Liew et al. [14], the optimal raw material-to-solvent ratio for the extraction of kaffir lime peel was 1:30 g/mL. This was because when the volume of solvent increased, the contact area between solute and solvent increased, resulting in a greater mass transfer or driving force to extract bioactive compounds from the plant cells to solvent [14]. Then, the mixture sample was vortexed by using a vortex machine (Wiggins, Vortex Model 3000) at 800 rpm for 2 min as the pre-treatment of extraction. Then, the mixture was immersed into an ultrasonic bath (Wiggins Eco Series, Model UE10SFD) with ultrasonic power at 40 kHz (Figure 1e). The extracts were subsequently centrifuged at 20 °C with 3500 rpm for 20 min by using a centrifuge model (MPW-352R), as shown in Figure 1f. Then, the extracts were filtered to separate the solid residue from supernatant layers. The supernatant was concentrated by using a rotary evaporator (Eyela OSB-2200) equipped with a vacuum pump (Figure 1h) at 25 °C and 25 rpm by using a polar solvent [15]. Meanwhile, for the non-polar solvent extraction a reciprocal shaker water bath (Yihder, Model BT-150D) (Figure 1i) was used at 60 °C for 1 h [18]. Both extracts were concentrated by using different techniques because the boiling point of petroleum ether is lower than that of methanol; hence, it can be removed from EOs even with the water bath. To ensure that the EOs quality was consistent across all batches, the extraction process applied the same raw materials, sample preparation techniques, and UAE parameter conditions. The EOs were kept in a vial and stored at 4 °C until analysis [3].

Scanning electron microscope (SEM)

The surface morphology of fresh *kaffir lime* powder before extraction and residual powder after extraction were analysed by using SEM (Model EM-30N COXEM).

Fourier transform infrared spectroscopy (FTIR)

The existence of functional group in the extracted *kaffir lime* EOs was characterised by using FTIR spectrophotometer (Perkin Elmer UATR Two) under wavelength ranges between 400 cm^{-1} and $4,000\text{ cm}^{-1}$.

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS, GC Agilent® 7890B, MS Agilent® 5977B, Agilent Technologies, Santa Clara, CA, USA) was used to determine the chemical constituents of *kaffir lime* EOs. Firstly, the EOs were mixed with methanol in the ratio of 2:3. Head column pressures for the HP-5ms (30m x 0.25mm x 0.25µm film thickness) panel were 70kPa. GC-MS was acquired on the following conditions: Carrier gas He; flow rate 1.0 ml/min; split 1:100; injection volume 1.0 µl; injection temperature 280 °C; oven temperature progress included an initial hold at 70°C for 2 min, then increased to 7 °C/min to 320 °C for 1 min. The temperature of ion sources was maintained at 250 °C. The mass spectrum was obtained by electron ionisation at 70 eV, and the detector operated in scan mode 30 to 500 Da atomic units [18]. Chemical constituents of EOs were identified by comparing retention indices with mass spectral database libraries National Institute of Standards and Technology (NIST) [2]. Chromatograms from GC and GC-MS captured the existing sample oil constituents in terms of peak area count.

Phytochemical screening

Qualitative phytochemical analysis was performed to detect the presence of chemical constituents in extracted *kaffir lime* EOs, including alkaloids, flavonoids, terpenoids, saponins, tannins, phenols, and proteins as follows:

Test for alkaloids

10% KI was mixed with 5% iodine for the preparation of the iodine test reagent. Next, 2 to 3 drops of EOs were added to 1 mL reagent and then boiled in a water bath until the colour disappeared. The alkaloid content in the

sample was indicated by the formation of a blue-black colour once the mixture cooled down [19].

Test for flavonoids

The intense yellow colour was formed when a few drops of EOs were added to 2 mL of 2% of NaOH solution. Following that, a few drops of diluted HCl resulted in the solution turning colourless. The formation and disappearance of intense yellow colour during the alkaline reagent testing indicated the presence of flavonoids in the extract [4].

Test for terpenoids

1 mL EOs was mixed with 2 mL chloroform and shaken well, followed by the addition of a few drops of concentrated H_2SO_4 . Salkowski test showed a positive result when a reddish-brown colouration of the interface or golden-yellow precipitate was formed [8].

Test for saponins

1 mL of EOs was mixed with 3 mL of distilled water and the mixture was vigorously shaken for 30 s. If persistent foam or bubbles were formed for several minutes, it indicated the presence of saponins in the extract [20].

Test for tannins

A few drops of EOs were mixed with 5 mL of distilled water and then boiled in a water bath. Following that, the cooled filtrate was mixed with 5 mL of 1% w/v $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. A positive result showed a blue-green, green-black, dark blue, blue-black or dark brown formation [21].

Test for phenols

2 mL of 5% w/v aqueous $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution was mixed with a few drops of EOs. The formation of a blue colour indicated a positive result [19].

Test for proteins

1.5g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 6 g of $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$, and 2 M NaOH were mixed and then added with 1000 mL distilled water for the preparation of Biuret test reagent. Then, 2 to 3 drops of EOs were mixed with 1 mL of reagent. The formation of a purple solution indicated that the result was positive [22].

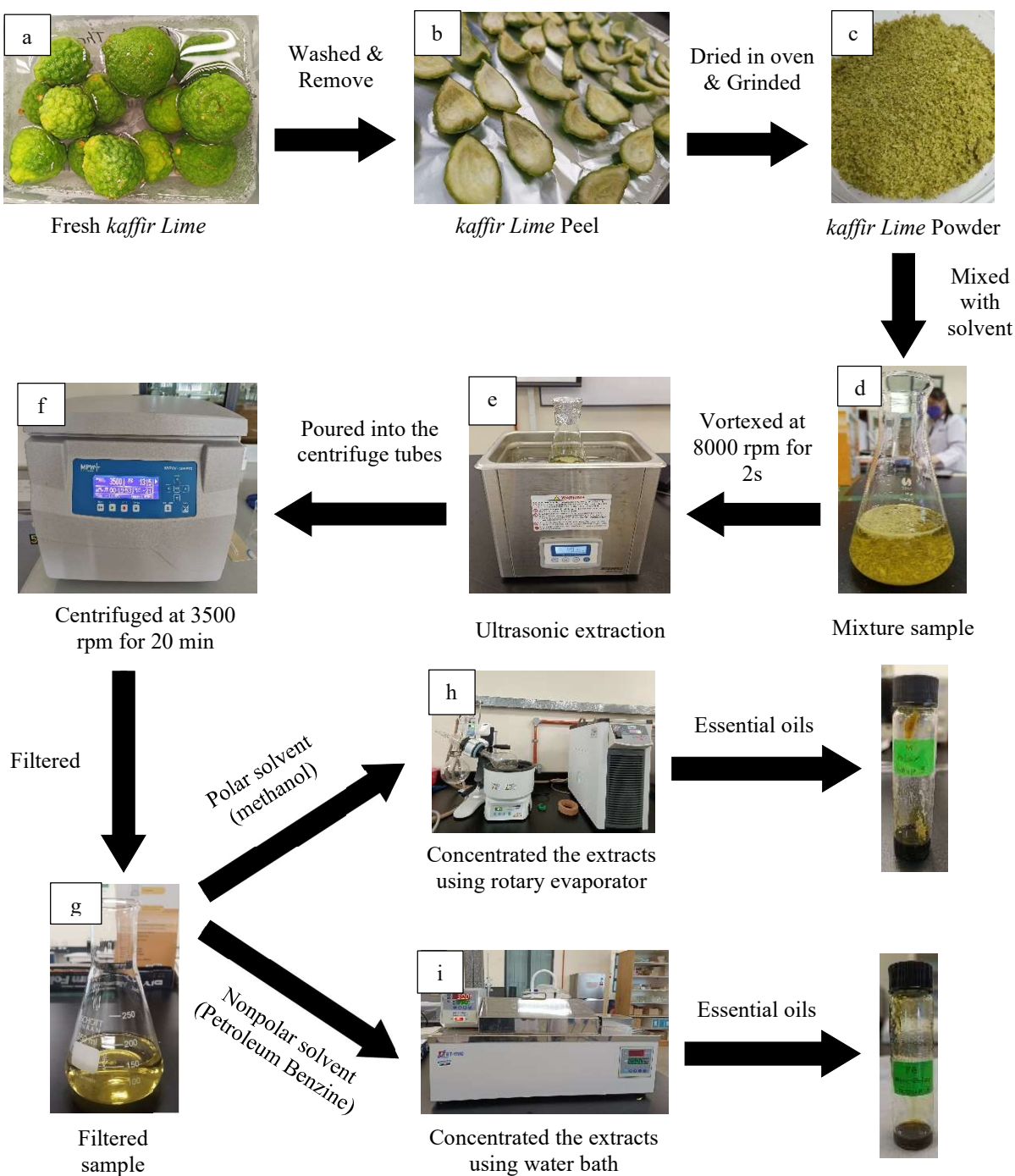


Figure 1. Schematic diagram of the extraction of the *kaffir Lime* essential oil using different polar solvents

Analytical method

The performance of the different polar solvents used in the extraction was evaluated according to the percentage yield of EOs obtained by calculated using Equation (1). The weight of extracted oil was based on fresh *kaffir*

lime peels. The density of *kaffir lime* oil is in the range of 0.871 g/ml [3]. From that, the weight of the obtained essential oil can be calculated by multiplying the density of the *kaffir lime* oil (0.871 g/ml) with the volume (ml) of EOs obtained.

$$\% \text{ Yield} = \frac{\text{Weigh of essential oil obtained (g)}}{\text{Weigh of fresh Kaffir Lime peel used (g)}} \times 100\% \quad (1)$$

Results and Discussion

Percentage yield of EOs obtained

Table 1 shows the percentage yield of EOs obtained from extraction with methanol and petroleum benzene solvents. The mass and percentage yield of EO extracted by methanol (6.62 g, 9.43%) was greater than the EO extracted by petroleum benzene (1.66 g, 2.36%). This could be explained by the fact that the EOs were organic constituents, which were more likely to dissolve in organic or non-polar solvents by following the concept of like dissolving like [23]. Solutes or bioactive compounds from *kaffir lime* peels tended to dissolve more easily in the solvent close to its polarity, and thus increased the yield of EOs [24]. The dielectric constant (ϵ) is the important key parameter to measure the interaction between solute and solvent, which would affect the percentage yield of extracted EOs [25]. Non-polar solvents are those with dielectric constants lower than 15 [26]. Therefore, methanol is a polar protic solvent with a dielectric constant of 32.7 [27] while petroleum benzene is a non-polar solvent with a dielectric constant of 1.9 [26]. Polar protic solvents can be identified by the presence of hydrogen bonds such as

the hydroxyl group (OH), whereas non-polar liquids possess similar electronegativities between atoms, such as carbon and hydrogen [28]. As a consequence, the OH group of the methanol molecule enables it to attract polar solutes and form hydrogen bonds, and thus increases the oil production. A solvent that is more likely to dissolve the solute provides a better potential to break down cell walls and extract the oil; hence, increases the amount of oil extracted [28]. According to Taesotikul et al. [29], the methanol extract yielded the most EOs with $68.86 \pm 7.31\%$ as compared to the other solvent fractions such as butanol, hexane, aqueous, and dichloromethane during the maceration technique extraction of *C. hystrix* peels oil. Furthermore, Tir et al. [25] reported that the oil yield of dichloromethane was $55.50 \pm 0.56\%$, which was greater than the oil yield of hexane of $43.76 \pm 0.81\%$ for sesame seed oil extraction by using the Soxhlet method. This was due to the fact that opening the cell wall to allow for more complete solvent extraction of the cell contents reduced the difference in surface tensions on the phase boundary, and thus promotes phase separation [25].

Table 1. Percentage yield of EOs obtained from the extraction with solvent methanol and petroleum benzene

EOs	Methanol	Petroleum Benzene
Mass of the peels used (g)	70.20	70.20
Volume of EOs obtained (mL)	7.6	1.9
Mass of EOs (g)	6.62	1.66
Percentage yield (%)	9.43	2.36

Scanning Electron Microscope (SEM)

Figures 2a-c showed the SEM images of *kaffir lime* powder before extraction, residual powder after extraction by using methanol and residual powder after extraction by using petroleum benzene, respectively. Figure 2b and Figure 2c show a few porosities as compared to Figure 2a. The cavitation bubbles produced by ultrasound during the extraction process allowed the surface of *kaffir lime* peels to undergo implosion and

caused the porosity to form [30]. Meanwhile, the changing of the surface allowed the solvents entry the cellular channels of *kaffir lime* peels to extract the compounds [31]. The implosion that occurred at the plant cell wall was related to several extraction parameter conditions in terms of types of solvent used, extraction time, extraction temperature, ultrasonic power, and liquid-to-solid ratio of extraction [22].

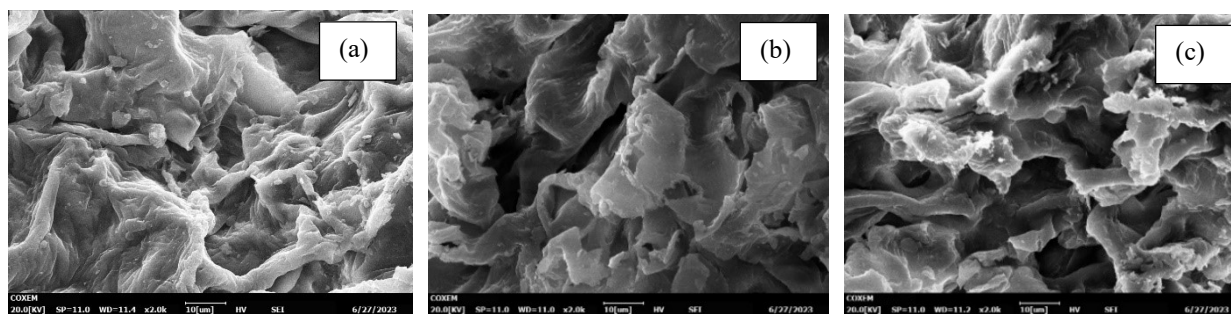


Figure 2. Photograph of SEM of (a) *kaffir lime* powder before extraction, (b) residual powder after extraction using methanol, and (c) residual powder after extraction using petroleum benzene

Phytochemical qualitative analysis

Phytochemical screening was performed to determine the secondary metabolites in the *kaffir lime* essential oils. Phytochemical qualitative analysis results are summarised in Table 2. The positive symbol (+) indicated the presence of phytochemicals while the negative symbol (-) indicated the absence of the phytochemicals in the EOs extracted by methanol and petroleum benzene. The difference in phytochemical results obtained might be due to polarity of the extraction solvent [22]. All secondary metabolites were present in the methanol extract Eos, except saponin and

protein. Meanwhile, only alkaloids and terpenoids were present in the petroleum benzene extract EOs. This was because the polar solvent had a higher ability to increase cell permeability and penetrate inside the cell of a plant as compared to the non-polar solvent, and thus extract more endocellular secondary metabolites. Meanwhile, the non-polar solvent only dissolved lipophilic compounds, including terpenoids, alkaloids, alkanes, waxes, colour pigments and sterols due to the zero-polarity index; therefore, extracting fewer secondary metabolites [32].

Table 2. Result of phytochemical qualitative analysis

EOs	Result of Phytochemical Qualitative Analysis						
	Alkaloid	Flavonoids	Terpenoids	Saponins	Tannins	Phenols	Proteins
Methanol	+	+	+	-	+	+	-
Petroleum Benzene	+	-	+	-	-	-	-

Fourier transform infrared (FTIR) spectroscopy analysis

Figure 3 shows the comparison of FTIR peak configuration of EOs by using different extraction solvents: (a) methanol and (b) petroleum benzene. Similar functional groups with similar infrared absorption band intensities could be seen in both FTIR spectra. The broad peak at 3025 cm^{-1} -3625 cm^{-1} was attributable to the O-H bond stretching, indicating the existence of phenolic compounds such as terpinen-4-ol, citronellol or linalool [33]. Besides, the varied peaks at 2834 cm^{-1} -2939 cm^{-1} represented a considerable asymmetric stretching of CH_3 , corresponding to an alkyl-saturated aliphatic group present in the compound [34]. The aldehyde, ester, carboxylic acid, or ketone

groups were responsible for the C=O stretching at 1717 cm^{-1} -1721 cm^{-1} [34]. Furthermore, the peaks at 1622 cm^{-1} -1624 cm^{-1} contain an alkenes bond, which was a C=C stretching of aromatic rings caused by the presence of terpenes or terpenoid compounds such as D-limonene or citronellal [33]. According to Figure 3, petroleum extracts had higher intensity across all spectrum bands than methanol extracts, except O-H stretching. The solubility of a solvent might affect intensity of the peak. The polarity of functional groups in EOs can differ and the solvent of choice can have an impact on solubility and interactions of the solute and solvent. Non-polar compounds are generally more easily dissolved by non-polar solvents such as petroleum benzene [24]. It can dissolve more readily in petroleum benzene if the

functional groups in the EOs are primarily non-polar or possess low polarity, which would result in larger intensity peaks in the FTIR spectra [35]. Comparatively, polar solvents like methanol might have a poorer affinity for non-polar functional groups, which might result in reduced solubility as well as lower oil yields [24]. Table 2 shows the fact that there were greater numbers of phytochemical groups found in methanol extracts.

However, some of these compounds might not have adequate IR-active functional groups to produce strong FTIR signals. A change in dipole moment in the process of vibration will affect the infrared absorption [36]. If there is little or no change in dipole moment, the radiation only interacts with vibration, with relatively little or no absorption. The intensity of absorption increases with the shift of dipole moment [36].

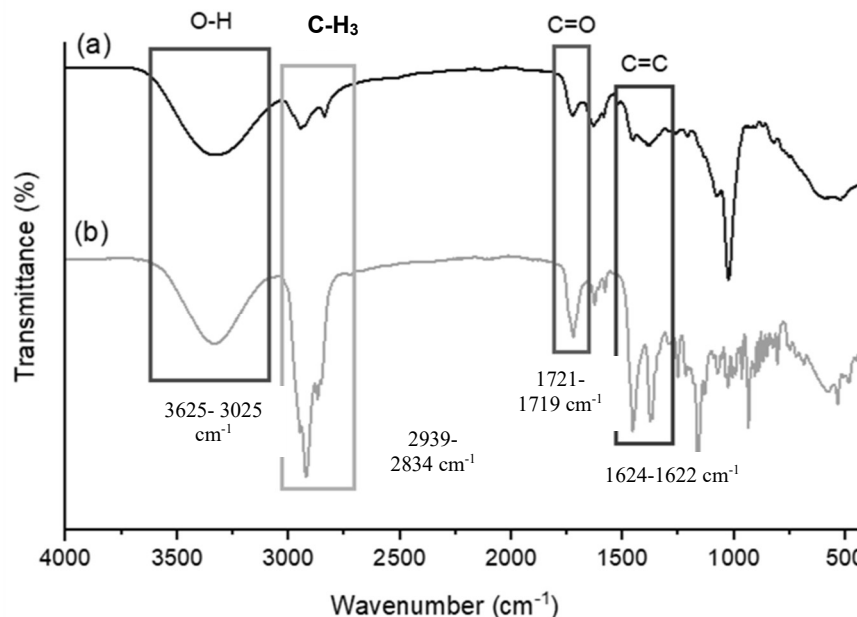


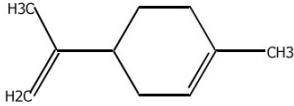
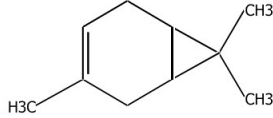
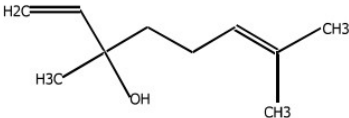
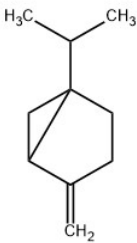
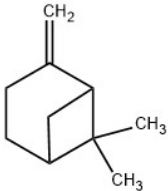
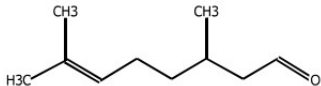
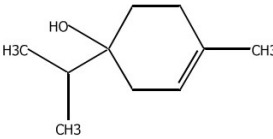
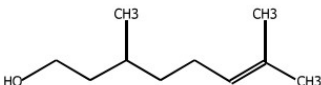
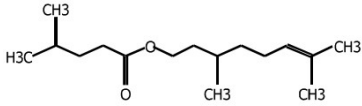
Figure 3. FTIR analysis of extraction of *kaffir Lime* essential oil using (a) methanol, and (b) petroleum benzene

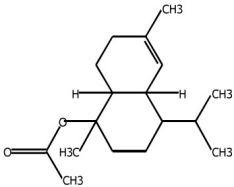
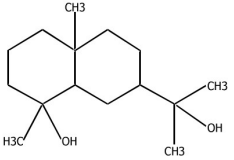
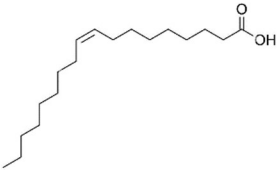
Gas chromatography-mass spectrometry (GCMS) analysis

Table 3 and Figure 4 tabulate the identified chemical compounds with the different concentrations that contributed to the total EOs extracted by using methanol and petroleum benzene. Generally, the main components found from the EOs included D-limonene, β -pinene, 3-carene, linalool, citronellal, terpinen-4-ol, and citronellol. As a result, D-limonene, 3-carene, citronellol, citronellal and β -pinene were found in the EOs extracted from the *kaffir lime* peels [10]. From the result obtained, Compound 1 was a naturally occurring bicyclic monoterpene, whereas Compound 13 was a

type of fatty acid. Both compounds extracted were considered non-polar compounds. However, the relative solubilities of Compound 1 and Compound 13 in both solvents might differ. Both compounds can only be solute in petroleum benzene. The terpenoid group was the majority chemical compound found in EOs. D-limonene could be considered the main component of methanol extract EOs, which provided a unique flavour, such as the smell of oranges. Previous research by [3] showed that the presence of β -pinene imparted a woody aroma to the oil, while citronellal indicated that the oil had antifungal properties and could; therefore, be used as an insect repellent.

Table 3. Chemical compounds in extracted *C. hystix* essential oil using methanol and petroleum benzine

No	Name of Compound	Structural Formula	Retention Time (min)	
			Methanol Extracts Oil	Petroleum Benzine Extracts Oil
1	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-		-	5.505
2	D-limonene		7.378	6.476
3	3-carene		7.690	7.005
4	Linalool		7.907	7.954
5	Sabinene		8.930	-
6	β -pinene		9.137	-
7	Citronellal		9.568	9.080
8	Terpinen-4-ol		9.879	9.646
9	Citronellol		10.548	10.595
10	Pentanoic acid, 4-methyl-, 3,7-dimethyl-6-octenyl ester		12.946	12,889

11	Tau-Cadinol acetate		16.038	16.059
12	7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1ol		21.035	21.014
13	Oleic Acid		-	29.960

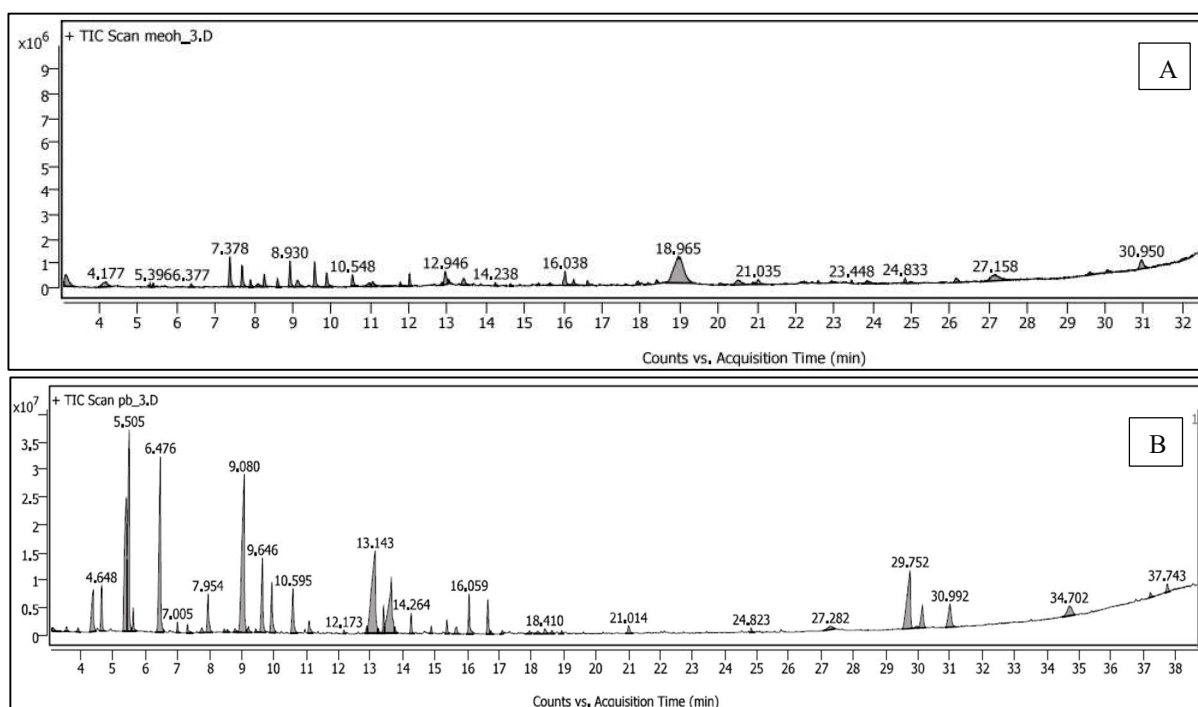


Figure 4. GCMS chromatogram of extracted *C. hystrix* essential oil using (a) methanol, and (b) petroleum benzene

Conclusion

The extraction of essential Oil *C. hystrix* (*kaffir lime*), respectively, with methanol and petroleum benzene by using UAE was successfully studied. The percentage yield of EOs obtained for methanol extract was higher than petroleum benzene extract, which was 9.43% and 2.36%, respectively. Methanol extracted more

compounds as compared to petroleum benzene due to the high polarity of solvent. Compounds that were extracted by using methanol were alkaloids, flavonoids, terpenoids, tannins and phenols while the compounds that were extracted by using petroleum benzene were only alkaloids and terpenoids. The screening phytochemical, FTIR and GCMS analyses proved that

D-limonene, β -pinene, 3-carene, linalool, citronellal, terpinen-4-ol, and citronellol were the main components found in *C. hystrix* essential oil.

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