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HIGH VALUED LIMONENE IN ESSENTIAL OIL EXTRACT FROM LIME PEEL WASTE FOR PERFUME INDUSTRY

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ABSTRACT

Limonene in essential oils is highly valuable attracts the great interest of consumers due to its specific fragrance. It is not surprising that many big perfume industries are looking for high purified limonene found in essential oils of certain fruits and flowers. This study investigates the extraction process of essential oil from lime peel waste. This study has used two extraction methods, i.e., the maceration method and the Soxhlet technique. Two types of organic solvent were applied, i.e., hexane and ethanol. This work studies the effect of extraction time, kind of organic solvent, and extraction method on extraction yield. Characterization studies as FTIR (Fourier Transform Infra-Red) Spectroscopy related to the chemical bonding of limonene in essential oil and GC-MS (Gas Chromatography-Mass Spectrometry) related to limonene fragmentation have also been investigated. The FTIR findings show C-H alkane, C-H aromatic, C-N aromatic, C-N aliphatic, and C=C functional groups concerning 2919.60 cm-1, 3091.83 cm-1, 1318.43 cm-1, 1232.98 cm-1, and 1606.39 cm-1, that is characteristics for limonene in essential oils. The GC-MS findings show the highest yield of limonene (45.87%) obtained by Soxhlet extraction using n-hexane followed by ethanol (42.75%) for 12h. Applying maceration for 12h, the highest yield of limonene (40.03%) using n-hexane followed by distilled water (33.10%) and ethanol (30.92%) was obtained by this study. It seems that the Soxhlet is better than the maceration method, and n-hexane is better than ethanol, except distilled water shows a different finding about its limitation for the Soxhlet method.

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INTRODUCTION

Limonene in essential oils possesses high value for the perfume industry due to its specific fragrance. Moreover, limonene is needed for expensive perfumes and cosmetics of high quality produced by established Western countries. Essential oils are found in certain plantations like flowers (lavender, magnolia, and rose), leaves (patchouli and cinnamon), wood (sandalwood and aloes), and fruits (orange, pomelo, and lime) (Baser & Buchbauer, 2010). Limonene is an organic component ($C_{10}H_{16}$) found in essential oil, with its structure illustrated in Figure 1.

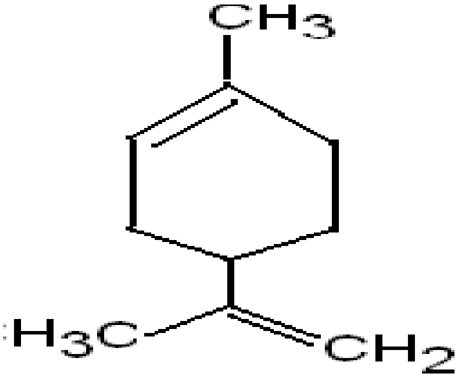

Figure 1 Limonene with its alicyclic monoterpene structure

Many chemical analysis of limonene in essential oil has been investigated (Lestari & Arreneuz, 2014; Sugiantoro et al., 2016; Wibaldus et al., 2016). S Darmasiwi et al., 2016 investigated limonene in the essential oil of pomelo peel applying chromatographic analysis, while Lestari and Arreneuz (Lestari & Arreneuz, 2014) identified limonene in the essential oil of local orange peel applying mass spectrometry. The results of the chemical analysis of limonene show that limonene is generally found as a major component together with other minor organic compounds in essential oils. Lestari & Arreneuz (2014) reported limonene (98.95%) and γ-mirsen (1.05%) in local lime, while S Darmasiwi et al., 2016 found limonene (41.98%) and β-mirsen (15.34%) in pomelo peel. Wibaldus et al. (2016) investigated limonene (26.04%), neral (10.40%), pinene (18.84%), citral (13.09%), and phellandrene (6.29%).

Lime (Citrus aurantifolia S) is found abundantly in many tropical countries. Lime is commonly applied for the food consumption of local people in a specific region. The high demand for lime fruits yields accumulated lime peel waste that generates the environmental problem. For that reason, the lime peel waste will be advantaged for its essential oil content (Hasibuan & Gultom, 2020). Due to its simple, fast, and low-cost method, organic solvent extraction with several modifications often attracts essential oil from the lime peel.

This study has applied maceration and Soxhlet solvent extraction for limonene isolation since it has some benefits in terms of simplicity, low cost, and being moderately fast. Issues of limonene and related products in essential oils have been discussed since many years ago. Chouchi and Barth (Chouchi & Barth 1994) applied gas chromatography to identify coumarine derivatives in lime peel. Limonene is one of the coumarine derivatives that is a volatile essential oil used in perfumes and cosmetics. Three years later, Reverchon & Marrone (Reverchon & Marrone, 1997) discussed supercritical CO_2 fluid to extract crucial oils about isolation, fractionation, and modeling based on solubility data. Salvador & Chisvert (Salvador & Chisvert 2007) comprehensively discussed analytical methods of perfume chemicals in cosmetics in relation to hydro distillation, steam distillation, solvent extraction, and supercritical fluid extraction and analysis of perfumes and perfumery raw materials, including natural and synthetic fragrances. On other occasions, Clarke (Clarke, 2008) reviewed in a book chapter about chemical composition and examination of essential oils for aromatherapy, including organic oils and liquid wax, and discussed quality control about analytical methods to ensure chemical purity. Afterward, Buckle (Buckle, 2015) overviewed analytical methods of essential oils related to gas chromatography, HPLC (High-Performance Liquid Chromatography), NMR (Nuclear Magnetic Resonance), optical rotation, refractive index, and infrared spectroscopy, as well as organoleptic nose analysis.

Although studies on essential oils, including limonene as a significant component from the perspective of chemical composition and examination as well as regulatory aspects, have already been reported in many publications, however, this study attempts to present a short technical paper about the isolation of essential oil from lime peel waste abundantly found in tropical countries applying two kinds of extraction method, i.e., maceration and Soxhlet extractions. This study aims to show that solvent extraction conducted at room temperature and atmospheric pressure used low-cost solvents (n-hexane, ethanol, and distilled water) and standard laboratory glassware. On the other hand, the distillation method needed heat energy to achieve boiling points of organic constituents. The supercritical CO_2 and fluid needed lower pressure to gain supercritical condition. Therefore, this study reported simple, low-cost, and fast methods for limonene isolation. The organic solvents for extraction are n-hexane and ethanol and the extraction time is varied for 6, 9, and 12h. The maceration extraction is stirred for 150 rpm. The effect of extraction variables in terms of extraction method, organic solvent, and extraction yield has been investigated. The characterization examination of essential oil extract from lime peel waste in the presence of limonene applying FTIR and GC-MS are also explored.

METHODS

Material and Equipment

This study used hexane and ethanol in analytical grade—distilled water used for lime peel waste extraction. Hot plate, analytical balance, Soxhlet device, reflux condenser, aerator, iron support rod and clamp, blender, oven, stirrer, glasswares, and rotary evaporator applied for extraction process. Pycnometer used for density measurement. Refractometer used for refractive index measurement. FTIR and GC-MS were used for the characterization study of essential oil extracted from lime peel waste. The FTIR is used to examine chemical bonding in essential oil. The GC-MS is used to identify limonene in essential oil.

Analysis of extraction yield, density, and refractive index

The yield analysis is related to the weight of essential oil extracted from lime peel waste to the importance of lime peel waste sample stated as "% Yield."

$$\%\ Yield = \frac{weight\ of\ essential\ oil\ extracted}{weight\ of\ lime\ peel\ sample} \cdot 100 \qquad (1)$$

The Pycnometer was used for the determination of the density of essential oil extract measured at 25°C. The density is calculated using equation (2).

$$Density = \frac{weight\ of\ essential\ oil\ in\ picnometer}{volume\ of\ essential\ oil\ in\ picnometer} \qquad (2)$$

The refractometer is used for refractive index measurement. The reading of refractive index for essential oil based on a stable reading of 25°C applying distilled water.

Preparation of material for extraction

The preparation of lime peel waste for the extraction process included: (i) collecting lime peel waste in a container with its initial weight of about 4 kg, (ii) drying the lime peel waste in an oven at 50°C for 7 – 8h until the weight was constant, (iii) powdering the dried sample applying a blender, and (iv) filtering the sample powder applying a 50 mesh filter and the final water content was found to be around 10%.

Extraction process

The extraction process was classified concerning the type of extraction method, extraction time, and type of solvent, as shown in Table 1. This study applied two methods of extraction, i.e., Soxhlet and maceration (Figure 1), extraction time varied for 6h, 9h, and 12h for each extraction method, and type of solvent varied for ethanol, n-hexane, and distilled water.

Characterization study

The characterization study of this investigation included (i) the FTIR and (ii) the GC-MS analyses.

FTIR analysis

The FTIR analysis is related to the examination of the functional group of limonene in essential oil attributed to C – H alkane, C – H aromatic, and C = C alkene at consecutive wave number intervals of 3000 – 2850 $cm\text{-}^{1}$, 3150 – 3050 $cm^{-1,}$ and 1680 – 1600 cm^{-1}.

GC-MS analysis

The GC-MS is a couple of gas chromatography. Its function is to separate organic components in essential oil based on different partition factors of organic components in two phases. The mass spectrograph is applied as detector based on mass fragmentation. The fragmentation pattern of organic components in essential oil is specific. Thus, the limonene component can be identified from its fragmentation pattern. In the mass spectrogram, mass fragmentations (m/z) are elevated from left to right, and the peak intensity describes its abundance. The quantitative analysis of gas chromatography is determined as component area divided by the area described % component.

$$\%\ component = \frac{component\ area}{whole\ sample\ area}.100 \qquad (3)$$

RESULTS AND DISCUSSION

Physical analysis of essential oil extract from lime peel waste.

The physical analysis in this investigation is addressed to the color of essential oil in given solvents (n-hexane, ethanol, and distilled water), the density, and the refractive index. The results are shown in Table 2. The specifications compared to the standard value recommended by ISO 3519:2005(E).

The physical analysis results are presented in Table 2 concerning color, density, and refractive index, showing no significant difference compared to the standard ISO 3519:2005 (E). It implies that the extracted result from lime peel waste in this investigation is identified as an essential oil from physical properties. The determination of density in this study applied eq. 2.

Extraction yield

The extraction yield is described as % Yield applying eq about the type of extraction, extraction time, and type of solvent, the extraction yield is defined as % Yield applying eq. 1., this study shows n-hexane produced the highest extraction yield at all given extraction times (6h, 9h, and 12h) applying the maceration method as shown by Figure 2. On the other hand, distilled water produced the lowest extraction yield using the maceration method. N-hexane and ethanol are known as organic solvents with nonpolar properties, while water is a polar solvent. Essential oils are organic constituents more likely to dissolve in organic or nonpolar solvent following the rule “like dissolving like.” According to the degree of nonpolarity, n-hexane has more nonpolar properties than ethanol with semi-polar properties. The assertive nonpolar behavior of essential oils causes more vital interaction with a high degree nonpolar solvent like n-hexane. The organic components of essential oils have nonpolar properties. The nonpolar interactions between organic constituents and nonpolar solvents are classified as Van der Waals due to induction and mesomeric effects. The mesomeric result is related to the resonance effect of an aromatic ring structure. Therefore, it is reasonable that n-hexane has more substantial extracting power on essential oils, as presented in Figure2.

Table 1 The extraction process is classified as the extraction method, extraction time, and type of solvent.

Extraction method	Extraction time (h)	Type of solvent
Soxhlet	6	n-hexane ethanol
	9	n-hexane ethanol
	12	n-hexane ethanol
Maceration	6	n-hexane ethanol distilled water
	9	n-hexane ethanol distilled
	12	n-hexane ethanol distilled water

Table 2 Results of physical analysis of essential oil extract from lime peel waste

Parameter	Essential oil extract from lime peel waste	Lemongrass oil ISO 3519:2005 (E)
Color	Yellow greenish in n-hexane Green yellowish in ethanol Yellow greenish in distilled water	Colorless tendency to yellow-greenish
Density (g/cc), 25°C	0.858	0.858 – 0.866
Refractive index, 25°C	1.476	1.474 – 1.477

(a) (b)

Figure 2 Equipment for extraction (a) maceration; (b) Soxhlet

A previous investigation showed that methanol has stronger extracting power on essential oil from a certain herbal than n-hexane, as reported by Ahmad et al. (Ahmad et al., 2010). However, Alhassan et al. (Alhassan et al., 2018) reported n-hexane as a suitable extracting solvent applying the maceration method. Alhassan et al. (2018) reported the essential oil yields obtained as 4.5% (maceration method) and 3.8% (Soxhlet method). The finding of Alhassan et al. (Alhassan et al., 2018) is in agreement with the finding of this study, although Alhassan et al. (Alhassan et al., 2018) applied a longer maceration extraction time (38h) compared to this study. Alhassan et al. (Alhassan et al., 2018) extracted essential oil from lemongrass leaves, while this study used lime peel waste. As shown in Figure 2, maceration extraction time looks consistently between % extraction yield of essential oil and extraction time for both n-hexane and water. However, there is a decline in extraction yield for ethanol that the % yield at 12h-extraction time is lower than that at 9h-extraction time. This phenomenon is related to the repulsion force between essential oil and ethanol molecules as the extraction time gets longer since ethanol has semi-polar behavior.

About Soxhlet extraction, this study also showed n-hexane had more substantial extracting power rather than ethanol, as shown in Figure3. Concerning Soxhlet extraction, there is a consistency between the % yield of essential oil and extraction time for both n-hexane and ethanol. The distilled water does not run correctly as an extractor in Soxhlet extraction. The hydrogen bonding of water molecules yields lower water vapor pressure causing circulation problems in Soxhlet extraction. Triesty and Mahfud (2017) reported that water could not be used in Soxhlet extraction for extracting essential oil from aloes plantations. Applying the Soxhlet extraction type, Ahmad et al. (Ahmad et al., 2010) investigated methanol had better extracting power than n-hexane for essential oil extraction from a given herbal yielding 14.18% yield for 12h-extraction time. However, this study yielded 6.15% extractant at the same time applying n-hexane. Tesfaye and Tefera (Tesfaye & Tefera, 2017) applied a solvent mixture of n-hexane and ethanol (40:60) for extracting essential oil from Neem seed using Soxhlet type and yielded 43.71% for 3h-extraction time. A mix of n-hexane and ethanol enhanced extracting power, probably due to increased nonpolarity. In general, this study shows a low yield of essential oil extract compared to the previous investigation results since the actual oil content in lime peel waste is relatively low. High actual oil content is found in lime fruit.

Furthermore, this study compares the extracting power between maceration and Soxhlet extraction methods concerning the % yield of extract obtained. Figure 4 shows this comparison in terms of organic solvents (n-hexane and ethanol) and duration of extraction (6h, 9h, and 12h). As shown in Figure 4, the Soxhlet method is more superior for essential oil extraction compared to that of maceration method for both solvents (n-hexane and ethanol) at given extraction time (6h, nine h, and 12 h). Figure 4 also shows n-hexane is more powerful than ethanol for solvent extraction as we have already discussed above.

Generally, a longer extraction process increased % yield of essential oil extracted except for ethanol, as shown by this study. The inconsistency between the time of extraction and % yield for ethanol has already been discussed above concerning the semi-polar behavior of ethanol. The report of Alhassan et al. (Alhassan et al., 2018) showed that the maceration method gave better extraction performance than that of the Soxhlet method for extraction of essential oil from lemongrass leaves. According to Alhassan et al. (Alhassan et al., 2018), the maceration method showed the optimum result, i.e., 4.5% yield for 38h duration time applying n-hexane. Longer extraction time using the maceration method yields much more attraction between molecules of both essential oil and n-hexane. Alhassan et al. (2018) conducted the work at temperature 65°C, while this study showed the extraction at room temperature (about 25°C). It is not surprising this study obtained a higher yield using Soxhlet method rather than maceration. On the other hand, the previous study obtained higher yield using maceration method (Alhassan et al., 2018). The moderate temperature (65°C) caused more chance for removal due to evaporation of yield together with organic solvent in dynamic Soxhlet extraction.

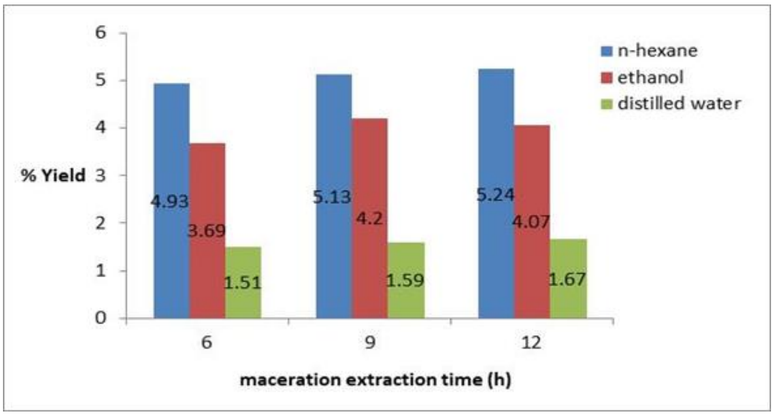

Figure 3 Extraction yield (%) of essential oil from lime peel waste using maceration method

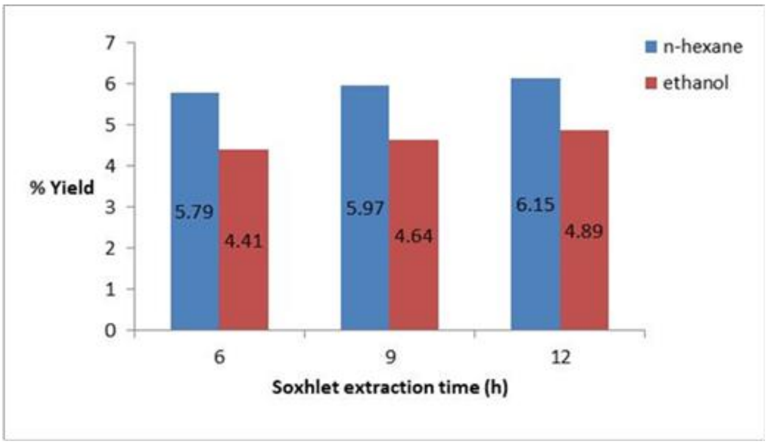

Figure 4 Extraction yield (%) of essential oil from lime peel waste using the Soxhlet method

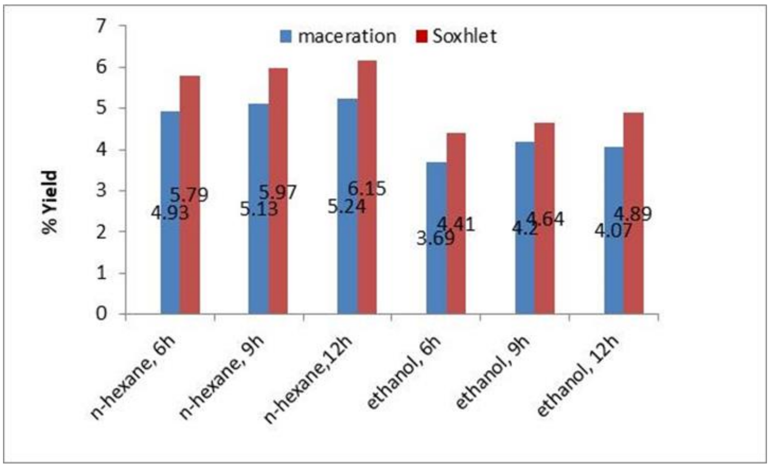

Figure 5 Extraction yield (%) of essential oil from lime peel waste with respect to Soxhlet and maceration methods. Extraction time 6h, 9h, and 12 h. Organic solvents n-hexane and ethanol.

Table 3 Functional groups of FTIR related to essential oil extract from lime peel waste.

Functional Group	Wavenumber (cm^{-1})	Wavenumber range (cm^{-1}) (Pavia et al., 2015)
C-H (alkanes)	2919.60	3000-2850
C-H (aromatic)	3091.83	3150-3050
C-N (aromatic)	1318.43	1350-1250
C-N (aliphatic)	1232.98	1250-1000
C = C	1606.39	1680-1600

This study shows a low % yield of essential oil extract from lime peel waste, either applying maceration or the Soxhlet technique. Nevertheless, this investigation is worth considering the beneficiation of accumulated lime peel waste for the perfume industry and to reduce environmental problems. Tropical countries with abundant fruit plantations may generate serious waste problems, and this is an effort to advantage its potency.

Characterization study

FTIR analysis

Since essential oil is an organic matter with a mayor content of limonene having alicyclic monoterpene structure and thus FTIR examination is the relevant analysis to investigate organic chemical bonding concerning C – H bonding in alkanes and aromatic and C = C bonding that possess specific range of wave number (Pavia et al., 2015).

Figure 5 shows the substantial functional groups of limonene existence in essential oil extract corresponding with C-H alkane, C-H aromatic, and C=C accordingly at ≈ 2919 cm^{-1}, 3091 cm$^{-1,}$ and 1606 cm^{-1}, respectively. The alicyclic monoterpene structure of limonene corresponds with the functional groups mentioned above. However, the C-N aromatic and C-N alifatik groups are related to other nitrogen organic compounds, such as a protein that existed in lime peel waste. The illustration of FTIR spectra in Figure5 is strengthened by the FTIR data in Table 3 that is in concordance with Pavia et al. (2015) report. Since FTIR energy is proportional to wavenumber, the energy of vibrational stretching of C-H aromatic is higher than that of C-H alkane, as shown in Figure5. According to the stereochemistry of organic compounds, the more rigid aromatic structure yields higher bond energy than that of more flexible alifatik alkane. Murnah, 2012 investigated the FTIR characteristics of essential oils in tuber roots and reported some functional groups in the wavenumber range of 1300 – 1730 cm^{-1}. The absorption of – CH3 was observed at 1310 cm^{-1} and 1380 cm^{-1}, and aromatic absorption peak at 1475 cm^{-1}, while the C = O bonding was observed at 1705 – 1725 cm^{-1}. Bending vibrations of C - CH and C – C were observed at 1118 cm^{-1} and 1165 cm^{-1}. A fascinating FTIR investigation combined with chemometric analysis was reported for essential oils in certain plants (Agatonovic-Kustrin et al., 2020). The stretching vibration of C – H (≈ 2900 cm^{-1}) was concordance with the phenomenon of this study related to an alkane, and the C = O stretching band observed at 1740 cm^{-1} for terpenoid agreed with the findings of (Murnah, 2012). The broad peak at ≈ 3400 cm^{-1} attributed to O – H stretching was found in lavender and rosemary plants. In plants, FTIR characteristics of essential oils are generally attributed to C – H, C = O, C = C, O – H, and – CH_3 functional groups.

GC-MS analysis

The gas chromatography in coupling with mass spectrograph as a detector is useful for limonene identification in essential oils since limonene is the remarkable and critical component for Perfume industry. The gas chromatography may separate organic components based on different partition coefficients between two liquid phases. Therefore, the eluent may contain selective components as a result of the distribution pattern. As the detector, the mass spectrograph may break the organic compound into fragments with increasing mass numbers. The retention time of the limonene component in lime peel is << 3 min. as shown by Figure 6. However, the retention time of limonene in local lime (Citrus nobilis) was reported around 10 – 15 min. (Sugiantoro et al., 2016). The difference in retention time is probably affected by different mobile and solid phases in the chromatographic column and the separation method used and type of organic solvent.

Moreover, the limonene compound has the highest concentration among other organic components in the lime peel, as shown by Figure 6 and strengthened by reported data in Table 4. Previous reports found limonene the most abundant component in lime fruit (Darmasiwi et al., 2016; Sugiantoro et al., 2016; Wibaldus et al., 2016). The identification of limonene in the chromatogram (Figure 6) was justified by the fragmentation pattern in the mass spectrograph. The number of components, as shown in Figure 6 is listed in Table 4 provided with concentration data.

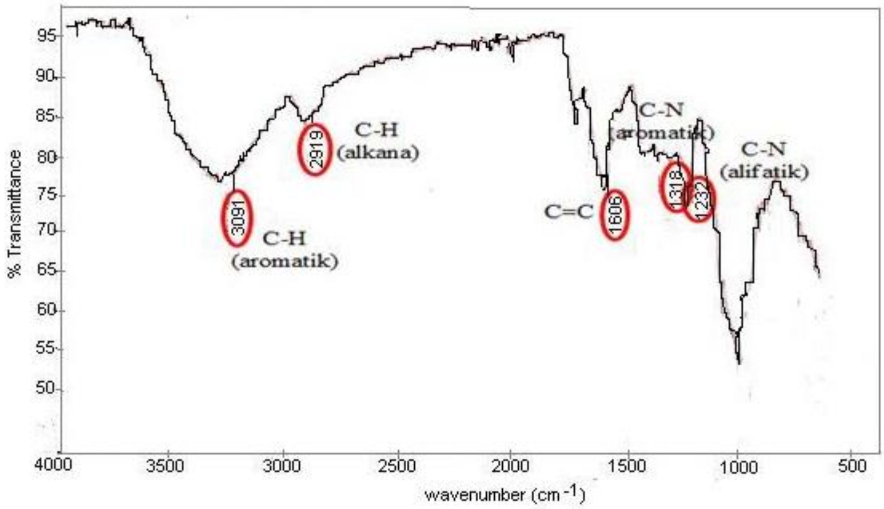

Figure 6 FTIR spectra of essential oil extract from lime peel waste

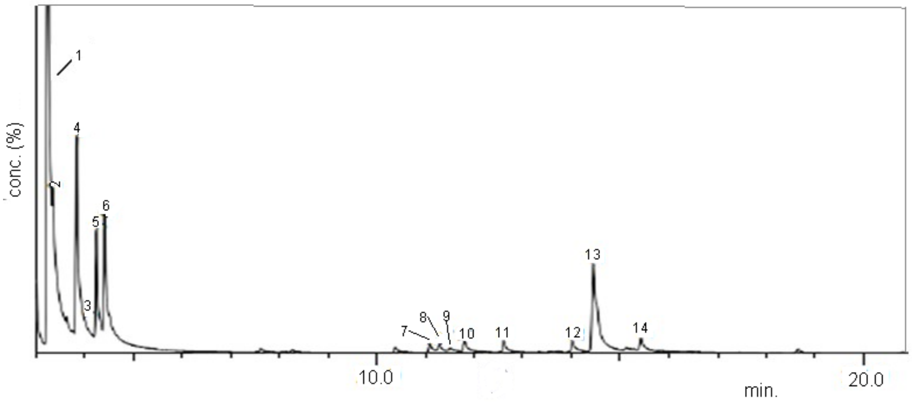

Figure 7 Chromatogram of essential oil extract from the lime peel. GC-MS Soxhlet extraction 12h. n-hexane

Table 4 Organic components in essential oil extract from lime peel waste. Soxhlet extraction. 12h. n-hexane

Number of components	Name of components	Conc. (%)
1	D-Limonene	29.89
2	D-Limonene	13.63
3	D-Limonene	2.35
4	Γ-Terpinene	15.42
5	o-Cymene	10.83
6	α-Terpinolene	12.85
7	1-Terpinenol	0.61
8	Fenchyl Alcohol	0.84
9	Caryophyllene	0.60
10	Terpinen-4-ol	1.17
11	β-Terpineol	2.45
12	β-Citral	1.28
13	α-Terpinenol	6.41
14	α-Citral	1.66

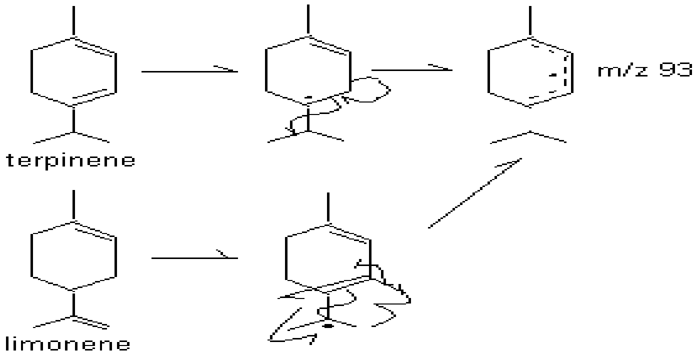

Figure 8 Fragmentation mechanisms of limonene and terpinene yielding C_7H_9 fragment (Thao et al., 2007)

The highest content of limonene obtained by this study is found to be 29.89 % (Table 4). Njoku and Evbuomwan (et al., 2014) found a limonene content of 17.07 % in Nigerian lime peel applying the distillation method. Different separation methods and lime regions may affect the content of limonene. Terpinene placed in the second-highest position after limonene (Table 4) has the same molecular formula as limonene, i.e., $C_{10}H_{16}$. Therefore, both limonene and terpinene undergo similar initial fragmentation producing C_7H_9 fragment (*m/z* 93) in electron ionization mass spectrometry (Figure 7).

Figure 8 shows another chromatogram of essential extract from lime peel applying ethanol for 12h using the Soxhlet method. The component data are listed in Table 5 provided with retention time. As shown in Table 5, the execution of limonene was still contaminated with cyclohexene assumed as the solvent used in the gas chromatographic column yielding a high concentration (42.75%). Using ethanol as extract solvent, the alcohol derivate, i.e., the eucalyptol, was executed from the chromatographic column at 3.39 min. Eucalyptol as one organic component in essential oil was produced if ethanol was used as extract solvent. However, it was not produced using n-hexane as extract solvent, as shown in Figure6 and Table 4. This finding shows the significant difference between solvent extraction of n-hexane and ethanol in the Soxhlet method applied for essential oil extraction from lime peel waste.

As caryophyllene was found in essential oil extract (Table 4) using n-hexane probably, there occurred no interaction between crucial oil and n-hexane during the Soxhlet or gas chromatographic process yielding caryophyllene as a new fragment that the mechanism is still unknown. Solvent extraction, either using n-hexane or ethanol in the Soxhlet method generating derivative of terpene such as terpinen, terpineol, and terpinolen and derivative of alcohol in fragments in Table 4 and 5. It is probably due to any interaction of organic components in essential oil and solvents used in Soxhlet extraction and chromatographic process at moderate temperature. Any process in solvent extraction and chromatographic column may involve the formation of alcohol derivatives and unsaturation compounds through a complex mechanism.

Other illustrations (Figure 9) show chromatograms of essential oil extract from lime peel applying GC-MS with maceration method using n-hexane solvent for 12h (Figure 9a), ethanol solvent for 12h (Figure 9b), and distilled water for 12 h (Figure 9c). With all solvents (n-hexane, ethanol, and distilled water), the limonene is the most major organic component in essential oil extract using the maceration method with its concentration above 30 %.

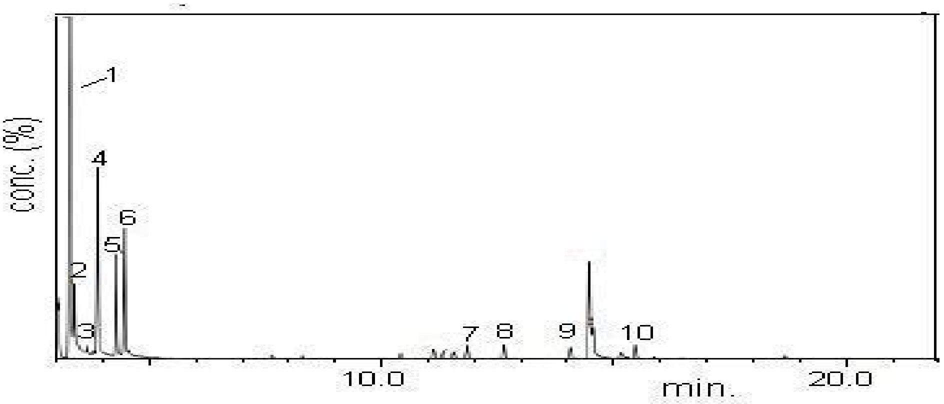

Figure 9 Chromatogram of essential oil extract from the lime peel. GC-MS. Soxhlet extraction 12h. ethanol

Table 5 Organic components in essential oil extract from lime peel waste. Soxhlet extraction. 12h. ethanol

no	Component name	Conc.(%)	Retention time (min)
1	D-Limonene, cyclohexene Eucalyptol	42.75	3.29
2	Pinene	6.05	3.39
3	Terpinene	1.45	3.65
4	o-Cymene	11.74	3.87
5	α-Terpinolene	7.19	4.27
6	4-Terpineol	9.28	4.45
7	β-Terpineol	1.21	11.83
8	β-Citral	1.42	12.63
9	trans-Citral	1.22	14.05
10		1.58	15.46

Table 6 Organic components in essential oil extract from lime peel waste. Maceration extraction. 12h. n-hexane

no	Component	Conc. (%)	Retention time (min)
1	D-Limonene	24.80	3.28
2	D-Limonene	12.60	3.38
3	D-Limonene	2.63	3.65
4	Terpinene	18.39	3.87
5	o-Cymene	6.63	4.27
6	1-Terpinenol	0.66	11.11
7	Caryophyllene	0.67	11.53
8	Terpinen-4-ol	1.21	11.82
9	β-Terpineol	1.55	12.63
10	γ-Terpineol	5.54	14.54
11	trans-Citral	1.82	15.45

Table 7 Organic components in essential oil extract from lime peel waste. Maceration extraction. 12h. ethanol

no	Component	Conc.(%)	Retention time (min)
1	D-Limonene	30.92	3.27
2	α-Pinene	2.13	3.65
3	γ-Terpinene	21.13	3.86
4	o-Cymene	8.70	4.27
5	α-Terpinolene	15.56	4.44
6	Terpinen-4-ol	0.80	11.83
7	β-Terpineol	0.91	12.63
8	β-Citral	0.82	14.05
9	γ-Terpineol	4.65	14.53

Table 8 Organic components in essential oil extract from lime peel waste. Maceration extraction. 12h. distilled water

no	Component	Conc.(%)	Retention time (min)
1	D-Limonene, Cyclohexene	33.10	3.23
2	1,8-Cineol	2.39	3.36
3	γ-Terpinene	11.76	3.84
4	o-Cymene	4.73	4.25
5	α-Terpinolene	5.45	4.47
6	Acetic acid	4.78	8.18
7	Cyclohexene, methanol Cyclohexene, methanol	24.87	14.45
8	Dioic acid	2.64	14.52
9		1.94	18.67

As already mentioned above, limonene is a significant component of the parfum industry. Usually, limonene is found in a mixture with cyclohexene after being executed from the column at around the same retention time (Sugiantoro et al., 2016). According to Sugiantoro et al. (2016), limonene has undergone biotransformation to form unsaturated constituent assumed to be due to resonance effect in mass spectrometer integrated with gas chromatography. p-Cymene is a monoterpene compound has anti-microbial properties related to its role in biological respiration (Table 6).

Moreover, the caryophyllene as a monoterpene derivative (Table 6) found in a minor amount (0.67%) is beneficial for human health relieving painful feelings. Another component, i.e., Terpinen-4-ol, is good as aromatherapy for anti-obesity.

It is apparent that almost all minor components yielded by the maceration method, such as cymene (6.63%), caryophyllene (0.67%), and terpinen-4-ol (1.21%), have therapeutic effects for remedial or healing. As it is known, essential oils have two advantages, i.e., for aroma/odor in the perfume industry and therapy in medicine.

The number of components in Table 6 is related to the number in chromatogram shown in Figure 9a referred to maceration method using n-hexane. Furthermore, the number of components in Table 7 is associated with the number in chromatogram shown in Figure 9b referred to maceration using ethanol. The number of components in Table 8 is related to several chromatograms shown in Figure 9c referred to maceration using distilled water.

Several remarkable components executed from the chromatographic column using ethanol solvent in 12h maceration should be noted in Table 7 related to its benefit besides limonene for the perfume industry. Those components are α-Pinene (2.13%) that has pharmacological properties concerning anti-tumor, anti-microbial, and anti-malarial effects; γ-Terpinene (21.13) with its perfume properties and pleasant odor; then α-

Terpinolene (15.56%) that besides it gives woody aroma, it also has a therapeutic effect on the central nervous system and a small concentration of β-Terpineol (0.91%) with its antioxidant and anti-cancer activities as a therapeutic agent.

Concerning their retention times, in general, the components of alcohol derivatives tend to be executed after 10 min. While the unsaturated components in the –ene form tend to run out earlier from the column, as shown in Figure 9b. Figure 9a also shows similar patterns in Figure 9b that components with –ene form executed earlier than alcohol derivative components. The difference of retention times of chromatographic column for organic components in essential oil extract corresponds with a difference of partition coefficients belonging to those components.

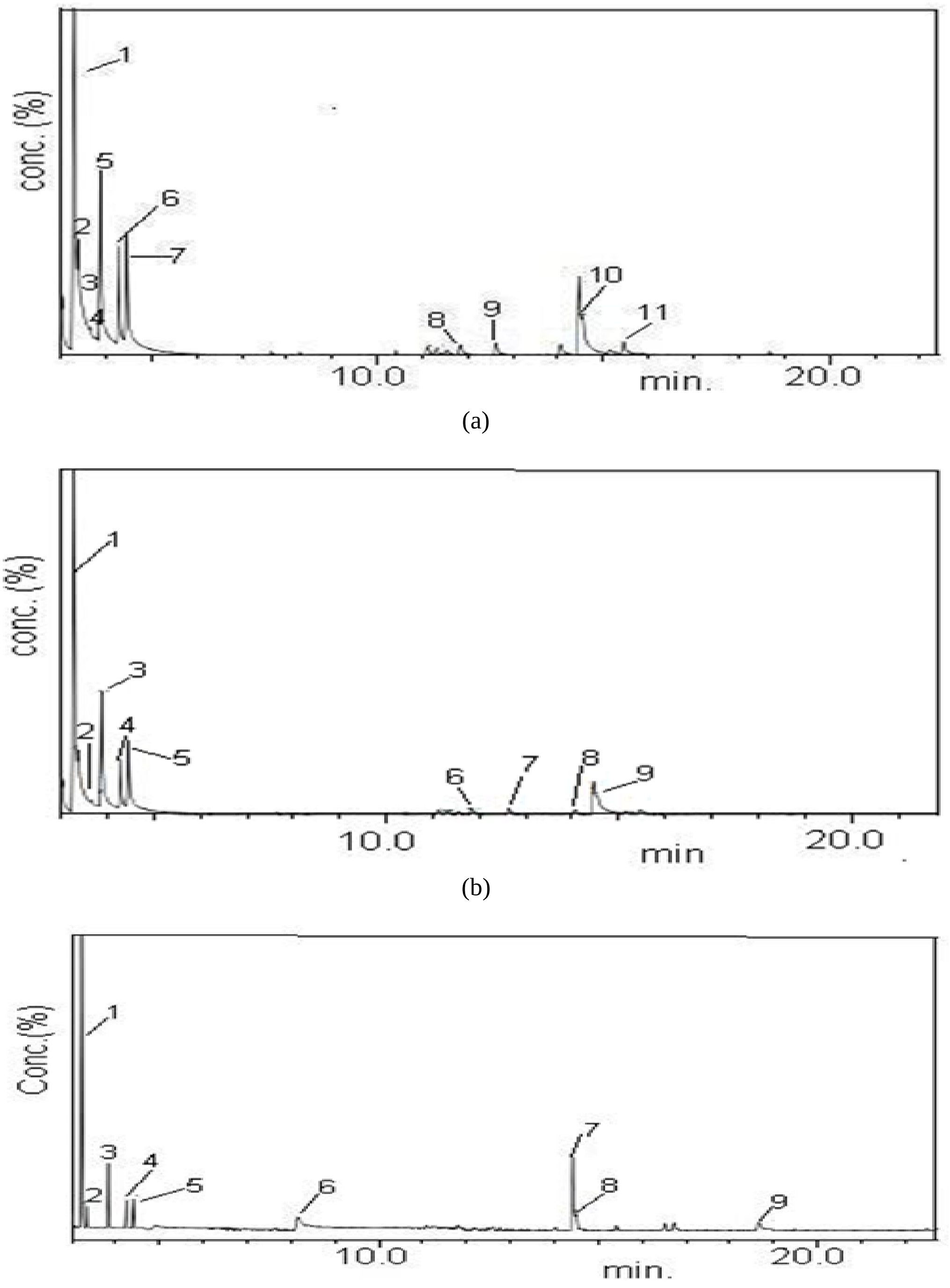

Figure 10 Chromatograms of essential oil extract from the lime peel. GC-MS. Maceration extraction 12h. n-hexane (a). ethanol (b). distilled water (c)

However, Table 8 is slightly different from the earlier two tables (Table 6 and 7) using the maceration method. Table 8 shows fewer organic components in essential oil extract, including limonene, cineol, terpinene, cymene, and terpinolene. The rest are organic solvents used in a chromatographic column such as cyclohexene and methanol. It indicates that those components in essential extract possess lower interaction with distilled water than the more vital interaction between organic components and organic solvents (n-hexane and ethanol). The degree of interaction during the maceration process affected the number of components of essential oil passing the chromatographic column. Figure 9c shows the chromatogram of crucial oil extraction using distilled water in the maceration method.

Nevertheless, the deeper study regarding substantial organic components in essential oil extract from the standpoint of the degree of interaction in relation to retention time in column chromatography and the benefit of minor components or by-product components in an essential oil is discussed in this study gives valuable information.

CONCLUSION

This study obtains a new finding on a lab scale of limonene identification in essential oil extract from lime peel waste. Nevertheless, the lab-scale conclusion is advantageous because it needs a simple, fast and economical method that can be developed to a larger industrial scale for export importance in the forthcoming. Discussion on the interaction between organic components and solvent used in extraction affected retention time in column chromatography. The benefit of minor or by-product components gives a new insight supporting the strength of this study.

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