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Contributed Paper

# Supercritical Fluid Extraction and Gas Chromatographic-Mass Spectrometric Analysis of Terpenoids in Fresh Kaffir Lime Leaf Oil †

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## ABSTRACT

Fresh Thai kaffir lime leaves were subjected to extraction by supercritical carbon dioxide yielding yellow clear oils which were then analyzed by gas chromatographic-mass spectrometric technique (GC-MS). The conditions of supercritical fluid extraction (SFE); pressure, temperature, extraction time, and type and percentage of modifier which gave the highest yield of the oil were 3,000 psi, 55°C, 15 min, and 10% isopropanol, respectively. Results from GC-MS analysis revealed 21 identified terpenoids which were classified in the groups of monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes, each having the number of 3, 5, 9, and 4, respectively. Citronellic acid (4.5%), nerolidol (2.14%), δ-cadinene (1.49%), citronellal (1.41%) and citronellol (1.39%) were found as the major constituents in this SFE extract of fresh kaffir lime leaves.

**Keywords:** terpenoids, SFE, GC-MS, kaffir lime leaves

## 1. INTRODUCTION

Kaffir lime (*Citrus hystrix* DC, Rutaceae) is a Southeast Asian citrus plant with very strong fragrance [1]. Kaffir lime leaves and fruits are frequently used as a condiment in various Thai and Malaysian recipes to add a tangy flavor to soups and curries [2]. They are also useful for treatment of colds, congestion, and cough. By taken internally, kaffir lime leaves and fruits are a digestion stimulant which alleviates flatulence and indigestion, and are used to promote regularity in the case of blocked or infrequent menstruation. For medicinal uses, they are well known as a blood purifier, an antioxidant with cancer-preventing properties, and are used to treat high blood pressure [3]. Moreover, kaffir lime leaf oil possesses some important bioactivities, such as antioxidant [4], antileukemic [5], antitussive, antihemorrhage, antioxidative stress properties

[6], and antibacterial properties [7]. Nowadays, essential oil of kaffir lime is in demand as a fragrance in the food, perfumery, and cosmetic industries [8,9].

Supercritical fluid extraction (SFE) has received much attention in foods, pharmaceuticals, and cosmetic industries [10,11] due to its several advantages in the extraction of natural products from plant samples. The combined liquid-like solvating capabilities and gas-like transport properties of supercritical fluids make it particularly suitable for the extraction of diffusion-controlled matrices such as plant tissues [12]. Pressure and temperature which are the most significant factors affecting on solvent strength of a supercritical fluid can be easily tuned by changing the applied pressure or temperature [13]. Carbon dioxide, the most commonly used supercritical fluid, has the additional advantages of being non-flammable, fairly non-toxic, chemically inert, available at high purity levels, cost-effective and easily removed from the extract following decompression [14].

Due to its relatively low critical temperature (31.1°C), thermal sample decomposition is reduced. However, pure $CO_2$ has a polarity comparable to liquid pentane and is, therefore, best suited for lipophilic compounds. Alternatively, addition of a small percentage of some organic solvents such as methanol, called "modifier", to the fluid usually enables it to extract more polar compounds. A variety of analyzes, ranging from non-polar to polar, can thus be quantitatively extracted. Some previous report exhibited that by using supercritical $CO_2$, extraction can be performed in a shorter time under mild conditions and the yield of volatile analyzed was increased [15]. The method was found to be more selective and particularly efficient for the isolation of terpenoid compounds such as α-thoujone and limonene from some herbs [11,12]. Additionally, a study on extraction of essential oil from Amomum *(Amomum krevanh Pierre)* had shown that the amounts of 1,8-cineole and β-pinene recovered by supercritical $CO_2$ were higher than those obtained by conventional solvent extractions [16].

Terpenoids constitute the most abundant and structurally diverse group of plant secondary metabolites [17]. They play important roles in direct and indirect plant defense against herbivores and pathogens, and are used as natural flavor and aroma compounds[18]. Terpenoids are normally produced in vegetative tissues, flowers, and roots [19].

In this research, SFE with $CO_2$ was applied to extraction of essential oil from kaffir lime leaves. The optimal parameters of the technique, such as pressure, temperature, extraction time, and percentage of modifiers of SFE were investigated. The kaffir lime leaf oils obtained from SFE were analyzed and their terpenoid components were identified by gas chromatography-mass spectrometry (GC-MS).

## 2. MATERIALS AND METHODS

### 2.1 Sample Preparation

Kaffir lime leaves were collected from a local orchard in Chiang Mai province, located in the north of Thailand. Upon arrival at the laboratory, mature leaves were sorted out. The mature leaves were washed under running tap water and dried by air. Then, the fresh leaves were ground just before extraction in a blender (Y46, moulinex blender, Spain) for 10 s to an approximate particle size of 0.5 mm. The ground leaf samples were then left to dry at room temperature for 12 h.

### 2.2 Supercritical Fluid Extraction

Supercritical $CO_2$ extraction was carried out using SFX-220 extraction system (ISCO, Lincoln, NE, USA). Pure $CO_2$ was supplied by using a syringe pump (ISCO Model 100DX, USA). Sample cell was filled with 2.5 g of ground kaffir lime leaves and 1 μl of internal standard (2,6-dimethylpyridine). Kaffir lime oils were extracted from kaffir lime leaf samples by varying parameters consisting of temperature (35, 45, 55, and 65°C), pressure (2000, 3000, 4000, and 5000 psi), extraction time (5, 10, 15, 20, and 25 min) and % of modifier including, methanol, ethanol, and isopropanol (1%, 5%, and 10%). All extracts were trapped by bubbling the $CO_2$ effluent in a glass tube containing methanol:dichloromethane (3:1, v/v). After extraction, a recieving solution was adjusted to 200 μL by vacuum evaporation using a rotary evaporator (Büchi, R-124, Switzerland). The yields were evaluated gravimetrically.

### 2.3 GC-MS Analysis

GC-MS analysis was performed on a gas chromatograph-mass spectrometer (Agilent 6890 and HP 5973 mass-selective detector, Agilent Technologies, USA) equipped with a fused silica capillary column, AT-5MS with dimension of 30 m × 0.25 mm i.d. × 0.25 mm film thickness (Agilent Technologies). GC-MS was operated under a temperature program which was started at 60°C and ramped to 246°C at 3°C/min. The injection temperature was 250°C with an injection volume of 1 μL in the split mode with a split ratio of 10:1. The quadrupole temperature was 150°C and the transfer-line temperature was 280°C. A mass range of *m/z* 29-550 was acquired. Helium was used as carrier gas and was maintained in a constant pressure mode. Identification of volatile constituents in kaffir lime leaf oils was performed by comparison of their mass spectra with those of the database using W8N08 and NIST 98 mass spectral libraries. The identifications were confirmed by comparison of their Kovat retention indices (KI), relative to $C_8$-$C_{22}$ n-alkanes.

### 2.4 Statistical Analysis

The statistical significance was assessed by students *t*-test and confidence limits were added at $P < 0.05$. Results were expressed as mean ± standard deviation (S.D.).

## 3. RESULTS AND DISCUSSION

### 3.1 Optimization of the SFE Parameters

Different SFE extraction parameters were used to study the effect of the pressure, temperature, extraction time, and percentage of modifiers on the composition and yield of the kaffir lime leaf oil. To analyze the effect of each parameter on the composition of the SFE extracts, four groups of key fragrant component representatives including monoterpenes (β-(Z)- and β-(E)-oicimene), oxygenated monoterpenes (citronella and citronellol), sesquiterpenes (caryophellene and δ-cadinene) and oxygenated sesquiterpenes (nerolidol and elemol) were considered. The quantity of all identified components was investigated by using a percent relative peak area as shown in Table 2.

#### 3.1.1 Pressure Effect

The major volatile constituents of kaffir lime leaf oils were significantly influenced by the extraction pressure, as can be seen in Figure 1A. At 3000 psi, most volatiles (β-(Z)- and β-(E)-oicimene, citronellol, δ-cadinene, nerolidol, and elemol) were resulted in high peak area ratios and slightly decreased when the extraction pressure was increased.

### 3.1.2 Temperature Effect

The peak area ratios of some major volatile constituents of kaffir lime leaf oils obtained by varying the temperature of SFE parameters are shown in Figure 1B. The temperature at 55°C gave the highest peak area ratios of major volatile compounds, but at 65°C peak area ratios of these volatiles significantly decreased. Analysis of the effect of temperature on the composition of the extracts indicated that higher temperatures resulted in lower peak area ratios of the volatiles due to the decrease of fluid density and thus reduced extraction efficiency.

### 3.1.3 Extraction Time Effect

The results of varying the extraction time of SFE from 5 to 25 min are shown in Figure 1C. At 15 min, most volatiles (citronellal, citronellol, δ-cadinene, nerolidol, and elemol) were resulted in high peak area ratios. Extraction time longer than 15 min did not increase peak area ratios of most volatiles.

### 3.1.4 Percentage of Modifiers

The results of kaffir lime leaf oils extract with different modifiers are shown in Table 1. The highest extraction efficiency was obtained when 10% of isopropanol was used as a modifier to the supercritical $CO_2$

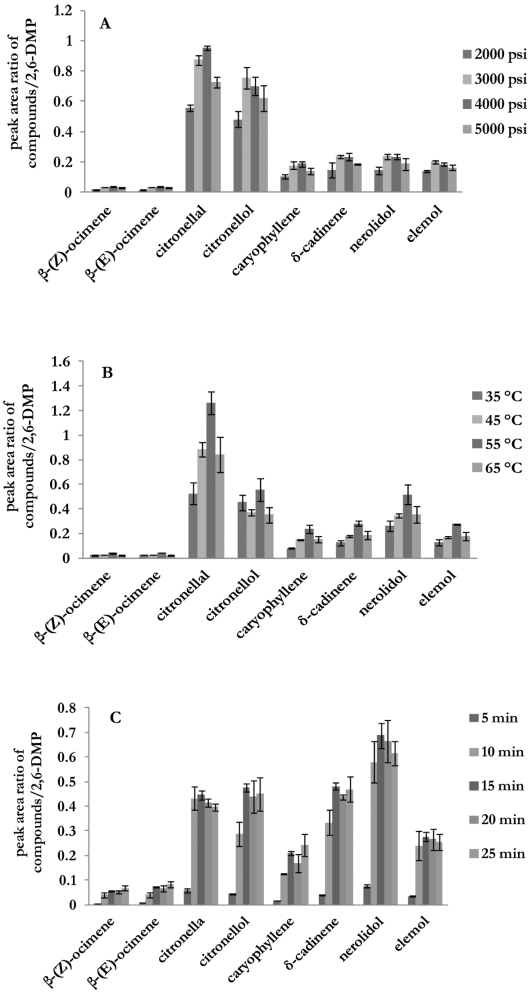


**Figure 1.** Effect of (A) pressure, (B) temperature, and (C) extraction time on peak area ratio of the major volatile constituents in kaffir lime leaf oils.

**Table 1.** Percentage yields of kaffir lime leaf oils obtained by SFE.

| Modifier | Percentage of modifier(v/v) | Yield(%) ± SD |
|---|---|---|
| non-modifier | 0% | 0.15±0.03 |
| isopropanol | 1% | 0.23±0.03 |
| | 5% | 0.27±0.02 |
| | 10% | 0.33±0.03 |
| ethanol | 1% | 0.22±0.03 |
| | 5% | 0.22±0.03 |
| | 10% | 0.17±0.03 |
| methanol | 1% | 0.17±0.03 |
| | 5% | 0.06±0.02 |
| | 10% | 0.05±0.01 |

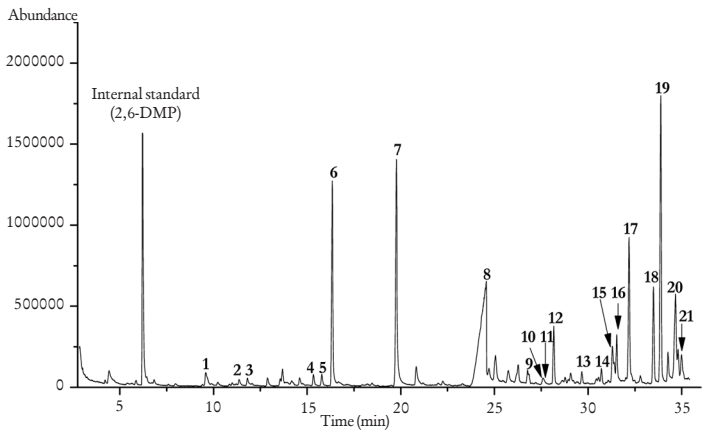


**Figure 2.** GC-MS chromatogram of terpenoid constituents in kaffir lime leaf oil obtained by SFE.

fluid, giving the highest yield of extraction (0.33%). Extractions of kaffir lime leaf oils with modifiers are obvious that using different modifier concentrations did not allow the extraction of any new volatiles. Methanol and ethanol yielded lower concentrations of constituents than those extracted with isopropanol. (Table 2.)

### 3.2 Volatile Constituents Analyzed by GC-MS

Kaffir lime leaf oils obtained by supercritical fluid extraction with $CO_2$ using the optimal condition were analyzed by GC-MS. The GC-MS chromatogram of terpenoid constituents in kaffir lime leaf oil obtained from SFE is shown in Figure 2. Twenty-one terpenoid compounds and their relative amounts in the oils extracted with non-modified $CO_2$ and 10% of modifiers are shown in Table 2. Terpenoids that contained in kaffir lime leaf oil were β-myrcene, β-(E)-ocimene, β-(Z)-ocimene, (Z)-epoxy ocimene, (E)-epoxyocimene, citronellal, citronellol, citronellic acid, α-copaene, β-cubebene, β-elemene, β-caryophyllene, α-humulene, germacrene D, α-muurolene, α-farnesene, δ-cadinene, elemol, nerolidol, spathulenol, and globulol. Citronellal, caryophyllene, citronellol, and

**Table 2.** Composition of kaffir lime leaf oils extracted by SFE with different modifiers.

| Peak no. | Compound | $T_r$(min) | I[a] | I[b] | % relative abundance | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 0% modifier | 10% methanol | 10% ethanol | 10% isopropanol |
| 1 | β-myrcene | 9.59 | 992 | 991 | 0.10 | - | - | 0.14 |
| 2 | β-(E)-ocimene | 11.35 | 1037 | 1037 | 0.04 | - | - | 0.04 |
| 3 | β-(Z)-ocimene | 11.80 | 1050 | 1050 | 0.06 | - | - | 0.06 |
| 4 | (Z)-epoxyocimene | 15.31 | 1129 | 1125 | 0.16 | 0.17 | 0.14 | 0.10 |
| 5 | (E)-epoxyocimene | 15.77 | 1139 | 1139 | 0.20 | 0.18 | 0.15 | 0.10 |
| 6 | citronellal | 16.31 | 1152 | 1153 | 1.41 | 1.91 | 1.81 | 1.33 |
| 7 | citronellol | 19.73 | 1228 | 1226 | 1.39 | 3.22 | 3.21 | 0.87 |
| 8 | citronellic acid | 24.41 | 1335 | 1340 | 4.50 | 11.10 | 7.30 | 2.52 |
| 9 | *a*-copaene | 26.23 | 1376 | 1377 | 0.20 | 0.15 | 0.12 | 0.26 |
| 10 | β-cubebene | 26.75 | 1389 | 1388 | 0.13 | - | 0.07 | 0.12 |
| 11 | β-elemene | 26.84 | 1391 | 1391 | 0.08 | - | 0.08 | 0.09 |
| 12 | β-caryophyllene | 28.15 | 1422 | 1419 | 0.57 | 0.32 | 0.34 | 0.68 |
| 13 | α-humulene | 29.65 | 1458 | 1455 | 0.12 | - | - | 0.15 |
| 14 | germacrene D | 30.69 | 1483 | 1485 | 0.15 | 0.82 | - | 0.16 |
| 15 | *a*-muurolene | 31.38 | 1499 | 1500 | 0.19 | 0.43 | 0.29 | 0.17 |
| 16 | *a*-farnesene | 31.50 | 1502 | 1506 | 0.45 | 0.34 | 0.31 | 0.34 |
| 17 | δ-cadinene | 32.16 | 1519 | 1523 | 1.49 | 1.39 | 1.24 | 1.31 |
| 18 | elemol | 33.46 | 1552 | 1550 | 0.85 | 1.18 | 1.03 | 0.65 |
| 19 | nerolidol | 33.83 | 1561 | 1563 | 2.14 | 3.21 | 2.61 | 2.00 |
| 20 | spathulenol | 34.62 | 1581 | 1578 | 0.97 | 1.34 | 1.17 | 0.82 |
| 21 | globulol | 34.93 | 1589 | 1585 | 0.20 | 0.43 | 0.32 | 0.19 |

[a] Retention indices using a AT-5MS column
[b] Retention indices from KI and literatures

β-myrcene were found as the main constituents in these SFE extracts the same as those reported in the kaffir lime leaf oil extracted by steam distillation [20,21]. Previous researches showed that citronellal and citronellol had high repellent effectiveness against mosquitoes [22]. β-Caryophyllene and nerolidol were also found in essential oil of cinnamon *(Cinnamomum osmophloeum)* twigs which exhibited excellent anti-inflammatory activity [23].

## 4. CONCLUSION

The optimal SFE condition for kaffir lime leaf oil was the use of pressure at 3000 psi, temperature at 55°C, extraction time at 15 min, and type and percentage of modifier at 10% isopropanol that gave the highest yield of extraction (0.33%). All kaffir lime leaf oils showed a pale yellow color. GC-MS analysis of these kaffir lime leaf oils resulted in 21 compounds of terpenoids in groups of monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes. Citronellic acid (4.50%), nerolidol (2.14%), δ-cadinene (1.49%), citronellal (1.41%) and citronellol (1.39%) were the major terpenoid constituents in the kaffir lime leaf oils extracted by supercritical $CO_2$.

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