The major and minor components of Kaffir Lime (*Citrus hystrix* DC) essential oil in the steam distillation process

T N T An¹,², T T K Nguyen¹,², C K Van¹,², H L T Anh³, L V Minh⁴, N V Ay⁵,∗

¹NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
²Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
³Mientrung Institute for Scientific Research, Vietnam Academy of Science and Technology, Vietnam
⁴Research Center of Ginseng and Medicinal Materials, National Institute of Medicinal Materials, Ho Chi Minh City
⁵College of Agriculture, Can Tho University, Can Tho City, Vietnam

*Corresponding author: nvay@ctu.edu.vn

Abstract: Essential oils are natural products used in many fields, including perfumes, cosmetics, aromas, spices and nutrition. Kaffir Lime (*Citrus hystrix* DC), which belongs to the Rutaceae family, is a prominent tree in the southwest region, particularly in An Giang province (Vietnam). Moreover, Kaffir Lime is known as a potential candidate for aromatherapy and cosmetics, a natural alternative for insect repellents, an antioxidant and spice for food. Therefore, the purpose of this study is analyze the phytochemical content of Kaffir Lime essential oil using the steam distillation method. The process of steam distillation was conducted by continuously applying heat onto 500 g of imported Kaffir Lime peels. After 15 min of condensation, the yield of essential oil extraction achieved 1.6%. The essential oil was primarily composed of monoterpenoid hydrocarbon, aldehyde and ester with sabine (22.875%), β-pinene (33.939%), D-limonene (15.847%) and β-citronellal (14.791%) being the main components. Other ingredients included α-pinene (3.099%), β-myrcene (0.836%), eucalyptol (0.354%), γ-terpinene (0.476%), 4-terpineol (2.246%), α-terpineol (1.426%) and linalool (1.139%). The presented extraction process as well as phytochemical profiles of Kaffir Lime suggested further studies on potential bioactivities of these constituents.

1. Introduction

Essential oils (EOs) are oily liquids existing in various plant parts such as stems and leaves as a secondary metabolite [1–5]. The tropical environment in Vietnam favours the growth and development of plants, especially essential oil-bearing ones, promoting their use in the pharmaceuticals, food and cosmetics [6–11]. Therefore, studies on EOs and their relevant extraction processes are gaining currency in scientific literature.

Citrus family, which is one of the world's leading commercial fruit trees, is also known as a significant plant producer of EOs. Kaffir Lime (*Citrus hystrix* DC), from the Citrus family is a native Vietnamese Lime species grown endemically in the Seven Mountains (An Giang province, Vietnam). It is a small to medium woody plant with round, rough, green fruits, which is commonly used in Southeast Asian cuisine, especially in Thai cuisine [12]. In addition, Kaffir Lime has long been used in folk medicine as a medicinal plant [13], due to the antifungal, antibacterial, antioxidant, antiviral, anti-
infectious properties against leukemia cells and cytotoxic activity, as shown in previous studies [19-20]. Unfortunately, despite the widespread use of the fruits, its potential has not been fully exploited. To be specific, while the fruit flesh is widely used for consumption and juice production, the peels are often discarded, producing tremendous amount of agricultural wastes [26]. Therefore, it is proposed that fruit peels of Kaffir Lime should be utilized as a source for EOs extraction.

Traditionally, the recovery of EOs was realized by performing hydrodistillation (HD) using their peels as starting material. Due to the sensitivity of EOs to temperature and chemical changes, the standard HD may pose some disadvantages in terms of low extraction efficiency, compound fluctuations, thermal degradation of unsaturated substances and esters [14]. Therefore, numerous extraction techniques have been developed, including supercritical fluid extraction [15], steam distillation [7], ultrasound-assisted extraction [16], and microwave-assisted hydrodistillation (MAHD) [10]. In the present study, steam distillation was used as an effective extraction method with lowered energy consumption while increasing yield and maintaining essential oil properties. The consistency of the EOs can be calculated by examining gas chromatography-mass spectrometry (GC-MS) analysis. Camphene, α-pinene, β-pinene, mycrene, sabinene, limonene, γ-terpinene, terpinolene, copaene, linalool, citronellal, citronellol, terpinen-4-ol, geraniol were the main compounds in Kaffir Lime [7, 17-18].

The purpose of this study is to present an extraction process Kaffir Lime peels EOs for efficient recovery and preservation of EOs quality, and to identify EO chemical components.

2. Materials and methods

2.1. Plant materials
Kaffir Lime fruit were collected in An Giang province (Vietnam) (10°22’52.02”N, 105°25’11.58”E). Fresh, ripened berries with smooth surface were selected as starting materials, thoroughly washed and had stalks and flesh removed to afford the peels. Obtained peels were pureed in a uniform size of > 2mm. This is justified by the fact that very small material becomes sticky when distilling, making the penetration process of steam and diffusion of essential oil difficult. Raw materials after pre-treatment was placed into distillation equipment.

2.2. Steam distillation
The steam distillation process was used to obtain EO from Kaffir Lime and the Clevenger device was employed in this study. Unlike hydrodistillation, steam distillation involves an advantage of no immediate soakage of raw materials in water. When steam passes through the organic material, the vesicles that contain EOs are forced to release EOs without causing any damage to the components.
An amount of 500 g of finely ground lemon peels were placed in a separate 1000 mL flask and 1500 mL of water was added to the other flask. The water container was heated on a stove and covered by a heating layer. The volatile essential oil evaporated in the form of the steam, passed through the condenser and accumulated. When the difference in reading essential oil volume was remained constant, the distillation process was stopped. EOs after extraction was dehydrated with sodium sulfate. EOs were stored in dark-colored glass bottles for GC-MS analysis.

2.3. GC-MS analysis
Gas Chromatography-Mass Spectrometry (GC-MS, GC Agilent 6890 N, MS 5973 inert, Agilent Technologies, Santa Clara, CA, USA) has been used for content analysis of Kaffir Lime EO. First, 25 μL of EOs was mixed with 1 mL of n-hexane (Sigma-Aldrich, St. Louis, MO, USA), and then dehydrated with Na₂SO₄. Head column pressures for the HP5-MS panel were 9.3 psi. 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 250 °C; oven temperature progress included an initial hold at 50 °C for 2 min, then increased to 80 °C at 2 °C/min, 150 °C at 5 °C/min, 200 °C at 10 °C/min and reached 300 °C at 20 °C/min held for 5 min.

3. Results and discussion
After extraction process, Kaffir Lime EOs was obtained with the yield of 1.6%. The EO was liquid, pale yellow and had a characteristic citric aroma. However, compared to the previous study where EO was isolated from Kaffir Lime harvested from the area in Selangor (Malaysia). To be specific, Muhammad et al. (2013) carried out steam distillation with induction heating systems at varying extraction temperatures, resulting in the recovery performance of approximately 1.7%—3.5% [21]. On the other hand, result of current yield is similar with that of Kasuan, where a yield of 1.34% was obtained with the same extraction method and material source [18]. These differences may relate to environmental changes that plants are exposed to, such as light, temperature and humidity, significantly affecting the release of volatile compounds of the compound leading to variation in productivity and chemical composition.

Table 1 and Figure 2 demonstrated the chemical composition result of the Kaffir Lime EO obtained from GC-MS analysis. Retention time is the time at which the compound was observed from GC column
during elution. The results indicated the presence of eighteen different retention time values, corresponding to eighteen volatile compounds existing at different concentrations and contributing to the total essential oil. Kaffir Lime EOs were characterized by the abundance of various constituents including β-pinene (33.939%), sabinene (22.875%), D-limonene (15.847%), β-citronellal (14.791%), 4-terpineol (2.246%) and α-terpineol (1.426%). Compared to other Citrus EOs where limonene was often detected at the predominant component of at least 95%, C. hystrix EO contained limonene at a relatively lower content [22]. This finding was in line with another study where peel-extracted EO of C. hystrix collected in Sri Lanka was obtained by HS-SPME (Headspace Solid-Phase Micro Extraction) and its composition was analyzed. To be specific, a total of 45 compounds were detected with main components being citronellal (12.267%), α-pinene (9.244%), 3-carene (18.310%), D-limonene (11.538%), α-cadinene (4.290%), copaene (4.290%), linalool (4.020%), caryophyllene (3.988%) and γ-cadiene (3.544%), accounting for about 71% of the total detected compounds [17].

The intrinsic property variations of C. hystrix may be mainly due to different extraction techniques, storage conditions, fruit ripeness, environmental factors and different transmissions, crop varieties and different geographic and climatic regions.

Jantan et al. (1996) conducted an analysis of the chemical composition of EOs belonging to the Citrus family, showing that the bark of C. hystrix contained EO composed mainly of monoterpenes (97.2%). Of which, limonene (14.2%), β-pinene (39.3%), terpinen-4-ol (8.9%) and citronellal (11.7%) were the main components. Other monoterpenes with significant amounts were citronellol (3.0%), α-terpineol (3.0%), γ-terpine (2.4%), α-pinene (2.0%) and linalool (1.9%). Seventeen sesquiterpenoid compounds were identified in EO but in small quantities and only accounted for 2.6% of the essential oil content [23]. Hongratanaworakit and Buchbauer (2007) showed that EO of Thai C. hystrix was mainly composed of hydrocarbon monoterpenes with β-pinene and limonene (30.73%) being the main component. Other minor components were α-terpineol (8.35%), terpinen-4-ol (10.63%), γ-terpine (6.18%), terpinolene (5.09%) and α-terpine (5.09%) [24]. The abundance of monoterpenes was also observed in leaf EO of C. hystrix, in which oxygenated monoterpenes accounted for 86.15% of the total EO and the major component was found to be β-citronellal (66.85%) [25]. The present compositional results showed lower content of citronellal compared to results of aforementioned studies. However, the abundance of β-pinene, sabinene and limonene that are exclusively exist in the peels might confer the derived EO a dry, spicy and refreshing aroma.

Table 1. The main ingredients of Kaffir Lime EO by steam distillation

| Peak | R.T.   | Name      | Pct Total |
|------|--------|-----------|-----------|
| 1    | 7.230  | α-pinene  | 3.099     |
| 2    | 8.997  | Sabinene  | 22.875    |
| 3    | 9.102  | β-pinene  | 33.939    |
| 4    | 9.907  | β-myrcene | 0.836     |
| 5    | 11.538 | α-cymol   | 0.426     |
| 6    | 11.737 | D-limonene| 15.847    |
| 7    | 11.831 | Eucalyptol| 0.354     |
| 8    | 13.473 | γ-terpinene| 0.476    |
| 9    | 13.954 | unknown   | 0.441     |
| 10   | 16.097 | Linalool  | 1.139     |
| 11   | 19.203 | β-citronellal| 14.791  |
| 12   | 20.197 | 4-terpineol| 2.246     |
| 13   | 20.887 | α-terpinol| 1.426     |
| 14   | 27.663 | Copaene   | 0.547     |
| 15   | 28.123 | β-cubebene| 0.427     |
16 28.186 β-elemen 0.117
17 28.980 β-caryophyllene 0.444
18 31.866 Cadinene 0.571

Figure 2. Chromatogram of Kaffir Lime EO by GC-MS

4. Conclusions
An EO extraction process was presented utilizing the peels of C. hystrix fruits collected from An Giang province (Vietnam) as the source material and the obtained EO was characterized for chemical composition via GC-MS. Steam distillation of C. hystrix fruit peels produced EO with the yield of 1.6%. A total of 18 volatile compounds were identified, with the main components including β-pinene (33.939%), sabinene (22.875%), D-limonene (15.847%), β-citronellal (14.791%), 4-terpineol (2.246%) and α-terpineol (1.426%). Further studies should determine the bioactivities of specific compounds and establish optimization study to enhance the quality and stability of the EO.

5. References
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