Determine the components of Kaffir Lime Oil (Citrus Hystrix DC.) in the Microwave-assisted Extraction Process

T T Hien1,2, N T C Quyen1,2,3, P T H Minh3,4 and X T Le5,6,*

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; 3Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Vietnam; 4Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam; 5Department of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam

Email: tien.le@hcmut.edu.vn, labasm2013@gmail.com

Abstract. Essential oils (EOs) are a complex element consisting of dozens to hundreds of compounds different. The essential characteristics of essential oils are governed by the main components of essential oils including oxygen monoterpen, hydrocarbon monoterpen, oxygen sesquiterpene, carbonylic compound, phenol, fatty acids and esters, which determine the primary aroma of essential oil. Kaffir lime EOs (Citrus hystrix DC; Rutaceae) is increasingly being used as a flavoring in perfume, cosmetic industries and food. Therefore, the purpose of this study is to extract essential oils from Kaffir lime to determine the components that create this fragrance. Kaffir lime EOs is obtained from 100g of fresh citrus peels of Citrus hystrix DC by microwave extraction method in 60 minutes and 450W power. The quality of Kaffir lime peel oil is assessed based on the chromatography of the compound present by the gas chromatography–mass spectrometry (GC-MS). Shell analysis led to the identification of twenty-six compounds that make up 100% of the essential oil and yield is 6%. The main compounds in kaffir lime shells such as β-pinene (45.206%), α-pinene (2.365%), D-limonene (18.358%), citronellal (17.745%), terpinen-4-ol (4.9964%).

1. Introduction

Essential oils (EOs) are widely used in everyday life, most notably as components of disinfectants and insecticides in the cosmetics industry such as various cologne waters, bath lotions, hair lotions, shampoos. [6]-[10]. Kaffir lime was used as the primary source of raw materials and originated in the province of An Giang, Vietnam. Lemon is also a well-established ingredient of extensive use in traditional Chinese medicine and is known as with various names, depending on the type as Citrus aurantifolia or lemon peach.
Given the abundant source of raw materials, the production of lemon-derived products, especially the lemon essential oils, has become rapid. According to many studies, the antioxidant, and antimicrobial properties of lemon EOs are widely used in food (confectionery, drinks, etc.) in medicine and are being studied for the treatment of various diseases, improving immunity and digestion. Lemon oil may come from various parts of the plant, such as leaves, stem, shoots, but mainly in lemon peel. Different extraction methods have been developed and applied in practice in industrial applications regarding essential oil production, including solvent extraction methodology, steam distillation methodology, compression methodology, and microwave methodology [11]-[15].

Today an alternate approach has been developed for the extraction of natural products using microwave technology. Because of the heating efficiency, faster heat transfer and eco-friendliness, the process of using microwaves to assist the process of extracting essential oils has become popular in experimental and industrial laboratory production. Microwave technology has been used in several industries, such as the manufacture of biodiesel, synthetic chemicals and food manufacturing [16],[17]. The use of microwave in extracting essential oils to help improve productivity and quality of essential oils, and shorten extraction time. Therefore, we choose this extraction method for of lemon essential oil. The aim of the research mentioned includes extracting EOs from Kaffir lime (Citrus hystrix DC) leaves in a specific optimal condition (60 minutes and 450W) using microwave extraction method and assessing the content of essential oil compounds obtained by the GC-MS method.

2. Materials and method

2.1. Materials
The fresh lime from Kaffir was collected from Vietnam's An Giang province (10 ° 22′ 52.02″ N, 105 ° 25′ 11.58″ E). To have the flesh separated, the fruits were sprayed with water and removed. Kaffir Lime peels are isolated from clean fruit and pureed before being brought into the extraction system by Solvent Free Microwave Extraction method.

2.2. Extraction method essential oil
Solvent Free Microwave Extraction (SFME): The extraction system extracts the Kaffir lime peels to obtain the EOs. The device consists of a MW71E Microwave Oven manufactured by SAMSUNG (Vietnam) that is called a heat source for the extraction process. In addition, the device is attached to the hydrodistillation apparatus (purchased by Bach Khoa Limited Liability Company, Vietnam) used to condense the extracted oil and water process and isolate it. Finally, the essential Kaffir lime oil is dried with sodium sulphate, which is bought from Sigma Aldrich (US). The criteria used were 60 minutes and 450W of electricity for 100 grams of Citrus hystrix DC fresh Kaffir lime peels.

2.3 GC-MS
Gas Chromatography-Mass Spectrometry (GC-MS) is used to evaluate all two extraction methods of the components found in EOs. 25 μL sample of 1.0 mL n-hexane essential oil and Na2SO4 dehydrated. Name equipment: GC Agilent 6890 N, MS 5973 inert. HP5-MS column, head column pressure 9.3 psi. Under the following conditions, GC-MS was obtained: carrier gas He; flow rate 1.0 mL/min; injection temperature 250°C, split 1:100; injection volume 1.0 μL. The development of the oven temperature involved an initial keep at 50°C for 2 min, an increase to 80°C for 2°C/min, an increase to 150°C for 5°C/min, an increase to 200°C for 10°C/min and an increase to 300°C for 5 min.

3. Results and discussion
GC-MS determined the chemical composition of EOs peels of the Kaffir lime EOs (Rutaceae). The findings of the study by GC-MS in Table 1 reveal that the oil obtained comprises twenty-seven major chemical
constituents including β-pinene (45.206%), α-pinene (2.365%), D-limonene (18.358%), citronellal (17.745%), terpinen-4-ol (4.9964%), γ-Terpinene (1.034%), α-Terpineol (1.768%), β-Citronellol (1.241%), and the other minor compounds. The total amount of hydrocarbon monoterpenes is 100%. Because the maximum at 9,123 minutes has the greatest intensity, this indicates that β-Pinene is the most important component in kaffir lime essential oil (Figure 1). In addition, D-Limonene was found in this study, consistent with another study in which D-Limonene was also found in the exalted content of kaffir lime peels EOs. Also, the specific dosage of aldehydes has not been tested in this study although their role is the primary measure of the quality of the oil. Variations in production and composition of essential oils can be attributed to biological differences in plant materials, parameters of extraction as well as plant environment conditions [18].

Figure 1. GC chromatogram of kaffir lime essential oil

Typically, the compounds discovered in GC-MS analysis are hydrocarbons in nature. [19] In Malaysia's kaffir lime peels essential oil, the dominant components are sabinene (46.573%), β-pinene (13.509%), limonene (17.232%), myrcene (1.804%), citronellal (7.809%) and terpinene-4-ol (2.418%). In another report [20], abundant ingredients in kaffir lime essential oil are β-citronellol (11.03%), β-citronellol (11.03%) L-linalool (13.11%), citronelyl acetate (6.76%) and sabinene (5.91%). Several components with a compound of just > 1% are: components with only compound of just > 1 βre: components wti (-)-isopulegol (1.57%), cis-Linalol oxide (1.86%), nerolidol (1.11%), 4-terpeneol (1.52%), and trans-eneol (1.52%), colorless hydrocarbon. This difference can be explained by the geographical conditions, growth capacity, cultivars, types of plants, and time to harvest Kaffir lime, besides the conditions of extraction and collection of essential oils in each place may be different. More analysis of the α-limonene structure in Figure 2a demonstrates that this material is an aliphatic and colorless hydrocarbon.

Figure 2a revealed that the compound is a major component of C. hystrix suitable for studies of a species of Citrus, Citrus limon (L.) Burm. f. [21]. The previous study also reported to be the second most common ingredient in the EO of Citrus limon [22]. Some plants have had β-pinene, limonene and sabinene derived from EOs such as Lamiaceae [23].

Figure 2b showed that α-limonene is an aliphatic, colourless hydrocarbon. Limonene is also classified as stable monoterpenes and cyclic. Moreover, limonene can be extracted at high temperatures without
decomposition. Because limonene displays high volatility, strong oxidative property, low solubility in water, and antibacterial activity [20], it is proposed that essential pomelo oil may be used as a strong radical antioxidant, an antibacterial agent, and an anti-free agent [24-25]. Besides, Citronellal is known for its insect repelling capability.

| Peak | R.T. | Name                  | This study | Malaysia [19] | Indonesia [20] | Thailan [21] |
|------|------|-----------------------|------------|---------------|----------------|--------------|
| 1    | 7    | α -Thuene             | 0.156      | 0.233         | -              | 0.35         |
| 2    | 7.23 | α -Pinene             | 2.365      | 3.262         | -              | 4.3          |
| 3    | 7.816| Camphene              | 0.154      | 0.155         | -              | -            |
| 4    | 9.112| β-Pinene              | 45.206     | 13.509        | 1.24           | 24.62        |
| 5    | 9.896| β-Myrcene             | 0.909      | 1.804         | 1.27           | 1.92         |
| 6    | 10.62| Sabinene              | -          | 46.573        | 5.91           | 22.06        |
| 7    | 11.089| unknow                | 0.597      | -             | -              | -            |
| 8    | 11.758| D-Limonene           | 18.358     | 17.232        | -              | 19.29        |
| 9    | 13.452| γ-Terpinene          | 1.034      | 1.354         | -              | 0.4          |
| 10   | 14.299| cis-Linalool Oxide   | 0.905      | -             | -              | -            |
| 11   | 15.198| Cyclohexene          | 0.142      | -             | -              | -            |
| 12   | 15.261| trans-Linalool oxide | 0.604      | -             | -              | -            |
| 13   | 16.087| β-Linalool            | 0.92       | 1.156         | 13.11          | 0.97         |
| 14   | 18.597| unknow                | 0.21       | -             | -              | -            |
| 15   | 19.234| Citronellal          | 17.745     | 7.809         | 46.40          | 10.58        |
| 16   | 20.207| terpinen-4-ol       | 4.964      | 2.418         | 1.52           | 2.31         |
| 17   | 20.876| α-Terpineol         | 1.768      | 0.907         | -              | 1.55         |
| 18   | 22.664| β-Citronellol       | 1.241      | -             | 11.03          | 1.78         |
| 19   | 27.088| unknow                | 0.36       | -             | -              | -            |
| 20   | 27.663| Copaene              | 0.421      | -             | -              | -            |
| 21   | 28.123| β-Cubenbe            | 0.305      | -             | -              | -            |
| 22   | 28.186| β-Elemen             | 0.126      | -             | -              | -            |
| 23   | 28.991| Caryophyllene       | 0.402      | -             | -              | -            |
| 24   | 29.984| α -Caryophyllene    | 0.111      | -             | -              | -            |
| 25   | 30.779| Germacrene D       | 0.209      | -             | -              | -            |
| 26   | 31.877| β-Cadinene         | 0.535      | -             | -              | -            |
| 27   | 32.483| Elemol               | 0.254      | -             | -              | -            |
4. Conclusion
In this research, Microwave extraction process used to remove essential oil from the Kaffir lime peels. The oil collected has been tested by GC-MS for chemical composition. The yield of extraction achieved 6%. The findings of the study of GC / MS showed 26 prevailing components that exist in the natural oils of kaffir lime. The most abundant component is as β-pinene (45.206%), α-pinene (2.365%), D-limonene (18.358%), citronellal (17.745%), terpinen-4-ol (4.9964%). It can be inferred that the chemical composition of kaffir lime EOs (Rutaceae) peels corresponds with the climatic conditions in which they are produced. The EOs of kaffir lime peels from Vietnam is ideal for use as an antibacterial agent.

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