Extraction of Kaffir Lime (Citrus hystrix DC.) essential oil by steam distillation and evaluation of chemical constituents

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Abstract. Citrus hystrix which is commonly known as Kaffir Lime, is a plant with great potential for research and commercialization. C. hystrix essential oil is used as a fragrance to increase appetite in food, or sweet-smelling in cosmetics and antibacterial properties in the health field. Most research have focused on the peels, yet the EO content in C. hystrix leaves is also noteworthy. There are many essential oils extraction methods from conventional to advanced techniques. However, it is necessary to consider the influence of heat during heat transfer to constituents in materials, which easily decompose the volatile compounds contained in the essential oil. Steam distillation method can limit this disadvantage by separating the solvents and materials into two places that are only connected by a steam pipe. In this study, the steam distillation method was employed to extract the EOs from C. hystrix leaves. The chemical components in this EOs was evaluated by gas chromatography–mass spectrometry (GC-MS) method. C. hystrix leaves achieved an essential oil yield of 1.24%. Moreover, different compounds in Kaffir Lime EOs obtained from steam distillation method were identified, including β-citronellol, citronellyl acetate, linalool, caryophyllene, pinene, sabinene, and naphthalene. Findings from the present study provide helpful insights to effective essential oil extraction from C. hystrix leaves with high content of valuable phytochemical compounds.

1. Introduction

Nowadays, there are different methods of extracting essential oils (EOs) from various plants, including hydrodistillation [1–3], steam distillation [4,5], cold-pressed [6–8], modern methods include microwave [9–11], ultrasonic-assisted method [12,13], liquid compression extraction method [14], and supercritical carbon
dioxide extraction methods [15,16]. However, modern methods often bring expensive installation and operating costs on the scale of laboratory research and industrial production. Steam distillation, which is considered to overcome the limitations of modern methods, is also the most common method used in plants to produce essential oils [17,18]. Essential oils are produced by steam distillation entrainment of high purity, and the ability of compounds to decompose because of heat is meager. In this method, only water vapor is carried over the surface of the material. It draws the essential oil from the material into the environment, which is not directly in contact with the overheated water [19–21].

Essential oil extraction is widely performed in the Citrus genus. This plant is grown in many places with the tropical monsoon climate in Vietnam. In particular, Citrus hystrix DC, which is commonly known as Kaffir lime, with parts from leaves, fruits, seed, containing essential oils. Most production of essential oil was from the fruit. However, the plant leaves also contain valuable volatile compounds such as β-citronellol, citronellyl acetate, linalool, and caryophyllene, beside the main compounds such as α-pinene, eucalyptol, camphene, bicyclo hept-3-en-2-one, caryophyllene, endo-borneol, and bornyl acetate [22]. Furthermore, all of these components have antioxidant, antibacterial, and antifungal properties in folk medicine, food, and cosmetics as a natural fragrance source published by many scientific studies. [23–25]. Therefore, C. hystrix leaves can be considered as an alternative source for essential oil extraction.

Due to the advantages which has been discussed in the previous paragraphs, steam hydrodistillation has been selected to extract EOs from C. hystrix DC leaves in a specific optimal condition. The chemical content of the obtained oil was subjected to identification by the GC-MS method.

2. Materials and method

2.1. Raw materials preparation

C. hystrix was collected directly from the garden houses situated in An Giang province, Vietnam (10° 22′ 52.02″ N, 105° 25′ 11.58″ E). in February 2020. After collection, C. hystrix leaves are washed to remove foreign matter, discard crushed leaves during transportation, leaves are pests, and choose intact leaves, preferably mature ones. Leaves after processing will be pureed into the flask of the extraction equipment.

2.2. Chemicals and reagents

Chemicals used in this study included anhydrous sodium sulfate (Na₂SO₄) (Sigma-Aldrich Chemie, Co Ltd (USA). Deionized water is used as a solvent by the Milli-Q purification system (Millipore, USA) to extract oil from C. hystrix leaves.

2.3. Steam distillation extraction

The steam distillation process with the same glassware and identical operating conditions was utilized for traditional steam distillation (purchased in Bach Khoa Co., Ho Chi Minh City, Vietnam). But unlike the method of hydrodistillation, steam distillation does not demand that the raw material be submerged directly in water. The essential advantage of this method of extraction is that the substance and water are not in close interaction with each other, as seen in Figure 1. First, 100 g of C. hystrix leaves were imported into a flask separate from the water container. The steam created by boiling water passed through the material, vaporized and released the essential oil, then passing to the receiving vessel via the condensing system. The extraction process took 30 min, heated by an electric stove with a capacity of 500 W. Water in the obtained essential oil was removed by using anhydrous sodium sulfate, then stored in a closed vial, before GC-MS analysis. The yield of obtained oil from C. hystrix leaves (%) is determined using the following Equation (1):
Yield of *C. hystrix* leaves oil (%) = \[ \frac{\text{the amount of essential oil obtained (g)}}{\text{the amount of raw material (g)}} \]  

(1)

**Figure 1.** Model team distillation method to extract the EOs from the leaves of *C. Hystrix.*

2.4. Identification of components by GC-MS analysis
An Agilent Technologies HP7890A GC device combined with an Agilent Technologies HP5975C Mass Spectrum Detector (MSD) and a DB-XLB column (60 m, 0.25 mm, film thickness 0.25 μm, Agilent Technologies) were used to perform GC-MS analysis. The temperature of the injector and detector was set at 250 and 280 °C, respectively. The thermal improvement of the column temperature was started at 40 °C, accompanied by a 20 °C / min rise to 140 °C and a corresponding 4 °C / min rise to 270 °C. At a flow rate of 1 mL / min the carrier gas was helium. Splitting with the break ratio of 100:1 poured a 1 μL of essential oils. The parameters for MSD is as follows: 70 eV ionizing voltage, 40 mA emission current, 35-450 amu acquisitions sample mass range under maximum analysis. A homologous *n*-alkane series was used as a standard for determining the retention time indices (RI). The relative quantities of individual components were calculated without correction, based on the GC peak area (MSD response).

3. Results and discussion
Under extraction conditions of steam capacity at 500 W, extraction time of 60 min after 20 min of the first drop of water condensation, the obtained essential oil is a pure, pale yellow liquid with a slight aroma and is highly volatile, the density of essential oil is 0.8863 ± 0.002 (g/cm³), the volume of essential oil is measured to be 1.4mL. Therefore, the yield of essential oil calculated by Equation (1) was 1.24 %. The obtained essential oil is a pure, pale yellow liquid with a slight aroma and is highly volatile. The results of Nurhani Kasuan et al. showed that the yield was 0.43% for leaves and 1.34% for peels [26].
When comparing the yield of *C. hystrix* leaves EOs obtained from steam distillation (SD) with other extraction methods in previous studies, the results in Kasuan et al. [26], Dugay et al. [23], Ramadhan and Iftitah [27] and Wulandari [25], the yields were 0.18%, 0.66%, 0.55%, 0.85%, respectively. Thus, the steam distillation method is shown to be superior for higher yields of essential oils, which are contributed by a number of plant growth conditions such as species, climate, material movement. On the other hand, using the similar extraction method, the yield from peels material reached 1.8% [28]. Therefore, as compared to the peel, the leaves of *C. hystrix* also have essential oils, yet at a lower content than the peels trivial.

![Figure 2](image)

**Figure 2.** The chromatograms of compounds in essential oil from *C. hystrix* leaves by steam distillation.

| Peak | Retention time (min) | Name     | Content (%) | Structural formula |
|------|----------------------|----------|-------------|--------------------|
| 1    | 8.955                | Sabinene | 1.004       | ![Structural formula](image) |
| 2    | 11.737               | D-limonene | 0.401     | ![Structural formula](image) |
| Peak | Retention time (min) | Name                  | Content (%) | Structural formula |
|------|----------------------|-----------------------|-------------|--------------------|
| 3    | 16.097               | Linalool              | 1.622       | ![Linalool](image)  |
| 4    | 19.328               | Citronellal           | 86.89       | ![Citronellal](image) |
| 5    | 22.664               | β-citronellol         | 4.899       | ![β-citronellol](image) |
| 6    | 27.056               | Citronellol acetate   | 2.919       | ![Citronellol acetate](image) |
| 7    | 27.673               | Copaene               | 0.263       | ![Copaene](image)   |
| 8    | 28.06                | Geranyl acetate       | 0.036       | ![Geranyl acetate](image) |
| 9    | 28.123               | β-cubebene            | 0.23        | ![β-cubebene](image) |
| 10   | 28.185               | Cyclohexane           | 0.09        | ![Cyclohexane](image) |
| 11   | 28.991               | Caryophyllene         | 0.956       | ![Caryophyllene](image) |
| 12   | 31.877               | Cadinene              | 0.331       | ![Cadinene](image) |
| 13   | 33.194               | Caryophyllene oxide   | 0.36        | ![Caryophyllene oxide](image) |
The results of the GC-MS study in *C. hystrix* leaves oil obtained by steam distillation was shown in Figure 2 and Table 1. A total of 13 volatile components were identified, including citronellal (86.89%), sabinene (1.004%), linalool (1.622%), β-citronellol (4.899%), citronellol acetate (2.919%), caryophyllene (0.956%), and all the other compounds were below 1%. Due to the highest content, citronellal can be considered as the main ingredient of *C. hystrix* leaf essential oil. This result was consistent with the previous studies of Othman et al. (2016) [21], in which the essential oil extracted from Malaysian *C. hystrix* was also dominated by citronellal (61.0%–73.0%), along with β-citronellol (13.4%), limonene (5.9%), citronellyl acetate (2.0%), and sabinene (1.6%) as major components. Meanwhile, citronellal (72.4%), citronellol (6.7%) and citronellyl acetate (4.1%) were the main components of *C. hystrix* leaves oil, as reported by Agouillal et al. [22]. Therefore, it can be seen that the leaves of *C. hystrix* grown in Vietnam have a higher concentration of citronellal than other places, which may support the research of this compound in plant extraction for application in other fields such as medicine, food and cosmetics.

4. Conclusions
The present study employed steam distillation to extract essential oils from *C. hystrix* leaves and identify the phytochemical constituents of the obtained oil using GC-MS analysis. The essential oil yield achieved 1.24% with Citronellal as the main ingredient, accounting for 86.89% of the plant total chemical content. This study helped to determine the composition of the EOs of *C. hystrix* leaves and provides useful knowledge for subsequent studies.

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