Evaluate the chemical composition of Kaffir lime (Citrus hystrix) essential oil using the classical method

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Abstract: Natural compounds have received much attention and research in recent years since the industrial economy developed. Essential oils, which contain many volatile constituents and aromatic compounds, have been around for a long time but are still considered to this day thanks to their natural properties and their unique application. There are several ways of collecting essential oils from herbal plants, including ancient approaches to contemporary techniques using different sections of plants such as buds, vegetables and nuts. In this study, the leaves of Kaffir lime (Citrus hystrix) were used to investigate the yield of essential oil extracted by the classical distillation system (hydro-distillation). The obtained essential oil was then analyzed for the chemical composition by gas chromatography–mass spectrometry (GC-MS). A total of 100 g of Kaffir lime leaves, under the following conditions: 1:3 of ratio of material to water, 120 °C of the temperature within 60 min. The extraction process yielded 1.3 mL of essential oils. GC-MS analysis also identified 16 compounds in the essential oil, accounting for 99.99% of total oils. The main compounds included citronellal (85.4%), citronellol (6.8%), linalool (1.9 %), citronellyl acetate (1.7%), and other compounds with content less than 1.0% by hydro-distillation. The findings of chemical composition of Kaffir lime leaves EO suggest further studies on optimization the extraction process for a desirable phytochemical content.

1. Introduction
Over the years, the production of essential oils (EOs) from fruits, seeds, tubers, stems and leaves has significantly increased to become one of the world's most important natural sources. [1-3]. EOs are best known for possessing several biological activities, including fungicidal, bactericidal, and virucidal [4-7]. EOs are considered as medicinal and their aroma is used in preserving food and make anti-inflammatory [8], analgesic [9], and antispasmodic drugs [10]. To this day, these characteristics remain unchanged and also help scientists know more about molecular mechanisms behind the biological activities of essential oils work, especially their antibacterial activity.

EOs from fruit of the Citrus genus are the largest source of natural compounds worldwide [11,12]. In these EOs, bioactive compounds with various health properties, including antibacterial, antioxidiant, cellular, and anti-inflammatory activities [13,14]. Citrus EOs (especially Kaffir lime) are commonly...
used in the food and cosmetics industries to manufacture perfumes, detergents, and products for body care. [15,16]. However, Kaffir lime EOs are often extracted from the fruit; whereas the EOs content in other plant parts such as leaves or stems remains limitedly known. Hydro-distillation, which extracts EOs using water, is a classical method of extraction that is still used at present and is widely applied in industry thanks to its easy operation and low cost, as compared to other methods such as ultrasonic and supercritical fluid extraction methods [28]. There have been many studies on the process of extracting EOs from herbs by hydro-distillation [17–21]. This proves that hydro-distillation method is highly preferred, bringing high efficiency and practical applications in the industry. In this study, the leaves of Kaffir lime (Citrus hystrix DC.) were hydro-distillated for EO extraction and the amount of volatile compounds in EO were analyzed using gas chromatography – mass spectrometry (GC-MS). The results of oil yield and phytochemical content were compared with previous studies.

2. Materials and Method

2.1 Plant sample preparation
Kaffir lime (Citrus hystrix DC.) leaves were collected from An Giang province, Vietnam (10° 22′ 52.02″ N, 105° 25′ 11.58″ E). The leaves were then washed with water several times before being placed in a non-hygroscopic container and stored in a cooler at temperatures below 10 °C (LC-1416B, Alaska, Vietnam). Prior to extraction process, the leaves were completely grounded as the reduced size would help increasing the surface area of the material, thus causing the extraction process to occur more rapidly and efficiently.

2.2 Extraction method
A total of 100 grams of grounded Kaffir lime leaves were placed in a 1000 ml volume flask attached directly to the Clevenger Apparatus (Vietnam) and heated by Manual Heating Mantle (1000.EU.05, 300 W, Glassco Laboratory Equipment Pvt. Ltd. India). The extraction process was performed at 120°C within 60 min of time. The critical oil was obtained and dehydrated with sodium sulfate (Na₂SO₄, Sigma Aldrich), then placed in a storage tank at 10 °C after the extraction process is finished. The extraction process using hydro-distillation was demonstrated in Figure 1. The yield of EO from Kaffir lime leaves (%) is calculated by the following formula (1):  

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\text{Yield of Kaffir lime leaves oil (%) = \frac{\text{the amount of essential oil obtained (g)}}{\text{the amount of leaves originally used (g)}}}
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![Figure 1. The process of extracting essential oil from Kaffir lime leaves](image-url)
2.3. Identification of components by GC-MS
GC-MS analysis was used for the study of components found in Kaffir lime leaves EOs. Name equipment: GC Agilent 6890 N, MS 5973 inert. HP5-MS column, head column pressure 9.3 psi. GC-MS was collected under the following conditions: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 μL; injection temperature 250 °C; an initial was kept at 50 °C for 2 min; an increase to 80 °C at 2 °C/min; an increase to 150 °C at 5 °C/min; an increase to 200 °C at 10 °C/min; and an increase to 300 °C at 20 °C/min.

3. Results and Discussion
The resulting Kaffir lime EOs was a light yellow colored liquid that has been dehydrated by the solid Na2SO4 with fresh aroma and citrus flavour. The conditions were set as follows: water volume and raw material weight in the ratio of 1:3 mL/g, the extraction time was 1 h and temperature of 120 °C, and the resulting oil yield reaches 1.3%. The EO yield in this study was also compared to previous studies. For example, Jean Waikedre et al. (2010) used hydro-distillation method to extract Kaffir lime EO recovered from leaves was 0.35% [24]. According to Wulandari et al. (2017), Kaffir lime leaves were dried and hydrodistillated with water at a ratio of 1/10 for 4 h, yielding 0.85% when the material is milled [25]. Similar to published scientific studies, Suhaila Mohd Sauid and his colleagues (2018) published a review of extraction methods for Kaffir lime EOs, in which upon the use of hydro-distillation method, the EO yield was found to be 0.83% (Yusoff & Sauid, 2016); 0.82% (Md Anjazi and Sauid, 2015; Zakaria & Sauid, 2016); 0.295% (Rosli & Sauid, 2016); 0.78% (Ismail & Saud, 2016) from fresh leaves and 0.818% (Zakaria & Saud, 2016) from dried leaves [9]. Overall, the EO yield obtained from Kaffir lime leaves by hydro-distillation method in the present study was relatively higher than previous studies.

The GC-MS analysis results (Table 1 and Figure 2) showed that in the Kaffir lime EO of this study, there are 15 volatile compounds accounting for 99.99% of the total essential oil content, with β-citral (85.436%) as the main component. This is similar to the study of Ibrahim Jantan et al. (1996) that in Kaffir lime leaf oil of Malaysia, in which citronellal was also the main ingredient accounting for 72.41% [26]. Also in the study of Fan Siew Loh et al. (2011) was cited Fazwa et al. (2005) reported that in 5 regions in Peninsular Malaysia, citronellal was the main constituent accounting for 40 to 81%, in addition to the other major components such as citronellol (from 2.1 to 23.7%), linalool (from 1.1 to 5.2%), citronellyl propionate (from 0.2 to 8.9%), and nerolidol (from 0.3 to 1.8%), depending on the development area [27]. This result is similar to Fan Siew Loh et al. (2011) in Kaffir lime leaves EO with 29 compounds, including β-citral 66.85% of the total oil and β-citronellol (6.59%), citronellol (1.76%), linalool (3.90%) and other compounds contain less than 2% [27].

In the study of Wulandari (2017), Kaffir lime essential oil consists of 5 compounds accounting for 100% and citronellal is the main compound accounting for 80.86% [25]. Besides β-citral, essential oils from lemon leaves in this study also showed other ingredients such as: β-citronellol (6.808%), β-linalool (1.891%), citronellyl acetate (1.705%) and 11 other compounds accounting for less than 1% are presented in Table 1. In contrast, there are several studies showing other results. Meanwhile, in the study of Jean Waikedre (2010) showed that the essential oil had 33 compounds, accounting for 89% of the total amount of EO with the main ingredient terpinen-4-ol for 13.0% [23]. In 2012, Zaibunnisa A. Haiyee and colleagues showed that there are 17 compounds, however cyclohexanol was the main ingredient with 45.83% of the total EO content [24]. This comparison shows that hydro-distillation method is a useful for extraction process of the Citrus EOs. This result also shows that in this study, the content of citronellal is appropriate as compared to the previous studies.
Table 1. The content of compounds in *Kaffir lime* leaves EO by hydro-distillation method

| Peak | R.T. (min) | Compounds     | Content (%) | Structural formula |
|------|-----------|---------------|-------------|--------------------|
| 1    | 8.976     | Sabinene      | 0.744       | ![Sabinene](image)  |
| 2    | 9.06      | β-pinene      | 0.311       | ![β-pinene](image) |
| 3    | 11.747    | D-limonene    | 0.34        | ![D-limonene](image) |
| 4    | 16.118    | β-linalool    | 1.891       | ![β-linalool](image) |
| 5    | 18.607    | Cyclohexanol  | 0.657       | ![Cyclohexanol](image) |
| 6    | 19.36     | β-citronellal | 85.436      | ![β-citronellal](image) |
| 7    | 22.685    | β-citronellol | 6.808       | ![β-citronellol](image) |
| 8    | 27.077    | Citronellyl acetate | 1.705 | ![Citronellyl acetate](image) |
| 9    | 27.683    | α-copaene     | 0.211       | ![α-copaene](image) |
| 10   | 28.144    | β-cubebene    | 0.133       | ![β-cubebene](image) |
| 11   | 28.206    | β-elemene     | 0.092       | ![β-elemene](image) |
4. Conclusion
In this study, the EO of Kaffir lime was analyzed for its chemical composition by the classical distillation system (hydro-distillation). For 100 g of Kaffir lime leaves, provided the material-to-water ratio is 1:3 (g: mL), the temperature is 120 °C, 60 min, essential oil yields reached 1.3 %. Besides, the study described the main compounds present in the EO using GC-MS. The Kaffir lime EO comprised of 20 ingredients with citronellal as the primary constituents (85.4%), following by citronellol (6.8%), linalool (1.9 %), citronellyl acetate (1.7%). The findings revealed the chemical components of Kaffir lime leaves EO that have been influenced by production methods as well as the local environment, which is essential for further optimization studies for a desirable content of phytochemicals in the Kaffir lime EO.
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