Application of green technology in the process of extracting essential oil from Vietnam's Kaffir lime (Citrus hystrix) leaves

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Abstract: In recent years, microwaves are considered a source of green materials in the manufacturing process. Thanks to its outstanding thermal and time capability, it was applied to many different areas of experimental and production scale. In this research, we optimized the yield of essential oils obtained from the Kaffir lime (Citrus hystrix) leaves in Vietnam by using the heat from the microwave oven extraction method. Researchers used heat from the microwave to extract polar compounds in plants because it is a green technique that improves the yield and quality of the essential oil extracted from the extraction process. Kaffir lime essential oil has many different effects in the food and cosmetic field because of its scent and antioxidant properties. The chemical composition of Kaffir lime leaves essential oil in this study was carried out by gas chromatography–mass spectrometry (GC-MS) method which showed that 19 constituents are accounting for 99.99% of the total essential oil with β-citronellal is the main constituent accounting for 81.23%, followed by β-citronellol (5.76%), citronellyl acetate (4.26%), linalool (1.85%), caryophyllene (1.51%) and other compounds accounted for less than 1.0% at 100 grams of Kaffir lime leaves. These compounds are known as effective antibacterial and antioxidant agents. In the ratio of raw materials to water (1:3 mL/g), extraction period (60 min), and microwave power (450W), optimum extraction conditions for the extraction of essential oil of Vietnam's kaffir lime (Citrus hystrix) leaves were achieved.

1. Introduction
In recent days, new research trend is focusing on environmental protection, including energy saving, reducing the amount of solvent used and reducing the amount of waste released into the environment. These trends have attracted researchers to focus on introducing new techniques such as: ultrasonic compound extraction (UE) [1, 2], using, supercritical carbon dioxide extraction (SCDE) [3, 4] or using microwave
support as a source of heat during extraction (WE) [5-9]. In the essential oil extraction process, extraction time, solvent selection, energy consumption, essential oil yield and quality require consideration. These factors have helped green technology which essential oils was extracted by microwave-assisted extraction method, has many advantages over traditional methods such as hydrodistillation (HD) or steam distillation (SD) [10-14].

Kaffir lime, known scientifically name as *Citrus hystrix* DC, is a member of the genus *Citrus* with oranges, grapefruit, tangerines, and lemons [15]. This is a tree commonly grown in the Mekong Delta provinces of Vietnam in recent years. In addition to the fruit, the leaves of Kaffir lime are also of special interest due to the special aroma of the leaves, and are used extensively in foods of Southeast Asian countries. Kaffir lime leaves play an important part in folk medicine with many health benefits known as stuffy nose, cold treatment, cough and healthy teeth. The above uses are made possible thanks to the biological activity of Kaffir lime leaf extracts known through scientific research conducted by scientists around the world [16–20]. In addition to the extracts in Kaffir lime leaves, an indispensable valuable component, essential oil, which contains many aromatic compounds in Kaffir lime leaves. These aromatic compounds can start from terpinen-4-ol, β-pinene, α-terpineol, 1,8-cineole and citronellol, which are active antibacterial, antioxidant and anti-proliferative activities [21-25].

The aim of this research is to use microwaves to extract essential oils from Kaffir lime leaves according to some parameters which have not been used by recent studies. The resulting essential oil was then analyzed for its phytochemical composition and compared with other studies in the world.

2. Materials and Method

2.1 Raw Materials and Chemicals

Kaffir lime leaves were collected for the extraction of essential oils from the garden in An Giang province, Vietnam. Then, it is washed several times with distilled water to remove the outer layer of dirt, which is acquired during growth and harvest. Next, 100g on sample material is kept in an environment without humid by an airless plastic bag for once extraction. The size of the material affects the yield of Kaffir lime leaves oil obtained from the extraction, in this study, it was chosen the smallest size is grinded.

Sigma Aldrich (US) procures anhydrous sodium sulfate (Na₂SO₄). Deionized water is used as a solvent by the Milli-Q purification method (Millipore, USA) to remove oil from the Kaffir lime leaves.

2.2 Extraction with Microwave

The method to Kaffir lime leaves oils extraction by microwave, which was carried out by a domestic microwave oven MW71E (SAMSUNG). This device is fixed with the Clevenger apparatus, and is illustrated in Figure 1. The operation power of this MW71E is 100-800W with a 250v-50Hz power source. The microwave method was run with conditions include water to leaves ratio was 1/3 (g/mL), power of microwave was 450 (W) and time of extraction according to 60 (min) [26]. The flask consists of 100 g of deionized water Kaffir lime leaves and was put in the MW71E microwave oven. A Clevenger apparatus located outside the oven, on the top, which was used to contain the lemongrass oils in extraction process.
2.3 Analysis of Sample

The Kaffir lime leaves oil yield extracted from the extraction process after removal of water by Na₂SO₄ was analyzed to estimate the influence of microwave during the essential oil extraction process. The Kaffir lime leaves oil yield per each run that was determined by equation (1):

\[ Y = \frac{V}{m} \times 100\% \]  

(1)

where, \( Y \) is a yield of oil was obtained (%), \( V \) is the column height volume of Clevenger apparatus obtained (mL), and \( m \) is the amount of leaves used (g). To analyze the ingredient contained in Kaffir lime leaves oils, the method GC-MS is used. Code appliances: GC Agilent 6890N, MS 5973 inert. HP5-MS column, head column pressure 9.3 psi. The conditions for obtaining GC-MS results are as follows: Helium is a carrier gas; injection volume 1.0 μL; flow rate 1.0 mL/min; injection temperature 250°C, split 1:100; oven temperature progress consist of 50°C for 2 min, then increased up to 80°C at 2°C/min, followed by 150°C at 20°C/min and maintained at 300°C for 5 min. The compounds were determined by comparing retention indices with Wiley library and published mass spectra.

3. Results and Discussion

The factors affecting the extraction process of the essential oil (EO) from Kaffir lime leaves have been optimized as follows: the raw material is utilized by grinding to the smallest size, the water content and the amount of material used was 1: 3 (g / mL), the extraction time is 60 (minutes) when the power reaches 450 (W). The Kaffir lime leaves essential oil is obtained from the extraction process to give a pale yellow color, freshness, light scent and citrus-like smell. The method of using microwaves to extract essential oils from Kaffir lime leaves has almost no extensive research, therefore, in this study, the yield of essential oils collected by microwave extraction method will be compared with other studies by various methods as shown in Table 1.
In this study, under the above extraction conditions, the yield of essential oil from Kaffir lime leaves was 1.3 (mL / g), indicating that the yield was higher than other studies by the other methods. Specifically, the study of Kasuan et al. (2009) showed that when Kaffir lime leaves were extracted by hydrodistillation method (HD), the yield was 0.18%, while that of steam distillation method (SD) was 0.48%. This yield was increased until the study of Waikedre and colleagues published in 2010, the yield increased to 0.66% when they used Kaffir lime leaves from New Caledonia. Using the same hydrodistillation method, in 2017 Ramadhan et al., the Kaffir lime leaves essential oil yield was only 0.5% at Brawijaya University. However, at the same time, the same method, Wulandari and colleagues extracted the essential oil from Kaffir lime leaves to achieve the highest yield of 0.85% with the material was milled. In addition, for the supercritical carbon dioxide extraction (SCDE) method in the study of Norkaew et al. (2013), the result is 0.33%. At the same time, in Master's thesis of Rosli and Zakaria (2016) as cited in the 2018 of Suhaila Mohd Saud, it shows that the method of using ultrasound support extraction (UE) for the yield reaches 0.295% and 0.818%, respectively. In general, in the methods of extracting essential oils, the method of using microwaves as a source of heat for the extraction process is most effective.

| Method | HD | SD | SCDE | UE | UE | HD | HD |
|--------|----|----|------|----|----|----|----|
| Kasuan (2009) | 1.3% | 0.18% | 0.48% | 0.66% | 0.33% | 0.295% | 0.818% |
| Kasuan (2009) | 0.18% | 0.48% | 0.66% | 0.33% | 0.295% | 0.818% |
| Waikedre (2010) | 0.66% | 0.33% | 0.295% | 0.818% |
| Norkaew (2013) | 0.66% | 0.33% | 0.295% | 0.818% |
| Rosli (2016) | 0.66% | 0.33% | 0.295% | 0.818% |
| Zakaria (2016) | 0.66% | 0.33% | 0.295% | 0.818% |
| Ramadhan (2017) | 0.66% | 0.33% | 0.295% | 0.818% |
| Wulandari (2017) | 0.85% | 0.85% |

Moreover, the Kaffir lime leaves EO was analyzed for the content of volatile compounds by GC-MS method. The results showed that there are 19 components accounting for 99.99% of the total essential oil with β-citronellal as the main ingredient accounting for 81.23%, followed by β-citronellol (5.76%), citronellyl acetate (4.26%), linalool (1.85%) α-Caryophyllene (1.74%) and other compounds make up less than 1.0% with 100 grams of Kaffir lime leaves was displayed in Table 2 and the chromatogram in Figure 2. In addition, volatile compounds in this study are compared with other studies on other methods. In the study of Wongpornchai et al (2016) also used microwave support for the extraction process, headspace solid phase microextraction (SPME) showed that the main component is still β-Citronellal (48.20%), β-Citronellol (14.25%), Citronellyl acetate (7.78%), and β-Linalool (5.13%), the remaining compounds account for less than 1.0%.

For hydrodistillation, the results of the study suggested that β-Citronellal compound is the main ingredient in essential oil from Kaffir lime leaves. Specifically, in the study of Siew Loh et al (2011), β-Citronellal content accounted for 66.85%, in Haiyee's study (2012) accounted for 17.51%, reaching 61.7% in the study of Othman et al (2016) and this content increased to 80.86% by Wulandari (2017). However, in Waikedre's study et al. (2010) showed that Terpinen-4-ol and β-Pinene are the two main components, similarly, Haiyee's (2012) report showed that Cyclohexanol is the main component accounting for 45.83%. For the steam distillation (SD) method, Tinjan et al. (1971) published that cho β-Citronellal was the most valuable component with 74.8%, followed by Sabinene (2.1%), β-Linalool (3.6%), β-Citronellol (2.0%), and Citronellyl acetate (1.9%). This is similar when β-Citronellal content accounts for 66.9% in the research of Othman et al (2016). In 2013, Norkaew et al used supercritical carbon dioxide extraction (SCDE) method to extract essential oils, the results showed that the main compounds changed much compared to other methods with β-Citronellal (1.33%), β-Citronellol. (0.87%), and Nerolidol (2.0%).

Finally, Haiyee et al. (2012) used pressurised liquid extraction (PLE) method for extraction, the results showed that β-Citronellal accounted for 25.87%, β-Citronellol 4.02%, β-Linalool 3.22%, Naphthalene
2.18%. The β-Citronellal content in this study is higher than other studies due to the properties of the microwave, the microwave is more effective for oxygen-containing compounds in the molecular formula, so β-Citronellal content accounts for 81.427%. This has created an appropriate method in the condition of extracting the number of compound β-Citronellal.

### Table 2. The content of compounds in Kaffir lime leaves essential oil

| Peak | R.T.(min) | Compounds        | Molecular formula | Content (%) | Structural formula |
|------|-----------|------------------|-------------------|-------------|-------------------|
| 1    | 8.966     | Sabinene         | C_{10}H_{16}      | 0.608       | ![Sabinene Structure](structure.png) |
| 2    | 14.341    | Linalyl Oxide    | C_{12}H_{20}O_{2} | 0.243       | ![Linalyl Oxide Structure](structure.png) |
| 3    | 16.118    | β-Linalool       | C_{10}H_{18}O     | 1.848       | ![β-Linalool Structure](structure.png) |
| 4    | 18.597    | Cyclohexanol     | C_{6}H_{12}O      | 1.502       | ![Cyclohexanol Structure](structure.png) |
| 5    | 19.370    | β-Citronellal    | C_{16}H_{20}O     | **81.427**  | ![β-Citronellal Structure](structure.png) |
| 6    | 20.270    | Terpinen-4-ol    | C_{10}H_{18}O     | 0.35        | ![Terpinen-4-ol Structure](structure.png) |
| 7    | 22.685    | β-Citronellol    | C_{10}H_{20}O     | **5.762**   | ![β-Citronellol Structure](structure.png) |
| 8    | 26.439    | unknown name     |                   | 0.25        | ![Unknown Name Structure](structure.png) |
| 9    | 27.067    | Citronellyl acetate | C_{12}H_{22}O_{2} | **4.255**   | ![Citronellyl acetate Structure](structure.png) |
| 10   | 27.684    | α-Copaene        | C_{15}H_{34}      | 0.401       | ![α-Copaene Structure](structure.png) |
| 11   | 28.133    | β-Cubebene       | C_{15}H_{34}      | 0.216       | ![β-Cubebene Structure](structure.png) |
| No. | Retention Time | Compound                | Molecular Formula | Mass       |
|-----|---------------|-------------------------|-------------------|------------|
| 12  | 28.196        | β-Elemene               | C_{15}H_{24}      | 0.147      |
| 13  | 29.001        | α-Caryophyllene         | C_{15}H_{24}      | 1.743      |
| 16  | 31.887        | Naphthalene             | C_{10}H_{18}      | 0.535      |
| 17  | 32.483        | Elemol                  | C_{15}H_{26}O     | 0.321      |
| 18  | 32.797        | Nerolidol               | C_{15}H_{26}O     | 0.254      |
| 19  | 33.215        | Caryophyllene oxide     | C_{15}H_{24}O     | 0.137      |
Figure 2. The chromatograms of compounds in essential oil from Kaffir lime leaves
Table 3. Comparison of content of compounds in Kaffir lime leaves essential oil

| Compounds          | This study (%) | (2007) | (2010) | (2011) | (2012) | (2012) | (2013) | (2016) | (2017) |
|--------------------|---------------|--------|--------|--------|--------|--------|--------|--------|--------|
|                    | ME | SD | HD | HD | PLE | HD | SPME | SCDE | HD | SD | HD |
| Sabinene           | 0.608 | 2.1 | - | 0.2 | 0.18 | - | 0.58 | - | 1.6 | 0.2 | 3.21 |
| Linalyl Oxide      | 0.243 | - | - | - | - | - | - | - | - | - | - |
| β-Linalool         | 1.848 | 3.6 | 2.8 | 3.9 | 3.22 | 3.77 | 5.13 | - | 1.0 | 3.9 | 3.02 |
| Cyclohexanol       | 1.502 | - | - | - | 0.27 | 45.83 | - | - | - | - | - |
| β-Citronellal      | 81.427 | 74.8 | 2.7 | 66.85 | 25.87 | 17.51 | 48.20 | 1.33 | 61.7 | 66.9 | 80.86 |
| Terpinen-4-ol      | 0.35 | - | 13 | 0.34 | - | 1.42 | 0.46 | - | - | - | - |
| β-Citronellol      | 5.762 | 2.0 | 6.0 | 6.59 | 4.02 | 1.33 | 14.25 | 0.87 | 13.4 | 6.6 | - |
| unknown name       | 0.25 | - | - | - | - | - | - | - | - | - | - |
| Citronellyl acetate| 4.255 | 1.9 | 2.2 | - | - | - | 7.78 | - | 2.0 | - | - |
| α-Copaene          | 0.401 | 0.7 | 0.3 | - | 1.14 | - | - | 0.26 | - | - | - |
| β-Cubebeine        | 0.216 | 0.6 | - | - | 1.17 | - | - | 0.12 | - | - | - |
| β-Elemene          | 0.147 | - | - | - | - | - | - | 0.09 | - | - | - |
| α-Caryophyllene    | 1.743 | - | - | - | - | - | 0.61 | 0.68 | - | - | 1.33 |
| Naphthalene        | 0.535 | - | - | - | 2.18 | - | - | - | - | - | - |
| Elemol             | 0.321 | - | - | - | - | - | - | 0.65 | - | - | - |
| Nerolidol          | 0.254 | 0.2 | - | 0.04 | - | - | 0.34 | 2.0 | 1.2 | 0.1 | - |
| Caryophyllene oxide| 0.137 | - | - | - | - | - | 0.37 | - | - | - | - |
| α-Pinene           | - | 0.1 | 3.6 | - | - | - | - | 0.1 | - | - | - |
| β-Pinene           | - | 0.1 | 10.9 | - | - | - | 0.01 | - | 0.1 | - | - |
| p-Cymene           | - | - | 5.6 | - | 0.48 | - | 0.04 | - | 0.1 | - | - |
| Methyl-trans-9-     | - | - | - | - | - | - | - | - | - | - | 11.58 |

4. Conclusion

Citrus genus is the most common and valuable variety in Vietnam. In this study, the chemical composition of Kaffir lime leaves essential oil was performed using gas chromatography-mass spectrometry (GC-MS) method, which showed 19 constituents, including β-citronellal (81.23%), citronellol (5.76%), citronellyl acetate (4.26%), linalool (1.85%) and caryophyllene (1.51%). As previous studies have evidenced a wide range of biological activities of these compounds, their presence in such a high content in Kaffir lime leaves essential oil increases the importance of continuous cultivation and exploitation of the plant. Moreover, the optimum extraction conditions for the extraction of essential oil from Vietnamese Kaffir lime (Citrus hystrix) leaves were achieved in the ratio of raw materials to water (1:3 mL/g), extraction period (60 min), and microwave power (450W). This research can be considered as a supportive evidence for green technology, which is being promoted in environmental protection, into the laboratory.
Acknowledgements
This research is funded by the Science and Technology Program of Ministry of Education and Training (Vietnam) with Project code: CT2020.01

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