Yields and Composition of Persian Lime Essential Oils (Citrus latifolia) from Hau Giang province, Vietnam extracted by Three Different Extraction Methods

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Abstract. The essential oils (EOs) was extracted from Persian lime (Citrus latifolia) peels collected in Hau Giang province, Vietnam. Volatile compositions of EOs obtained by three extraction methods of hydrodistillation (HD), steam distillation (SD) and microwave extraction (ME) were analyzed and compared. The volatile compounds in EOs were analyzed based on gas chromatography-mass spectrometry (GC-MS). An amount of 3 ml/g, 2.3 ml/g and 4 ml/g of EOs was obtained from HD, SD and ME with twenty-one compounds were identified, accounting for 99%-100% of the total oil content. Limonene, β-pinene, α-pinene, γ-Terpine, α-citral, β-citral and Sabinene are the major compound in essential oil. However, there were qualitative and quantitative differences between oil samples obtained by the three extraction methods. The difference is probably due to the influence of different environmental factors and specific characteristics of each extraction method. The results of this study will provide information on the content and chemical composition of Lemon peels. C. latifolia and serve as a stepping stone for further research into the applications of the compound to cosmetic, food and pharmaceutical products.

1. Introduction

Recent interest in pharmaceutical research has shifted towards plant species that contain aromatic compounds and their extracts, which possess biological effects beneficial to human health and physical development. Essential oils could be obtained from a wide range of plant species and materials using various techniques [1-18]. In industrial production of essential oil, materials could be either fresh or partially dried and usually covers many species in the citrus family whose essential oil composition is predominated with D-limonene [19-21]. The compound has been long known for potent antibacterial and antioxidant properties and is facing with increasing demand originated from the consumption pattern that changes towards safe and green products.

Being endowed with abundant resources and great biodiversity, Vietnam has been a repository of many unique plant species applicable in production of natural products used in various industries. However, some species, such as those in the citrus family, have been demonstrated to have limited applications despite their great potential. One notable example is lemon, an abundant agricultural
product whose peels have not been exploited and utilized in commercial production of lemon essential oil, a valuable export good. Persian lime (Citrus latifolia), also known as seedless lime, is an important tree of the genus Citrus, the family Rutaceae, native to tropical and subtropical regions of Southeast Asia [22-26]. C. latifolia tree height can be up to 10 meters (33 feet) tall, but they are usually smaller. The branches form open and spiny clusters. The leaves are green, elliptical and shiny. The flower has a strong aroma and is white on the outside with a purple streak on the inside. On a lemon tree, flowers and fruits can simultaneously exist. The main constituents existing in Persian lime essential oil include Limonene (31.1 to 65.7%), β-pinene (5.1 to 13.1%) and γ-terpine (10.8 to 12.2%), which are the components playing an important role in determining its potent antibacterial and antioxidant capabilities [27-28]. In addition, there is a wide array of minor components whose concentrations were determined to be lower than 1.0% [29-31]. Atti-Santos et al (2005) conducted an extract of lemon oil from the hydrodistillation method (5.45% w/w) and supercritical carbon dioxide (7.93% w/w). At the same time, GC-MS analysis showed that α-pinene 1.9% and 2.7%, β-pinene 12.4% and 14.5%, D-Limonene 47.5% and 48.9%, γ-Terpinene 12.3% and 17% [32].

To obtain essential oil from Persian lime, a wide range of extraction methods could be applied including mechanical pressing, hydrodistillation, steam distillation, extraction by volatile solvents, extraction with supercritical fluid (CO₂) or microwave-assisted extraction. However, since molecules are sensitive to heat and susceptible to chemically induced denaturation [33-34], certain isolation processes may result in essential oil with impaired quality, low extraction efficiency and degradation of unsaturated compounds or esters. This highlights the role of the applied extraction technique in ensuring quality of the obtained essential oil and in considering economic feasibility of large scale production processes [35, 36].

In this study, different extraction methods including hydrodistillation, steam distillation and microwave-assisted extraction were compared to determine the most optimal method to produce C. latifolia essential oil with best efficiency and quality. The obtained C. latifolia essential oil was analyzed for volatile composition by GC-MS. The results are expected to contribute to further efforts in valorization of agricultural waste in production of EOs.

2. Materials and methods

2.1. Plant material
Persian lime was collected directly in garden houses in Hau Giang province, Vietnam in March 2020 and transported back to laboratory. Mature fruits with smooth, plump skin were collected and washed. Afterwards, peels were removed, pureed and transferred into the flask for extraction.

2.2. Hydrodistillation (HD) method
100g of raw pureed lemon zest was extracted using a Clevenger type device. Raw material was soaked directly in water. In this study, extraction was performed following a peel / water ratio of 1:3. The mixture was heated continuously until the no essential oil was observed in the condensing device. The resulting oil was then dehydrated with anhydrous sodium sulfate to remove water and kept in a dark vial and stored for chemical composition analysis.

2.3. Steam distillation method
The steam distillation procedure was performed similarly to the HD with identical peel / water ratio and operating conditions. Unlike the hydrodistillation process, steam distillation does not require the raw material to be immersed directly in water. The main feature of this extraction process is that the material and water are not in direct contact with each other. 200g of lemon zest was placed into a flask that was separated from the water container. Steam generated by boiling water passes through the material, entrapping the essential oil in the materials and passing through the condensing device to the receiving vessel. The heat source is an electric stove with a capacity of 500W. The extraction process took 3 hours. The distilled essential oil is filtered to remove water and anhydrous sodium sulfate and stored in a closed vial before GC-MS analysis.

2.4. Microwave-assisted extraction method
The microwave used for extraction was generated by Samsung (MW71E) microwave oven operating at 2450 MHz. The maximum power of the oven is specified as 800 W. The system consisted of a microwave oven connected to a Clevenger type device in which a 1000L flask containing materials and solvent was located inside oven cavity. The extraction process was performed with the peel/water
ratio of 1:3. A condensing device is placed on top, outside the oven, which was used to collect the extracted EOs. The microwave was propagated for 60 minutes at a fixed power of 450W. Essential oils after extraction were removed from water with anhydrous sodium sulfate and stored for further use.

2.5. Gas chromatography-mass spectrometry analysis
Chemical composition of the calamondin EO was determined by GC-MS analysis. A GC Agilent 6890 N instrument (Agilent Technologies, Santa Clara, CA, USA) was coupled with HP5-MS column and MS 5973 inert. The head column was 9.3 psi of pressure. The EO was added with n-hexane and dehydrated with Na₂SO₄. GC-MS were obtained 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. The flow rate was kept constant at 1 mL/min and the rate of division was 30. Thermal program for samples began at 50°C for 2 min, then continued to rise from 60 °C (20 °C / min), 150 °C (5 °C / min), 200 °C (10 °C / minute) and when reached 300 °C (20 °C / min) maintained for 5 min.

3. Results and discussion
All EOs samples extracted from C. latifolia peels using the three mentioned method were in the liquid state with color being either pale yellow or colorless. Figure 2 summarized the extraction efficiencies resulted from carrying out the three extraction methods. In this study, ME which have been recently used in extracting plant-derived essential oil produced different results, as compared to HD and SD [37]. Firstly, while the ME and HD methods were both performed at 1:3 peel / water ratio, the former only requires a shorter extraction time of 60 mins to reach the optimal performance. On the other hand, the SD method requires a relatively long extraction period of around 3 hours to obtain optimum performance. However, SD has economic benefits, as the mixture’s boiling temperature is lower than the boiling water temperature. In addition, it also allows the separation of the components in essential oils into separate parts with higher purity.

Microwave extraction uses microwave irradiation to speed up cell disruption by dramatically increasing the temperature and increasing pressure within the plant cell walls. As a result, the cell wall is broken and essential oil trapped inside the cell wall is released into the extraction solvent, shortening the extraction time. The essential oil yield obtained from HD, SD and ME processes were 3 ml/g, 2.3 ml/g and 4 ml/g respectively. This result is comparable to another study concluding that steam distillation process of essential oil for the same species in the Maringa region (Brazil) achieved an efficiency of 2.76% [29]. In contrast, higher yields on the same species were revealed from materials grown in the Cachoeira de Macacu area in Brazil at a yield of 1.4%, which was achieved via
hydrodistillation [27]. This difference can be attributed to a number of factors such as climatic and geographic conditions and the harvest and drying conditions.

Overall, considering the shortened extraction time and high oil yield, the present study proposed that ME is an effective extraction method for *C. latifolia* peels essential oil.

![Figure 2](image.png)

**Figure 2.** *C. latifolia* essential oil content is obtained by hydrodistillation, steam distillation and microwave extraction

In order to make accurate comparisons and assessments on the quality of the EOs product obtained, composition analysis of *C. latifolia* peel oil samples obtained using the three extraction methods was conducted by GC-MS. The analysis results of the sample obtained by steam distillation, hydrodistillation, and microwave extraction are shown in Table 1 and Figure 3.

Based on Table 1, it is shown that in *C. latifolia* essential oil, there are a total of 19-21 identified compounds accounting for 99% - 100% content of *C. latifolia* essential oil. The main components in EOs are hydrocarbon monoterpenes, which accounts for the highest content. The composition of the EOs obtained from all three methods was found to be almost similar in quality, albeit with some quantitative differences. From the chemical composition of Table 1, it is shown that essential oils obtained from hydrodistillation and steam distillation have a higher hydrocarbon proportion than the essential oil samples obtained by microwave extraction. Because the hydrocarbon is a less polar compound, it is less affected by microwave irradiation. Therefore, microwave irradiation could accelerate the heating of water much faster, resulting in lower limonene content than those of the other methods.

Apart from the rapid heating of water, the microwave-assisted method also incites the isolation of polar constituents (compounds containing oxygen) present in the plant material, as evidenced by the appearance of Terpineol and α-Terpineol at higher contents (0.259% and 0.711% respectively). As shown in Table 1 and Figure 3, all three methods were able to extract major ingredients such as Limonene (61.347%; 61.932% and 60.551%), β-pinene (11.016%; 9.693% and 9.335%), γ-Terpine (14.486%; 15.853% and 14.484%), α-pinene (2.003%; 1.659% and 1.424%), α-citral (1.743%; 1.404% and 2.584%), β-citral (1.572%; 1.214% and 2.132%) and Sabinene (1.627%; 1.303% and 1.326%). Some other minor components were identified in this study with content <1%.

Previous studies have shown that *C. latifolia* essential oils, obtained with hydrodistillation were predominantly constituted by D-limonene (51.64%), β-pinene (12.79%), β-thujene (14.85%), and γ-terpinene (12.8%) [38]. Although the current composition results of *C. latifolia* essential oils share qualitative similarities to previously published data [39], there are some minor quantitative differences, possibly originating from variations in growing conditions [40]. To demonstrate, compositional analysis of a lemon essential oil sample from Cuba revealed obtain major compounds being Limonene (55.6%), γ-terpinene (11.8%), α-terpinene (6.6%), and cis-p-terpinol (2.2%) [30]. In another study where the bark portion of Indian *C. latifolia* was extracted for essential oils and analyzed, it was found that Limonene (49.2%), β-pinene (9.2%) and α-terpinene (12.1%) were the main constituents [31]. Raddatz-Mota et al. (2019) conducted steam extraction and chemical analysis on the essential oils from lemon from Mexico, revealing that the main components accounted for more than 90% of the volatile content, suggesting that the aromatic structures are similar between the studied radicals. The dominant chemical compounds were pinene (from 11.7% to 12.6%), γ-Terpine (from
10.5% to 11%, limonene (from 44.8% to 47.6%), α-citral (from 3.9% -4.4%) and β-citral (from 3% -3.3%) [41].

Table 1. Chemical compositions of the *C. latifolia* EOs extraction by hydrodistillation, steam distillation and microwave extraction

| Compounds (%) | Hydrodistillation | Steam distillation | Microwave extraction |
|---------------|------------------|--------------------|----------------------|
| β-Thujene     | 0.518            | 0.414              | 0.348                |
| α-Pinene      | 2.003            | 1.659              | 1.424                |
| Sabinen       | 1.527            | 1.303              | 1.326                |
| β-Pinene      | 11.016           | 9.693              | 9.335                |
| β-Myrcene     | 0.891            | 0.769              | 0.798                |
| α-Terpinen    | 0.349            | 0.283              | 0.303                |
| p-Cymene      | 0.708            | 0.724              | 0.911                |
| D-Limonene    | 61.347           | 61.992             | 60.551               |
| γ-Terpinene   | 14.486           | 15.853             | 14.484               |
| Cyclohexene   | 0.575            | 0.557              | 0.564                |
| Terpineol     | 0.226            | -                  | 0.259                |
| α-Terpineol   | 0.464            | 0.249              | 0.711                |
| β-Citral      | 1.572            | 1.214              | 2.132                |
| α-Citral      | 1.743            | 1.404              | 2.584                |
| δ-Elemene     | -                | 0.153              | 0.139                |
| Neryl (S)-2-methylbutanoate | 0.486 | 0.799 | 0.731 |
| β-Elemene     | 0.119            | 0.198              | 0.217                |
| Caryophyllene | 0.453            | 0.593              | 0.696                |
| α-Bergamotene | 0.836            | 1.037              | 1.152                |
| β-Bisabolene  | 0.682            | 0.973              | 1.21                 |
| Germacrene B  | -                | 0.135              | 0.124                |
Figure 3. GC-MS chromatogram of the EOs extracted from *C. lafolia* by (A) Hydrodistillation, (B) Steam distillation and (C) Microwave extraction.
4. Conclusions
The extraction of EOs from Persian lime (C. latifolia) was performed by three different methods in order to obtain the oil with high yield and enriched content of bioactive compounds within a shortened amount of extraction time. Overall, microwave-assisted extraction resulted in much shorter extraction times and high oil yield (4 ml/g) in comparison with hydrodistillation method (3 ml/g) and steam distillation (2.3 ml/g). In total, 19 - 21 components were determined using GC-MS, accounting for 99% - 100% of the total EOs. The main components of C. latifolia EOs are mainly monoterpenic hydrocarbons such as Limonene, β-pinene, γ-Terpineine, α-pinene and Sabinene. However, lemon essential oil extracted by hydrodistillation method also contained a high content of α-Pinene, β-Pinene, D-Limonene, γ-Terpineine, as compared to the other two methods. Comparing compositional results obtained using the three extraction methods suggests that the selection of extraction technique plays a vital role in determining the quality of the obtained EOs.

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