Comparative study of Mandarin (Citrus reticulata Blanco) essential oil extracted by microwave-assisted hydrodistillation, microwave extraction and hydrodistillation methods from Tien Giang, Vietnam

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Abstract. The mandarin (Citrus reticulata Blanco) essential oil is well-known for bactericidal, antioxidant and anti-cancer properties. Thus, an effective extraction method is required to obtain high oil yield with an abundant content of bioactive compounds. In the present study, the chemical profiles of essential oils extracted from the peels of Mandarin via hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD) and microwave extraction (ME) were reported in this study. Extraction techniques were carried out at their optimal conditions and the results were compared with each other. Gas chromatography-mass spectrometry (GC-MS) was employed to perform compositional determination. A total of 5 components was determined, accounting for almost 100% of total oil content. The extraction efficiencies were 3.6%, 6.8%, and 5.5% respectively obtained from HD, MAHD and ME. The results showed that MAHD seemed to result in higher yield and essential oil with identical chemical composition and quality in comparison with those of other methods, suggesting that MAHD was a potential alternative method to HD to reduce the time and cost of the extraction process.
1. Introduction
Advancements in natural products play a key role in physical and biological sciences and their applications bring new avenues in interdisciplinary fields [1-5]. Natural products also contribute in biomedical, health, nutrition, and other interrelated sciences by bringing the discovery of new compounds, implications regarding bioactivities of existing compounds and development of new techniques [6-10].

The Citrus family has been known as a potential material for production of highly valued products applicable in medicinal and nutritional applications [11-13]. Citrus includes 2000 species distributed worldwide except in cold areas. In Vietnam, the tropical and subtropical climate have given rise to the wide cultivation of citrus varieties, making the production of citrus-derived products a promising industry. The mandarin tree, scientifically named Citrus reticulata Blanco, is a fruit tree belonging to the genus Citrus, Rutaceae family, native to India and China. Mandarin essential oils have been used in many products such as cosmetics, pharmaceuticals, beverages, and as flavouring agents in foods [14, 15]. The mandarin essential oils are also known for bactericidal, antioxidant and anti-cancer properties [16, 17]. Composition wise, most major compounds in the mandarin essential oils are found to be in the terpene group while some other minor compounds could be either sesquiterpenes or oxygen containing constituents such as alcohols, aldehydes and esters [18].

In Vietnam, different varieties and types of mandarins are widely present across the regions. Tangerine trees are woody plants with, medium, strongly dispersed branches. Stems and branches have thorns and shed when reaching a certain age. Essential oils mostly accumulate in peels and leaves of the plant. The mandarin essential oil is conventionally extracted using hydrodistillation (HD). However, high temperatures and longer reaction times cause certain disadvantages in terms of feasibility and quality of the obtained product and limit the applicability of this method at larger scales. These disadvantages have been improved with recent development of microwave-assisted hydrodistillation (MAHD) and microwave extraction (ME), by increasing the extraction efficiency while reducing the heat and shortening the extraction time [19].

Therefore, in this study, we explored the possibility of using microwave-assisted extraction and the combined extraction technique in isolating essential oils from mandarin peels. The extraction yields and essential oil compositions, determined by gas chromatography-mass spectrometry (GC-MS), obtained in three methods were compared with literature Identification of an economically feasible extraction method that can result in acceptable yield and products of sufficient quality greatly contributes to valorization of abundant agricultural commodities and reduction of environmental impact accruing from processing activities [20].

2. Material and Methods

2.1. Plant materials
In this study, fresh and healthy mandarin fruits were collected in Tien Giang province (10°25′13.04″N, 106°17′48.64″E), Vietnam in April 2019. Prior to the extraction process, the fruits were washed and peeled to separate the outer part of the mandarin from the flesh. The peels were 10% (w/w) of the entire fruit weight. Anhydrous sodium sulfate (Na₂SO₄) and distilled water at analytical grade were used for extraction.

2.2. Hydrodistillation process (HD)
Mandarin peels (100 g) were finely ground into 1-3 mm of size using Sunhouse electric blender (Model SHDS5322) and placed in the flask (1000 mL) at an ingredients/solvent ratio of 1:4. The mixture was then distilled for 125 min using a Clevenger type device (Figure 1). The resulting essential oil was a mixture of essential oils and water, which was then deposited in two layers of liquid. The pure essential oil was obtained by anhydrous with Na₂SO₄ and stored in a dark bottle.

2.3. Microwave extraction process (ME)
The microwave oven was used for microwave irradiation is MW71E operating at maximum 800W. First, 100 g of pureed ingredients was put in a flask, which was located inside the cavity of the microwave oven. In the next step, microwave irradiation took place with the fixed power of 470 W for 90 min. A Clevenger system was located outside the microwave chamber to continuously condense water vapor, the condensate was continuously brought back to the extraction flask. The essential oil mixture was
removed by water with Na$_2$SO$_4$ and the chemical composition of the oil was isolated and then analyzed by GC-MS.

2.4. Microwave-assisted Hydrodistillation (MAHD) process

In this routine, microwave extraction first took place, followed by the conventional hydrodistillation process. The same microwave oven that was previously used in ME process was employed (Figure 1). The microwave irradiation was performed first at capacity of 450 W for 1 min. Afterwards, instead of microwave irradiation normal heating conditions took place at 120°C for 2 h. The essential oil is collected in an amber-brown bottle, dehydrated with anhydrous Na$_2$SO$_4$ and stored at 4°C in a refrigerator until analysis.

![Figure 1. The process of extracting mandarin peel essential oil](image)

2.5. GC-MS Analysis

Chemical composition of the mandarin fruit oil was determined by GC-MS analysis using GC Agilent 6890 N instrument coupled with HP5-MS column and MS 5973 inert. The pressure of the head column was 9.3 psi. 25 µL of essential oil was added with 1.0 mL n-hexane and dehydrated with Na$_2$SO$_4$. The flow rate of was constant at 1 mL/min. Injector temperature is 250°C and the rate of division is 30. Thermal program for samples: 50°C kept for 2 min increased by 2°C/min to 80°C, continued to increase by 5°C/min to 150°C, continued to increase by 10°C/min to 200°C, increase 20°C/min to 300°C hold for 5 min.

3. Results and Discussion

The essential oils of tangerine peel are extracted from the above three methods was in liquid state with light yellow color. The optimal factors influencing the extraction process including the ratio of material/solvent, time, temperature and capacity are shown in Table 1. Reported efficiencies were expressed as the volume of the essential oil per gram of fresh plant material. Comparison between recovery performances of the three methods is shown in Figure 2.

In HD process, the optimal oil yield of 3.6% was achieved at a ratio of 1: 4 and under 125 min. For comparison, the combined method seemed to result in significantly higher oil yield, at 6.8%, achieved after 120 min of extraction. The ME processed gave the essential oil with lower yield, at 5.5%, however at much shorter extraction time, at only 90 min. This could be explained by the longer time needed to heat the material in combined and HD technique to achieve yield equivalent to that of ME. Since the mandarin peel lacks oil sacks or glands, heating the whole material through microwave irradiation is more effective in inciting the release of oil. In addition, microwave irradiation increases the rate of cell
breakage by suddenly elevating the temperature and internal pressure of plant cells. As a result, the cell wall breaks down and releases essential oils trapped inside the cell wall into the extraction solvent [21]. Appropriate microwave irradiation capacity is very important to ensure a time-efficient extraction process. However, the amount of heat should not be too high, otherwise causing volatile compounds to degrade.

**Table 1.** The optimal parameters of mandarin peel essential oil extraction process.

| Extraction methods        | Material and water Ratios (g/mL) | Time (min) | Temperature (°C) | Power (W) |
|---------------------------|----------------------------------|------------|------------------|-----------|
| Hydrodistillation          | 1:4                              | 125        | 115              | -         |
| Combined extraction method | 1:4                              | 120        | 120              | 450       |
| Microwave extraction       | 1:3                              | 90         | -                | 470       |

**Figure 2.** Performance of mandarin peel essential oil extracted by hydrodistillation process (HD), Microwave-assisted Hydrodistillation MAHD and Microwave extraction (ME) methods.

All identified components existing in the mandarin peel essential oils obtained through three different extraction methods and the chromatograms were summarized in Table 1 and Figure 3. All three methods seemed to produce essential oils which were qualitatively similar in composition, while some quantitative differences were observed. Five components were identified with the main components of mandarin peel essential oil being mostly monoterpenic hydrocarbons. Results of GC-MS chromatographic analysis Table 2 shows that HD and ME method gave compositions having four main compounds: α-Pinene (0.54%, 0.375%), β-pinene (0.414%, 0.284%), β-Myrcene (1.405%, 1.461%) and Limonene (97.64%, 97.88%) for HD and ME respectively. On the other hand, five compounds were identified in mandarin peel essential oil extracted by the combined method including α-Pinene (0.518%), β-pinene (0.317%), β-Myrcene (1.104%), Limonene (97.94%) and sabine (0.122%). The abundance of limonene in the current study is similar to the report of Sultana et al. (2012) where essential oil extracted from Indian mandarin by HD method was analyzed and exhibited Limonene content of 87.45% [22]. Similarly, Minh Tu et al. (2002) showed that Limonene (95.1%) content was the main constituent existing in essential extracted from Vietnamese C. reticulate Blanco [23]. The chemical structure of α-Pinene, β-pinene, β-Myrcene, Limonene and sabine were shown in Figure 4.

Another study involving mandarins grown in Burundi showed that fifty-eight components, accounting for 97.2% of the total volatiles have been identified in the essential oil. Of which, Limonene is the most prominent component (84.8%), followed by α-terpinene (5.4%), myrcene (2.2%) and α-pinene (1.1%) [24]. Similarly, in Algerian mandarin tree, Limonene (67.04%) was also the main component among 24 detected compounds [25]. It is clear that the Limonene content in the current study is richer than the previous reports. The discrepancies could be attributable to a number of factors
including varieties, geographical location, season and species. Environmental factors, such as soil type and climate, extraction methods and genetic factors also play important roles in determining the chemical composition.

Overall, from the results of extraction efficiency and chemical composition analysis, MAHD can be considered as the most effective among the three extraction methods, with 6.8% of oil yield under the following conditions: 4:1 mL/g of water: raw material ratio, 450 W of pressure, 120 °C of temperature within 120 min. These conditions help minimizing heat and extraction time, as compared to ME and HD. The resulted mandarin peel oil has high yield (6.8%) and high content of valuable compounds, namely, Limonene (97.94%).

Table 2. Volatile components of mandarin peel essential oil by GC-MS.

| Peak | R.T. | Name       | HD (%) | ME (%) | Combined method (%) |
|------|------|------------|--------|--------|---------------------|
| 1    | 7.261| α-Pinene   | 0.54   | 0.375  | 0.518               |
| 2    | 8.997| Sabinene   | -      | -      | 0.122               |
| 3    | 9.081| β-pinene   | 0.414  | 0.284  | 0.317               |
| 4    | 9.949| β-Myrcene  | 1.405  | 1.461  | 1.104               |
| 5    | 11.925| Limonene  | 97.64  | 97.88  | 97.94               |

Figure 3. Chromatogram of mandarin peel essential oil extracted by (a) Hydrodistillation, (b) Microwave extraction and (c) Microwave-assisted Hydrodistillation.
Figure 4. Structure of monoterpene hydrocarbons compound identified in mandarin peel essential oil.

4. Conclusion
In this study, the combined method was compared with HD and ME in terms of yield and chemical composition of mandarin peel essential oil. The combined method gave an improved yield (6.8%) in comparison to HD (3.6%) and ME (3.6%) methods. Chemical compositions of essential oil samples produced using the three methods exhibited no clear qualitative, with 4 main compounds detected in HD and ME-extracted oils (e.g. α-Pinene (0.54%, 0.375%), β-pinene (0.414%, 0.284%), β-Myrcene (1.405%, 1.461%) and Limonene (97.64%, 97.88%) and 5 main compounds for combined method (e.g. α-Pinene (0.518%), β-pinene (0.317%), β-Myrcene (1.104%), Limonene (97.94%) and sabinene (0.122%). The results suggest the use of the combined method in isolating essential oils from mandarin fruit peels. Improved yield observed in the combined method indicate that the technique has distinct economic advantages and should be taken into consideration in further studies.

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