Optimization of green mandarin (Citrus reticulata) essential oil extraction using microwave-assisted hydrodistillation and chemical composition analysis

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Abstract. Citrus reticulata Blanco. peel possess various phytochemical compounds which exhibit high-value biological activities. However, the microwave-assisted technique has not been experimentally adopted in essential oil extraction from Citrus reticulate. Therefore, the present study attempted adopt this microwave-assisted hydrodistillation method to extract the essential oil from the mandarin fruit peel and improved extraction method specifications. Fresh ground peels were used as the material and underwent three extraction cycles. Technical conditions for optimal oil yield consisted of raw material/solvent ratio of 1:3 (g/ml), microwave power of 600W, and time extraction of 55 min. After extraction, essential oil products were analyzed for composition on the GC-MS method. Obtained essential oils featured main ingredients including limonene (97.688%), sabine (0.12%), β- myrcene (1.394%), (Z)-β-pinene (0.273%) and 1R-α- pinene (0.525%). These results suggest the use of Citrus reticulata essential oil in manufacture of antibacterial, antioxidant agents and in cosmetics, food, and pharmaceuticals.

1. Introduction
Today, the demand for essential oils and aromas (for cosmetics) in the world is increasing due to the trend of using natural compounds [1-3]. Meanwhile, Vietnam is one of the places where the tropical climate is favorable for cultivating essential oil-bearing plants. Essential oils are refined liquid, most commonly obtained by distillation by steam or water, extracted from leaves, stems, flowers, bark, roots, or other components of plants. The composition of the essential oil includes numerous volatiles including monoterpenes, hydrocarbons sesquiterpenes, and their oxygen derivatives whose content may vary depending on many factors such as plant variety, plant part, growing habitat or separation methods [4-6]. Mandarin (Citrus reticulata Blanco.) is a fruit tree belonging to the genus Citrus, family Rutaceae, and originating from India and China [7-9]. In Vietnam, the plant is widely grown with many different varieties and types, mainly for its fruit [10]. Mandarin trees have thorny stems and branches with the globular fruit slightly flattened with color varying from green-yellow to green [11]. Mandarin peel essential oil contains many valuable chemical compounds, with limonene accounting for at least 85% of oil content, along with sabine, α-pinene, β-pinene, α-phellandrene, β-phellandrene, (Z)-β- ocimene and terpinolene with a content of lower 2.0% [12-14]. Besides strong antimicrobial, antifungal and antioxidant properties, tangerine peel oil has been demonstrated to inhibit cancer cell growth [15].
Conventional methods to obtain essential oils from mandarin peels include traditional methods such as steam distillation, which have disadvantages regarding energy consumption and long extraction time [16]. Recent developments in oil extraction have proposed the method of ultrasound extraction, supercritical fluid extraction, and microwave-assisted hydrodistillation extraction [17]. Of which, microwave-assisted hydrodistillation has been shown to be more efficient in terms of time and solvent consumption [18]. To our knowledge, although there are many studies of tangerine peel extract, the microwave assisted technique has not been experimentally adopted in extraction of this valuable EOs [19-20].

In this study, EOs was extracted from Citrus reticulata peels using microwave-assisted hydrodistillation and its oil composition was analyzed by the gas chromatography method (GC-MS). The extraction process was investigated with respect to three main experimental parameters consisting of material/water ratio, microwave power and extraction time. The study results are expected to aid in opening up the potential of Citrus reticulata oil in cosmetic, food and pharmaceutical industry.

2. Materials and Methods

2.1. Plant material
Healthy and ripened green mandarin fruits with smooth and plump skin were collected from Cho Lach district (10°15′ 53″ N, 106° 7′ 48 ″E), Ben Tre province, Vietnam. After washing the fruits, the fruit peels were separated from the flesh, pureed and stored in a low-temperature cooler.

2.2. Extraction of EOs by microwave assisted hydrodistillation (MAHD)
Microwave is an electromagnetic wave that propagates at the speed of light. Electromagnetic waves with a frequency range of 300 MHz to 300GHz can generate heat when penetrating and interacting with molecules such as water in materials. In this study, the MW71E microwave oven (SAMSUNG, Vietnam) is connected to a Clevenger type device to operate the hydrodistillation as shown in Figure 1. Raw materials and distilled water were added to a 1000 mL storage container and installed in the microwave compartment. The essential oil exits outside, entice the steam to the condensing system or dissolving into water that is covering the outside of the material. The essential oil samples were collected and dehydrated with anhydrous sodium sulfate, and stored in amber vials until analysis. Parameters include the ratio of materials/water, the capacity and extraction time surveyed by one-factor-at-a-time method.

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

2.3. GC-MS analyses
A GC Agilent 6890 N instrument coupled with HP5-MS column and MS 5973 inert was used to determine chemical composition of obtained essential oil. 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 Na2SO4. The flow rate of was constant at 1 mL/min. Injector temperature is 250 °C and the rate of division is 30. The thermal program is as follows: 50 °C maintained in 2 min, then increased by 2 °C / min to 80 °C, 5 °C / min to 150 °C, 10°C / min to 200°C, and finally increase by 20 °C / min to 300 °C and maintained for 5 min.
3. Results and Discussion

3.1. The effect of power, shell ratio/distilled water, and extraction time

The effect of microwave power, material ratio and solvent and extraction time on the EOs yield was shown in Figure 2. Firstly, in terms of microwave power levels, the EOs yield varied peaked at 6.8% at 600 W and reduced to 4% at 700 W [21].

Secondly, in terms of material ratio and solvent, it was shown that when using the ratio of material/water of 1: 2, the amount of attar obtained was 6.8%. And when the ratio was increased to 1: 3, the amount of obtained EOs also increased with a yield of 7%. However, further increasing the ratio past 1:3 seemed to cause the extraction efficiency to reduce. Specifically, when increasing the ratio to 1: 4, 1: 5 and 1: 6, the performance of essential oil in turn decreased to 4.8, 4.6 and 4.4% respectively [22]. According to the above results, selecting a 1: 3 ratio (shell/water) is optimal for the extraction process.

Finally, in terms of extraction time on the efficiency of the EOs extraction, it was shown that the amount of EOs increased rapidly in the first 30 minutes, reached the efficiency of 4%, then increased slowly and stable for the next 25 minutes and peaked at 55 minutes with 7% efficiency [23]. Determination of the appropriate distillation time is essential in order to obtain the most maximum essential oils and the highest economic efficiency. Therefore, the optimal distillation time with essential oil samples is 55 minutes. The average yield of tangerine peel oil is 7%. The final essential oil was a transparent liquid having a characteristic aroma of fruit.

![Figure 2](image-url)

Figure 2. The effect of (A) power to extract, (B) shell ratio/distilled water and (C) extraction time on the yield of *Citrus reticulata* Blanco, peel essential oils.

3.2. Chemical composition of mandarin peel EOs

The retention time and content of each component in tangerine peel essential oil samples are shown by the results of GC-MS analysis in the Table 1. The yield of mandarin peel essential oil obtained from microwave-assisted hydrodistillation extraction was 7% [24]. This oil contained 5 monoterpene hydrocarbons, amounting to 99.727% of the oil content. The chromatogram of the mandarin peel essential oil in Figure 3 shows that there are 5 peaks at different sizes, corresponding to 5 compounds shown in Table 1. The main constituents were limonene (97.688%), sabinene (0.12%), β- myrcene (1.394%), (-)-β-
pinene (0.273%) and 1R-α-pinene (0.525%). No clear peaks attributing to sesquiterpenes, esters, ketones and aldehydes were spotted. The high content of limonene is consistent with Javed et al. (2014), which found that the main ingredients in tangerine peel essential oil were limonene (87.45%) and α-terpineol (12.55%) [25]. However, in another report, Minh Tu et al. (2002) has shown that there are at least 43 compounds identified from tangerine peel essential oil obtained by cold pressing, some of which were limonene (95.1%), myrcene (2.0%), α-pinene (0.5%) and β-pinene (0.4%) [26]. Sultana, Ali, and Panda conducted essential oil extraction by hydrodistillation method, the volatile oil chemical composition of Citrus reticulata Blanco fruit (Rutaceae) of Delhi region is composed mainly of monoterpene (99.1%) formed limonene (92.4%), γ-terpene (2.6%) and β-phellandren (1.8%) [11]. The composition of EOs is a characteristic that depends on the initial plant source, the method of oil extraction, the environment factor, the time of harvest, humidity, temperature, as well as used plant parts, resulting in differences in performance as well as volatile composition in tangerine peel oils in different areas [7, 27-28]. The limonene has been suggested to be responsible for strong inhibitory properties of essential oils against food-borne bacteria such as Escherichia coli, Bacillus subtilis, Aspergillus flavus, Staphylococcus aureus and Penicillium digitatum [29]. Tao, Jia, and Zhou (2014) found that limonene is a potential antifungal agent against P. italicum and P. digitatum [30]. Therefore, limonene is the most important component that determines the quality of the Citrus reticulata essential oil, suggesting the application as additive in food, soap, perfume to create odors and aromas [31].

| Peak | R.T.  | Name          | Molecular formula | CAS number   | Content(%) |
|------|-------|---------------|------------------|--------------|------------|
| 1    | 7.24  | 1R-α-pinene   | C_{10}H_{16}     | 7785-70-8    | 0.525      |
| 2    | 8.976 | Sabinene      | C_{10}H_{16}     | 3387-41-5    | 0.12       |
| 3    | 9.06  | (−)-β-pinene  | C_{10}H_{16}     | 127-91-3     | 0.273      |
| 4    | 9.917 | β-myrcene     | C_{10}H_{16}     | 123-35-3     | 1.394      |
| 5    | 11.956| Limonene      | C_{10}H_{16}     | 138-86-3     | 97.688     |

4. Conclusions
Numerous methods have been employed to extract the high-value essential oil in Citrus reticulata Blanco peel. However, microwave-assisted hydrodistillation has not been studied in terms of its extraction efficiency. The study was the first to optimize the parameters of microwave assisted hydrodistillation
method to achieve the highest extraction efficiency of mandarin essential oil. The optimal oil yield was 7%, corresponding with the peel/water ratio of 1:3, the microwave capacity of 600W and the extraction time of 55 minutes. The chromatographic analysis allowed qualitative and quantitative determination of 5 components with the main components of limonene (97.688%), β-myrcene (1.394%), 1-α-pinene (0.273%), sabine (0.12%) and 1R-α-pinene (0.525%). These results have proposed an extraction method for mandarin fruit essential oils with the aim of supporting the feasibility for scaling up the extraction at a low cost within a shortened amount of time.

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