Microwave-assisted hydrodistillation and determines volatile components of essential oils from Calamondin (Citrus microcarpa) shells

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Abstract. Since the industrial economic development, natural compounds have received much attention and study in recent years. Essential oils (EOs) that contain many volatile constituents and aromatic compounds have been around for a long time but are still considered today due to their natural characteristics and unique application. In this study, the leaves of Calamondin (Citrus microcarpa) shells were used to investigate the yield of essential oil extracted by the Microwave-assisted hydrodistillation (MAHD). Calamondin essential oil is determined by the microwave assisted hydrodistillation method and analyzed by GC-MS. Extraction essential oil Calamondin with MAHD in optimal conditions for 45 min, 300W capacity and 1:3 shells/water ratio. The main components of volatile compounds in Calamondin essential oils include limonene (96.039%), β-myrcene (0.953%), 1R-α-pinene (0.266%), sabinene (0.55%), 1,6-cyclodecadiene (1.611%), Y-eudesmol (0.207%), β-eudesmol (0.232%), α-eudesmol (0.185%). The oil produced by microwaves assisted hydrodistillation (MAHD) method was found to have higher levels of compounds and significant economic values.

Keywords: Microwave-assisted hydrodistillation, Volatile components, Essential oils, Calamondin (Citrus microcarpa), shells

1. Introduction

Essential oils (EOs) are aromatic compounds that are widely used in the pharmaceutical, perfume, food and other industries. Chemical composition of EOs could be made of up to 200 different compounds of which a large proportion of monoterpenic hydrocarbons, sesquiterpene and their derivatives such as esters, alcohols, and aldehydes [1-4].

Citrus microcarpa, which is also known as Calamondin, is a member of the Citrus genus of Rutaceae family. The EO derived from the plant is a transparent liquid which has a light yellow color and a very pleasant aroma. The main components existing in the Calamondin EO was found as Limomen, accounting for over 85% of total content, followed by IR-alpha-pinene, beta-pinene, sabinen, b-cocimen and linalool [5], [6]. It is the difference in chemical composition that may have contributed to the antibacterial as well as other biological activities of Calamondin EO [7] [13-21] [22-25].

Of all the wide range of EOs extraction methods developed over the past few years, microwave-aided hydrodistillation (MAHD) has been recognized as a highly promising extraction method which is capable
of producing high-quality oil yield within a shorter period of extraction time while lowering environmental impacts, as compared to other methods such as cold pressing and hydrolysis [32-34]. However, studies that employed this method to extract Calamondin EOs have remained lacking. With such attempt, this study aimed to utilize this method to extract EO from Calamondin peels and optimize the relevant parameters to achieve an optimal yield [35,36]. In addition, the composition of Calamondin EO was analyzed by gas chromatography with mass spectrometry (GC-MS) to determine the volatile components in EO.

Now, in the world, there are quite a number of studies on the benefits, chemical properties and biological properties of calamondin essential oils. The effective methods of exploiting these essential oils have also been studied and improved greatly towards modernization to minimize costs, time and bring about high efficiency. In Vietnam, there are also a lot of studies on calamondin. However, the studies on the extraction of these essential oils are very limited. Therefore, the use of microwave support method in the study of calamondin essential oil extraction very necessary.

2. Materials and Methods

2.1. Materials

Calamondin was selected and collected from Cho Lach District (10°15′53″N, 106°7′48″E), Ben Tre province, Vietnam. Ripe and smooth-skinned fruits were selected, washed, drained and removed stalks, pods and peels. Peels of Calamondin were then used to extract the EO.

2.2. Extraction of EO by microwave assisted hydrodistillation (MAHD)

The MW71E microwave oven (SAMSUNG, Vietnam) was connected to a Clevenger type device to operate the hydrodistillation. Samsung MW71E mechanical microwave oven has a capacity of up to 800W, divided by 5 different heat levels. Operating with 220V power source. Raw materials and distilled water were added to a 1000mL storage container and installed in the microwave compartment. Under the action of microwaves, the water in the plant cells was heated up, elevating the internal pressure dramatically and causing the tissues containing the EO to break. The EO after existing to the surrounding media was enticed by the steam to the condensing system or dissolved into water that was covered the outside of the material. The collected EO samples were dehydrated with anhydrous sodium sulfate, and stored in amber and preserved vials until analysis. The following parameters were assessed: the ratio of materials/water, the capacity and extraction time of the method of each individual variable.

The effect of power to extract: conduct experiments with 100g pureed calamondin peel, 1: 3 ratio of raw materials and distilled water. The extraction process is performed at power 300, 450, 600, 700W, within a 40 minute period. All experiments were performed three times over.

The effect of shell ratio/distilled water: With the optimal extracting capacity selected 300W, continue to fix the time of 40 minutes. Investigate the extraction of essential oils with shell material and water at 1: 2 to 1: 6 ratio (g / mL). Weigh and analyze the essential oil sample composition to compare the content and composition of essential oils in a different ratio to choose the optimal volume. All experiments were performed three times over.

The effect of extraction time: Fixed two elements of the extraction process with a capacity of 300W and the shell/water ratio and changed the extraction time in stages 5, 15, 25, 35, 45, 55 minutes. Time is started from when we get the first drop of small essential oil condensation down. All experiments were performed three times over.

2.3. Gas chromatography-Mass spectrometry (GC-MS) analyses

Chemical composition of the calamondin EO was determined by GC-MS analysis. A GC Agilent 6890 N instrument 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. The flow rate was kept constant at 1 mL/min. The temperature of injector was set at 250°C and the rate of division was 30. Thermal program for samples began at 50°C for 2 min, then continued to rise from 80°C, 150°C, 200°C and finally reached 300°C.

3. Results and Discussion

3.1. The effect of Extraction conditions

Figure 1 showed the effect of material size of the calamondin peel on the yield of EO after the extraction process. The yield obtained when using pureed material (2%) was almost twice as high as that of sliced
(1.2%) and raw (0.8%) materials. In general, as the material size reduced, the efficiency obtained as well as the weight of the EO was significantly changed. Results have shown that smaller raw materials have caused improvements in EO yield. With non-ground materials, the process of extracting essential oils involved osmosis and dissolution. Initially, water started penetrating from the outside into the cells, and dissolved the EO. Under the effect of heat, the oil and water, evaporated into the external environment, followed by the attraction of the EO to the container. When grinding the raw materials, oil-containing cells were broken, promoting the escape of oil into the outside environment. In addition, the extraction process involving ground material also gave the resulting EO with transparent color and more natural odor in comparison with those obtained using nonground materials, which yield oil with yellow color and unnatural odor due to the prolonged heat exposure.

Figure 1. The effect of size of peel

Determination of an optimal temperature was crucial to ensure economic feasibility of the extraction process. Figure 2 showed the effect of microwave power on yield of calamondin EO. The separation efficiency depended greatly on the density of the EO as well as the solubility of the constituents. As shown in Figure 3, increasing the capacity from 300W to 700W seemed to reduce the efficiency. As solubility of the EO greatly depended on temperature, which could hinder the entraining of oil with steam during the distillation process. In addition, high temperature may cause the quality of the obtained essential oil to degrade. As a result, the capacity of 300W was selected for the distillation of essential oils.

Figure 2. The effect of microwave power on EO yield.

Determining the amount of a suitable material for the distillation device is essential to fully utilize the instrument without compromising extraction efficiency. The degree of microwave influence of essential oil tissue types is not the same due to the construction of different tissues, as soon as the material is thinned. This result is reflected through the extraction period. We carried out the distillation of EO from calamondin
peel with different proportions of raw materials/water ranging from 1:2 to 1:6. Figure 3 indicated that as the ratio of raw material/water changed from 1:2 to 1:6, the recovery efficiency decline to the minimum yield of 0.4%. The ratio of raw materials/water of 1:3 produced the optimal recovery efficiency of 2%. However, when rising the ratio from 1:4 to 1:6, the efficiency of oil collection decreased significantly. In addition, when the ratio of water and raw materials, water is affected rapidly, the polar components (compounds containing oxygen) present in the essential oils are also affected by the microwave. In contrast, the hydrocarbon constituents are less susceptible to microwaves (due to their poor polarity), so their extraction is similar to that in normal steam distillation, but at a much faster rate because the water is heated rapidly by microwave. Therefore, the 1:3 ratio was used in subsequent experiments.

**Figure 3.** The effect of shell ratio/distilled water

Oil recovery efficiencies with respect to extraction times were illustrated in Figure 4. Under the action of microwaves, the water in the plant cells is heated up, the internal pressure increases suddenly causing the essential oil-bearing tissues to rupture. Essential oil escapes to the outside, attracts steam into a condensing system or dissolves into the organic solvent that is covering the material. The essential oil will escape outside during the period of peak and gradually decrease later in the distillation process because the essential oil decomposes when the heating time is prolonged. It was shown that when the distillation time was increased from 5 to 45 min, there was a significant increase in EO recovery. However, extending the distillation time over 45 min has caused a minor reduction in oil yield. Through the obtained results, it seems that the maximum oil recovery efficiency of 2% was achieved at 45 min. Therefore, 45 min was selected as the appropriate time for the optimal distillation process.

**Figure 4.** The effect of extraction time (min) on EO yield.
3.2. Determination of volatile components of EO

Determination of EO compounds were carried out by comparing the mass spectra and GC retention time with the standard spectra library (Table 1). The peak with the highest intensity in the chromatogram was the peak with 11.935 of retention time (Figure 5), which could be attributed to limonene (96.039%). Other identified components with lower contents were β-myrcene (0.953%), 1R-α-Pinene (0.266%), Sabinene (0.55%), 1,6-Cyclodecadiene (1.611%), Y-Eudesmol (0.207%), β-Eudesmol (0.232%), and α-Eudesmol (0.185%). A previous report on the chemical composition of Malaysian calamondin EO showed the dominant compounds included, β-sesquiphellandrene (18.3%) hedycaryol (19.0%), β-eudesmol (8.6%) and α-eudesmol (14.4%), which were more significant compared to the current results [37]. The major volatile components of the EO by cold pressing (CP), steam distillation (SD), and hot water heating (HWH) was limonene (91.15-92.70%), germacrene D (0.54-1.21%) and myrcene (2.08-2.46%). Meanwhile, the major compounds identified from whole fruit EO (HWH/SD) were limonene (89.28%), β-myrcene (2.72%), and α-terpineol (1.92%) by Chen et al. (2013) [38].

The composition of volatile compounds in EO can be influenced by environmental factors, time, extraction process and the geographical characteristics of the area. The difference between the concentration of EO and standard antibiotics can be explained by the fact that the active ingredients in the oil only include a part of the EO used and the concentration of active ingredients can be much lower than the standard antibiotics used [39]. EO contains a wide range of complex volatile compounds that can exhibit strong antimicrobial activity individually or by combining with different compounds [40]. In this study, α-pinene and limonene which were found to enrich in calamondin EO, was reported to show anti-fungal activity [41,42].

![Figure 5. Chromatography of Calamondin EO](image)

**Table 1. Chemical composition of EO from Calamondin peels microwave-assisted hydro-distillation**

| Peak | R.T. | Name       | Structural formula | CAS number   | Pct Total |
|------|------|------------|--------------------|--------------|-----------|
| 1    | 7.261| 1R-α-Pinene| ![structural_formula](image) | 7785-70-8    | 0.266     |
| 2    | 8.997| Sabinene   | ![structural_formula](image) | 3387-41-5   | 0.055     |
### 4. Conclusion

The Calamondin shell EOs was obtained from the microwave-assisted hydrodistillation method and its composition was analyzed by GC-MS. Ten volatile compounds have been identified, accounting for 99.383% of the total content of calamondin EOs. Optimal conditions of extraction included 300W, 1:3 shells/water ratio, 45 min and pureed ingredients. The main ingredient in parts of the occlusion was limonene (96.039%), followed by β-myrcene (0.953%), 1R-α-Pinene (0.266%), Sabinene (0.55%), 1,6-Cyclodecadiene (1.611%), γ-Eudesmol (0.207%), β-Eudesmol (0.232%), α-Eudesmol (0.185%).

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