Effect of microwaves energy on volatile compounds in Pepper (Piper nigrum L.) leaves essential oil

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Abstract. The Pepper (Piper nigrum L.) with spicy flavor, which is considered as one of the special spices in Vietnamese dishes. In the pepper contains a high content of essential oils contained in seeds and leaves, which not only have antioxidant and antibacterial resistance but also anti-Colon toxin, antidepressant, antifungal, analgesic, antidiarrhoeal. In the present study, we optimized the yield of essential oils obtained from Pepper leaves by Microwave-assisted hydrodistillation (MAHD) extraction method by examining the factors that affect the extraction process such as the size of leaves, the ratio of materials with solvent, time and microwave power. The highest essential oil performance achieved 0.94 mL/g by MAHD at grind size, extraction time (40 min), the ratio of water to raw materials (4:1mL/g), and microwave power (450W). A total of 22 volatile constituents were identified from the pepper leaves oil, with the major components being 3-Carene, D-Limonene, Caryophyllene, and β-Pinene.

1. Introduction

Essential oils, also known as etheric oils (volatile oil), are a complex mixture of volatile compounds produced by various organs of the plant including roots, stems, leaves, and flowers [1–6]. The plant materials could be subjected to either traditional hydrodistillation extraction or steam distillation...
extraction to isolate essential oils. However, the two methods share various limitations, including long extraction duration and low yield efficiency. Microwave-assisted extraction (MAE) is an emerging approach for isolation of natural compounds from plant materials. Previous studies have shown that the application of microwave in isolation of natural compounds resulted in reasonable outcome [7–10]. In MAE, the heating of the material was induced by ionic conduction and dipole rotation, caused by the electromagnetic field, resulting in elevation of temperature in the solution and enhanced transfer efficiency of target solute to outside solution. In comparison with traditional methods, MAE offers several advantages, including improved extract quality, higher yields, less time- and solvent-consuming.

*Piper nigrum* L., also referred to as black pepper, is an indigenous climber plant to South India whose height can reach 50–60 cm. Like another aromatic plant, black pepper is known for carminative, antioxidative, antiseptic and antimicrobial properties, finding wide use in traditional medicine for treatment of influenza, atrophic arthritis, apathy, and febricity. The plant could also be used as an antibacterial agent, nerve tonic and antitoxin. Industrial application of *P. nigrum* mainly revolves around its essential oil and are diverse, ranging from the manufacture of food, pharmaceuticals, perfume [11,12]. In this study, isolation of essential oils from *Piper nigrum* l. leaves harvested from the South of Vietnam was attempted. We adopted microwave-assisted extraction (MAE) to obtain *Piper nigrum* l. leaves oil and experimental design was performed to optimize experimental parameters relevant to the oil yields. We consider particle size of the materials, extraction time, microwave power, and the liquid/solid ratio as variables of interest. Moreover, volatile chemical compounds in essential oils obtained from *Piper nigrum* l. are determined by GC-MS.

2. Material and Methods

2.1 Plant Samples

The leaves of the raw pepper used for this experiment were harvested from Ba Ria Vung Tau Province, the Southeast Region of Vietnam. Leaves samples were washed several times in the water and one last time with distilled water from the double-distilled water distillation system (Labsil OPTI-D-4) at the laboratory of the NTT High-Tech Institute and preserved in coolers (LC-1416B, Alaska, Ho Chi Minh City, Vietnam) at temperatures of about 4–10°C. Samples selected for were cut into fibre about 2-3 cm in size and ground by the versatile grinder of Sunhouse (SHD 5328, Vietnam).

2.2 Microwave-Assisted Hydrodistillation

MAE process of essential oils from the leaves of *Piper nigrum* L. is depicted in figure 1. A home microwave oven (model - Samsung MW71E), combined directly to the Clevenger apparatus includes a steam condenser to condention steam with essential oils and a separator to separation water with essential oils (purchased by Bach Khoa Ltd., Ho Chi Minh City, Vietnam). Microwave oven operates at a maximum capacity of 800W, power source of 250v - 50Hz) and microwave irradiation frequency of 2450 MHz. For this extraction process, the cleaned and preserved pepper leaves were transferred to a 1.0 litre flask, set in the microwave compartment and connected to the Clevenger apparatus. During extraction, the heat of microwave assisted to the essential oil from pepper leaves was extracted and separated by separator from the 10minute survey period during the 60-minute extraction period. After extraction, the essential oil from the raw pepper leaves is dehydrated on anhydrous sodium sulfate (Na$_2$SO$_4$, Sigma-Aldrich, St. Louis, MO, USA) to remove excess water in the essential oil, then the pure essential oil is and stored in a dark bottle in the refrigerator at 10 °C.

2.3 The optimization of the essential oil extraction

In this process, four parameters are optimized include the material size (leaves, fiber cut, grinded), the ratio of water and raw materials (mL/g) (from 2:01 continuous until 6:01), extraction time (from 10 to
60 minutes, each experiment is raised to 10 minutes) and microwave power (from 150W to 600W, each experiment is separated by 150W) for the optimization of the essential oil extraction for microwave-assisted hydrodistillation (MAHD) method.

In order to evaluate the optimum between experiments, the yield of the *Piper nigrum* L. (pepper) leaves essential oil obtained was examined through equation (1).

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\text{Yield of essential oil was obtained} = \frac{\text{Volume of attained essential oil}}{\text{Amount of the raw materials used}} \quad (1)
\]

2.4 Identification of Components by Gas Chromatography-Mass Spectrometry (GC-MS)

After the process had been optimized, 25 µL of essential oil was then taken and mixed in 1.0 mL n-hexane and dehydrated with Na2SO4 for GC-MS analysis. GC Agilent 6890 N (Agilent Technologies, Santa Clara, CA, USA) was used as analysis instrument. The device was coupled with MS 5973 inert, HP5-MS column, head column pressure of 9.3 psi. Following conditions were imposed: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 µL; injection temperature 250°C. From the initial hold at 50°C for 2 min, oven temperature progressed to 80°C at 2°C/min, from 80°C to 150°C at 5°C/min, from 150°C to 200°C at 10°C/min, from 200°C to 300°C at 20°C/min and was maintained at 300°C for 5 min.

3. Results and Discussion

3.1 Effect of size material on Yield

Effect of the size of raw material on the extraction yield of the essential oil from the leaves of *Piper nigrum* L. at fix water to raw material ratio of 3:01 (mL/g) after 30 (min) with microwave power at 450 (W) is shown in Figure 1. According to Figure 1, essential oil obtained yield increases as raw material size decreases (from 0.3 mL/g at natural size up to 0.85 mL/g at ground size), but to a specific size this yield is no increases. This is explained by as the materials is cut, the contact surface between water and the surfaces is extended, facilitating water diffusion into the essential oil bags of the material, quickly pushing the oil outside under the effect of microwave leading to higher yield [13–15]. However, if the materials cut into tiny pieces, more essential oil will be lost in the cutting process, resulting in loss of efficiency of oils obtained. Consequently, the ground size of leaves was selected for use in subsequent experiments.

![Figure 1. Effect of the size of raw material on extraction yield](image)

3.2 Effect of Extraction Time on Yield

Figure 2 illustrates that pepper oil yield was constant when being extracted in 40 minutes. Visually, there was a progressive increase in the yield of pepper essential oil as the extraction time increased
from 10 to 40 minutes. At the extraction time of 10, 20, 30, and 40 minutes, the yield of pepper from the leaves extracted was 0.2 mL/g, 0.55 mL/g, 0.70 mL/g, and 0.90 mL/g, respectively. However, when the extraction time more than 40 minutes and up to 60 minutes, the pepper leaves oil yield decreases significantly [16]. MAHD could both reduce the extraction time and acceleration extraction of essential oils, thus creating a higher energy saving ability while still maintaining high oil yield [17]. At the same time, this increase in the yield thanks to water solvents, which have high polarity and high dielectric properties, contribute to higher heating rates. However, after 50 minutes, the decrease in essential oil yield could be attributable to evaporation of volatile components in plant material, caused by prolonged microwave and heat exposure [18], resulting in a reduction in the yield of pepper leaves as in previous studied [7-10]. Therefore, for MAHD in this study, 40 minutes was chosen as the optimal time to extract pepper leaves oil.

Figure 2. Effect of extraction time on extraction yield

3.3 Effect of Water to Raw Material Ratio on Yield

Effect of different water-to-pepper ratio on pepper oil yield was investigated with constant weight of 100g of raw material, extraction time at 40 min and microwave power of 450 W. Water quantity varies from 2:01 to 6:01 (mL/g). The yield is extracted from pepper leaves at the highest ratio at 4:01 (mL/g, 0.95 mL/g) while the lowest is at the ratio of 2:01 (mL/g, 0.6 mL/g). Typically, in the distillation process, water could both limit the metamorphosis of the material in the heated mixture and transport essential oils from the pepper leaves of the evaporation process. The use of a large amount of solvent may lead to increased energy consumption and prolong the condensation of the extract. In contrast, the volume of reduced water as a smaller solvent can make the extraction process not fully effective as the trend in Figure 3 [6,11]. In this study, it is noted that although there is an increase in yield, then the yield is reduced when the volume of water increases as a rule mentioned above, indicating that MAE requires less solvent to produce high yields of essential oils. Therefore, the optimal yield of the oil extracted from the pepper leaves has been obtained at the ratio of water to raw materials is 4:01 (mL/g) and this is used as a reference condition to study other parameters.
3.4 Effect of Microwave Power on Yield

Since microwave power could be converted into thermal energy in dielectric materials such as water, thus elevating the temperature of the mixture, it is a vital factor that often affects the efficiency of MAE. The influence of microwave power on extraction yield is shown in Figure 4 in which the extraction time and water-to-material ratio were set constant at 40 minutes and 4:01 mL/g, respectively. It is also revealed that the yield increases with increasing microwave power level until it reaches a certain peak, where it begins to decrease. The reason for this is that higher microwave power provided more energy for the mixture of water and pepper leaves, creating fast heat for enhanced extraction [23,24]. However, it can be seen that pepper oil yield begins to decrease when the microwave capacity continues to increase by more than 450W, which is evident when the capacity from 450W increases to 600W, the yield decreases from 0.95 mL/g down to 0.75 mL/g due to the influence of extreme microwave power, resulting in very high temperature and damaged produced compounds. Based on the above observations, 450W is defined as the optimal microwave power for extraction of essential oils from pepper leaves.

3.5. The Result of GC-MS

Using the optimum conditions of pepper leaves extraction, including the size is grinded with the ratio of water to leaves of 4:01 (mL/g), 450 (W) for microwave power and time extraction of 40 minutes, a
total of about 22 observed components accounted for 99.294% of the total essential oil content obtained and is presented in Table 1.

![Figure 5](Image)

**Figure 5.** The result of chromatography of pepper essential oil

| Peak | Retention time | Name                        | Content % |
|------|----------------|-----------------------------|-----------|
| 1    | 7.167          | Thujene                     | 0.297     |
| 2    | 7.397          | α-Pinene                    | 1.641     |
| 3    | 9.154          | Sabinene                    | 1.389     |
| 4    | 9.248          | β-pinene                    | 10.041    |
| 5    | 10.105         | Myrcene                     | 0.385     |
| 6    | 11.789         | o-Cymene                    | 17.968    |
| 7    | 12.082         | Limonene                    | 55.511    |
| 8    | 12.113         | Eucalyptol                  | 0.48      |
| 9    | 17.467         | trans-p-Mentha-2,8-dienol   | 0.316     |
| 10   | 18.304         | cis-Limonene oxide          | 1.121     |
| 11   | 18.481         | cis-p-Mentha-2,8-diene-1-ol | 0.647     |
| 12   | 18.638         | trans-Limonene oxide        | 1.146     |
| 13   | 23.041         | α-Terpineol                 | 0.411     |
| 14   | 23.428         | Myrtenol                    | 0.57      |
| 15   | 25.132         | cis-Carveol                 | 0.502     |
| 16   | 25.833         | trans-Carveol               | 0.46      |
| 17   | 26.502         | D-Carvone                   | 1.255     |
| 18   | 30.946         | Limonene-1,2-diol           | 0.453     |
| 19   | 31.939         | Nerol acetate               | 2.109     |
| 20   | 34.219         | α-Bergamotene               | 0.944     |
| 21   | 36.426         | β-Bisabolene                | 1.033     |
| 22   | 38.172         | Caryophylene oxide          | 0.615     |
Limonene is the main component in pepper leaves essential oil (55.51%). Furthermore, pepper leaves essential oil also contains o-Cymene (17.968%), β-pinene (10.041%), α-Pinene (1.641%), Sabinene (1.389%), D-Carvone (1.255%), Limonene oxide (1.146%), and β-Bisabolene (1.033%). The percentage of area of other observed volatile compounds is less than 1.00%. Besides the difference and abundance of component in pepper leaves oils, the most limonene component in essential oils can be explained by the effect of microwaves. Specifically, microwave radiation makes polar molecules containing oxygen, such as limonene molecules, separated and attracted to steam faster. This makes the polarized compounds in the oil bag easily separated from the material, resulting in higher concentrations. In contrast, hydrocarbons, non-polar, are less absorbed by magnetic waves and are therefore less likely to be separated.

4. Conclusions
The purpose of the present article was to apply conventional extraction methods from pepper leaves (Piper nigrum L.) in Ba Ria province, Vietnam was tested to extract the essential oil. The highest essential oil achieved at 0.94 mg/L. The optimal extraction conditions for the extraction of essential oil of pepper leaves were validated at extraction time (40 min), the ratio of water to raw materials (4:1 mL/g), and microwave power (450W). Microwave-assisted hydrodistillation (MAHD) method plays a vital role in the essential oil extraction processes due to decrease processing time, increase efficiency. Result of GC/MS revealed three predominant components existing in the pepper essential oils. The abundant element is Limonene (55.51%), followed by o-Cymene (17.968%), β-pinene (10.041%).

Acknowledgments
This study was supported by Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

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