Comparison of essential oil extracted from black pepper by using various distillation methods in laboratory scale

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Abstract. Black pepper is an indispensable spice in life—pepper oil used in various fields as pharmaceutical, cosmetic, repellent. Black pepper was picked in Vietnam and its essential oil was obtained by distillation methods. This study investigated the effect of several parameters such as material/water ratio, distillation time, the weight of the sample, and extraction power on the yield and the quality of black pepper essential oil from three distillation methods. The results showed that the hydrodistillation method reached the highest yield of 2.19% at conditions of 60 minutes extraction time and material/water ratio of 1:15 g/mL compare to the steam distillation method and microwave assisted hydrodistillation method. GC-MS analysis resulted in the identification of 70 components accounting for 99% of the total amount: β-caryophyllene, 3-carene, D-limonene, and β-pinene, are among the main compounds in black pepper essential oil.

1. Introduction

The black pepper plant, also known as “black gold” or “king of spices,” is the most commonly used spice in the world due to its commercial, economic, nutritional, medicinal value [1], human dietaries, preservative, and biocontrol agent agents [2]. Although pepper production in India is the largest globally, it also is cultivated in other tropical regions, such as Brazil, China, Indonesia, Madagascar, Malaysia, Mexico, Thailand, and Vietnam [3].

β-Caryophyllene is a natural sesquiterpene, one of the constituents that contribute to the aroma of pepper oil. Several biological activities have been attributed to β-caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, and local anesthetic activities [4]. Limonene, another primary compound found in black pepper oil, has well established chemopreventive activity against many chemically induced cancers in animal models, such as colon cancer, gastric cancer, and hepatic cancer with promising results [5, 6]. The antioxidant constituents found in the essential oil of black pepper include p-cymene, α-terpineol, while α-pinene, β-pinene, δ-3-carene, Limonene, and α-terpineol inhibit bacterial growth [1]. Gurdip Singh identified 49 components accounting for 99.39% of the total amount, and the major components were β-caryophyllene (24.24%), Limonene (16.88%), sabine (13.01%), β-bisabolene (7.69%), and α-copaene (6.3%) [7]. I. P. S. Kapoor analyzed pepper essential oil using the GC-MS analysis method showed 54 components representing about 96.6% of the total weight. β-
caryophyllene (29.9%) was found as the primary component along with Limonene (13.2%), β-pinene (7.9%), sabinene (5.9%), and several other parts [8]. T. Jagelle and W. Grosch (1999) indicated α- and β-pinene, myrcene, α-phellandrene, Limonene, linalool, methyl propanol, 3-methyl-butanal, butyric acid, and 3-methyl-butyric acid as key odorants [9].

Ferreira was studied the essential oil of black pepper in a fixed bed extractor using liquid carbon dioxide as a green solvent. The solubility of the oil in the solvent was determined using the dynamic method [10]. Kudaya Sankar used supercritical fluid carbon dioxide at a pressure of 8 and 10 MPa and two different temperatures, 40 °C and 60 °C. The result showed that pepper oil obtained with supercritical fluid carbon dioxide at 10 MPa and 60 °C was superior to the rest [11]. Kottarapat Jeena prepared black pepper oil by steam distillation (yield- 5-6%) was purchased from Kancore Ingredients Limited, Angamali, Kerala India. The color and appearance of black pepper essential oil were pale yellows [12].

Ying Wang (2008) has studied the essential oils of black pepper (Piper nigrum L.) from China that were isolated by hydrodistillation (H.D.) and microwave-assisted hydrodistillation (MHD) in yields of 3.1% and 3.8%, respectively. The optimum conditions for MHD were: Microwave delivered power 800 W, treatment time 5 min. and a 1/10 ratio of spice to water [13]. Andri C. Kumoro et al. (2009) indicated that the effect of process parameters, namely pressure (7.5, 10, and 15 MPa), temperature (303, 313, and 323 K), and particle size (0.5 mm, 0.75 mm, and whole berries), on the extraction rate was examined in a series of experiments conducted in a laboratory-scale apparatus [14].

The aim of this study is to investigate the optimal conditions in three distillation methods, including hydrodistillation, steam distillation, and microwave-assisted hydrodistillation to obtain the highest extraction yields and compared the composition of these methods.

2. Material and methods

2.1. Plant material

The materials used in this study were black pepper (Piper nigrum L.) seeds, which were grown, harvested, and processed in Chau Duc, Ba Ria-Vung tau Province, Vietnam. The seeds were collected in March 2019. Then, it was picked, naturally dried, and then ground using the grinder. Sodium sulfate was purchased from Merck, Germany. Water used in the research was distilled by the water distillation unit from India with a capacity of 4 L water in an hour. Sodium chloride was used to soak the solution in the hydrodistillation method.

Figure 1. Distillation models include (a) hydro distillation, (b) microwave-assisted hydrodistillation and (c) steam distillation
2.2. Distillation process

There were three distillation methods used in this study, including hydrodistillation, steam distillation, and microwave-assisted hydrodistillation that shown in Figure 1. The ground seed was added to the distillate flask. The extraction time is counted/started when the first drop of condensed essential oil is condensed and dropped into the oil extraction system. After the extraction process completes, the essential oil is recovered and dehydrated with sodium sulfate. Figure 2 illustrated the process extract essential oil from black pepper.

2.2.1. Hydrodistillation. An amount of 40 g of black pepper powder and 600 mL water was placed into a 1000 mL volumetric flask directly connected to the Clevenger apparatus. Sodium chloride was dissolved in water to a certain concentration and added to the flask containing the material, after being soaked with sodium chloride solution for the required time period and heated with the heater. The extraction time is started when the first drop of condensed essential oil is condensed and dropped into the oil extraction system. After the extraction process is completed, the essential oil is recovered and dehydrated with sodium sulfate and maintain at 10 °C in a cooler [15]. Factors affecting the extraction of black pepper essential oil, including a concentration in the sodium chloride solution, the ratio material to water, extraction time, were examined in this study. First of all, pepper powder was measured humidity by moisture analyzer. This step is performed for all of the methods. Concentration of the sodium chloride solution is ranging from 0 to 2.5% solution (wt/wt). After that, the ratio of raw material to solvent (1/10, 1/15, 1/20, 1/25) and extraction time (1-4 h) was studied. The single-factor method is used to optimize conditions for all methods. Each experiment was repeated three times, and the average value was recorded.

![Figure 2. Distillation process.](image-url)
2.2.2. **Steam distillation.** 300g pepper powder was placed in a flask connect with a bottom flask containing water in a vertical model. The water vapor produced in the bottle crosses the black pepper, charges essential oil to the condenser, where it is condensed. Then the oil is separated from water by decantation. After the extraction process is completed, the essential oil is recovered and dehydrated with sodium sulfate and maintain at 10°C in a cooler [16]. For the steam distillation method, heating capacity is proportional to the evaporation rate and condense rate of the solvent. The evaporation rate of the solvent was ranging from 0.05 to 0.13 mL/s. In addition, material feed ranging from 200-500 grams and extraction time (1-4 h) were also studied. Each experiment was repeated three times, and the average value was recorded.

2.2.3. **Microwave-assisted hydrodistillation.** The microwave oven was modified by drilling a hole at the top. An amount of 50 g black pepper powder and 50 mL water was added to a flask having a capacity of 1000 mL was placed in the oven and connected to Clevenger apparatus through the hole. During the process, the vapor passed through the condenser outside the microwave cavity, where it was condensed. After the extraction process is completed, the essential oil is recovered and dehydrated with sodium sulfate, and maintain at 10°C in a cooler [17, 18]. Factors affecting the extraction of black pepper essential oil by microwave-assisted hydrodistillation, including the ratio of material to solvent, power of microwave, and extraction time. First of all, the ratio of raw material to solvent (1/1–1/5 g/mL), extraction time (15-30 min), and power of microwave from 100 W, 200 W, 380 W, and 540 W were also studied. Each experiment was repeated three times, and the average value was recorded.

2.3. **Determination of the chemical composition of essential oils by GC-MS**

Today GC/MS techniques are widely applied and widely used in industries such as medicine, environment, agriculture, and food testing. In addition, the presence of spurious peaks in a spectrum adds to the risk of making false identifications. Perhaps worst of all, this uncertainty in the origin of mass spectral peaks leads to a general loss of confidence in the reliability of making identifications by GC/MS, especially for trace components in complex mixtures, a key application area for this technique [19-21]. GC-MS analysis was carried out in a Thermo Fisher Scientific Trace 1310 gas chromatography combined with TSQ 9000 Tandem mass spectrometer. The MS scanning (2 scan s⁻²) was performed in the mass range from 50-600 amu with electron impact ionization at 70eV. Helium was used as carrier gas in a split ratio of 1:250. The oven temperature was programmed as: (i) 50°C for 30 sec; (ii) rate of 5°C.min⁻¹ from 50°C to 180°C; (iii) rate of 20°C min⁻¹ from 180°C to 300°C and hold for 15 min.

2.4. **Extraction yield**

The yield of extraction was calculated by equation (1):

\[
\text{The yield of black pepper oil (\%)} = \frac{m_{\text{oil}}}{m_{s}} \times 100\%
\]  

Where:

- \(m_{\text{oil}}\) = weight of black pepper oil in g
- \(m_{s}\) = weight of dry material in g

3. **Results and discussions**

3.1. **Optimizing parameters to hydrodistillation method**

Extracted yield increase from 1.82% to 2.18% when the material to solvent increase from 1:10 to 1:15. Sample with ratio 1:15 and 1:20 gave the same highest yield. Thus, ratio 1:15 is selected as the optimum ratio for the extraction as it gives the maximum yield with the minimum usage of raw material. When adding salt to the extraction mixture, the solubility of some non-polar components of essential oil into the water medium could also be reduced. The time of the extraction increases; the yield of black pepper essential oil also increased. But the extracted yield increased insignificantly from 60 min distillation, whereas the obtained yield achieved 2.19%.
3.2. Optimizing parameters to the steam distillation method
When the evaporation rate increases, the yield of black pepper essential oil also increased. When the evaporation rate was 0.13 mL/s, the highest yield of oils was 1.16%. The evaporation rate increases, rising of the residence time of vapor, leading to more vapor and material interaction, the oil obtained higher. The longer the extraction time, the higher the essential oil extracted from the material, lead to increase extracted yield. The extraction yield was found to rising insignificant when prolonging time extraction past 45 minutes.

3.3. Optimizing parameters to Microwave-assisted hydrodistillation method
The power of microwave is a significant parameter effect on interaction compounds and others. Extraction yield enhanced with growth up the microwave power from 100 W to 580 W with 0.62% and 1.33%, respectively. Power at 540 W gave the highest yield oil (1.33%), but the essential oil is dark yellow due to a small amount of material being splashed into the flask and burned. Some compounds sensitive to temperature were thermal decomposition at high temperatures. Therefore, 380W was chosen as the optimum power for subsequent surveys. Extraction yield rising rapidly from 5 minutes to 15 minutes, this tendency fallen down with increasing extraction time. Thus, 15 minutes of treatment time is the optimum condition in this investigation.

3.4. Compare the distillation efficiency of the three methods with appropriate parameters.
Figure 3 shows the yield of the distillation methods with appropriate parameters at the laboratory scale. In all three methods, the factors that affect performance are nearly the same. But the hydrodistillation method is more efficient because it applies immersion with NaCl as an electrolyte, increasing the density and polarity of the water, making it easier for essential oils to separate from water, resulting in higher yield than other methods.

3.5. The result of GC/MS method
The results of the GC-MS analysis of the black pepper essential oil extracted at the optimized conditions were presented in Table 1. GC-MS analysis resulted in the identification of 70 components. Mono-
terpenes and sesqui-terpene hydrocarbons such as β-caryophyllene, 3-carene, D-limonene, β-pinene, and α-phellandrene are the main compounds in Piper Nigrum L. essential oils. The essential oils obtained from the Steam distillation method has more sesqui-terpene hydrocarbon than the hydrodistillation method, and the Microwave-assisted hydrodistillation method, with the major compound, is β-caryophyllene (29.86%). Besides, hydrodistillation obtains most monoterpenes, an amount of 46.35% monoterpenes presence in essential oil.

Table 1. A comparison with regard to the methods of extraction.

| No | Compounds          | HD   | SD   | MD   |
|----|--------------------|------|------|------|
| 1  | α-Thujene          | 0.10 | 0.07 | 0.529|
| 2  | α-Pinene           | 5.11 | 4.67 | 0.529|
| 3  | β-pinene           | 8.13 | 7.93 | 0.011|
| 4  | Sabinene           | 0.22 | 0.20 | 5.959|
| 5  | Camphene           | 0.16 | 0.12 | 0.049|
| 6  | δ-3-Carene         | 17.12| 15.49| 2.544|
| 7  | Limonene           | 15.51| 15.24| 13.163|
| 8  | β-Caryophyllene    | 24.34| 29.86| 25.694|
| 9  | α-Copaene          | 2.67 | 4.36 | 0.052|
| 10 | β-Myrcene          | 2.60 | 2.06 | 0.065|
| 11 | α-Phellandrene     | 5.37 | 4.23 | 1.047|
| 12 | Terpinolene        | 1.03 | 0.82 | 0.195|
| 13 | α-Humulene         | 2.48 | 3.03 | 1.810|
| 14 | α-Terpinene        | 0.14 | 0.07 | 0.09 |
| 15 | p-Cymene           | 0.14 | 1.42 | 2.136|
| 16 | α-Terpineol        | 0.24 | 0.05 | 0.225|
| 17 | Linalool           | 1.03 | 0.30 | 0.504|
| 18 | Caryophyllene oxide| 0.99 | 1.54 | 1.2  |

4. Conclusions
In this study, the conditions for optimal three methods, including hydrodistillation, steam distillation, microwave assisted hydrodistillation with a laboratory scale extraction of essential oil from Ba Ria Province, Vietnamese black pepper (Piper Nigrum L.) seeds were investigated. GC-MS analysis also was used to characterize the chemical composition of the produced oil. The hydrodistillation method's optimal conditions included extraction time of about 60 minutes, NaCl concentration of 2%, and the material-to-water ratio of 1:15 g/mL, giving the highest yield of 2.19%. Besides, optimal conditions in the steam distillation method included a material 500 g, extraction time about 45 minutes in the evaporation rate of 0.13 mL/s give the highest 1.57%. Optimal conditions in microwave-assisted hydrodistillation included extraction time of about 30 minutes, a material-to-water ratio of 1:4 g/mL, extraction power 380 W, and the highest yield of 1.4%. GC-MS analysis showed that the main constituent of pepper essential oil such as α-Pinene, β-pinene, δ-3-Carene, Limonene, β-caryophyllene, β-Myrcene, α-Phellandrene, Caryophyllene oxide.

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