The extraction of rosemary essential oil by microwave-assisted hydrodistillation method

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Abstract. Rosemary (Rosmarinus officinalis L.) has been a significant herb and was used as an antiseptic, astringent, antifungal, antibacterial, antioxidant, etc. This study determined the effect of material-water ratio, material size, distillation time, and power of microwave on the obtained yield of rosemary essential oil extracted by microwave-assisted hydrodistillation method. The results show the optimum extraction condition at 1:1 of feed-water ratio, 380W of distillation power, 25 minutes distillation, and 0.3cm of material size with the optimal extracted yield of 2.7%. The major components are α-pinene (22.277%), verbenol (16.488%), 1,8-cineole (16.256%) and geraniol (5.705%), which would be a potential material for cosmetic and pharmaceutical products caused their activities such as antioxidant, antibacterial, antiseptic, reduce anxiety.

1. Introduction

Rosemary (Rosmarinus officinalis L.) belongs to the Lamiaceae family, which comprises up to 200 general and about 3500 species [1]. Since antiquity, Rosemary has been a significant herb, and it was traditionally used as an antiseptic, astringent, and preservative in food processing before the invention of refrigeration [2].

The composition of rosemary essential oil was widely studied in the world in many different regions. Christiane Takayama (2015) suggested that main constituents were 1,8-cineole (28.5%), camphor (27.7%) and α-pinene (21.3%) [3]. Angioni (2004) showed that essential oil isolated from rosemary growth in Brazil had some major compounds such as α-Pinene, borneol, verbenone, and bornyl acetate [4]. Pitarokili (2008) mentioned that composition rosemary in Greece is 24.1% α-Pinene, 14.9% camphor, 9.3% 1,8-cineol, 8.9% camphene [5]. El-Massry (2008) studied in Egyptian rosemary, the composition of rosemary oil was 52.8% 1,8-cineol, 11.9% camphor, 10.2% α-pinene, 7.5% borneol [6]. Okoh (2010) investigated rosemary oil's antibacterial activity with various bacteria, such as S. aureus, B. subtilis, E. coli, and K. pneumonia [7]. Bozin studied the bioactivity of Camphor in rosemary on S. enteritis [8]. Pitarokili (2008) mentioned that ability of α-Pinene effect to S. sclerotiorum [6]. Özcan (2008) studied in rosemary oil has 44% p-Cymene, and it should be considered because of its antifungal activity [9].

Many different methods have used to obtain rosemary essential oil. In 2005, Maria Presti studied the quality of rosemary essential extracted from four different methods: traditional hydrodistillation (HD), supercritical fluid extraction, organic solvent extraction, and microwave-assisted hydrodistillation (MAD). The components of essential oil extracted by MAD are quite similar to other methods but MAD...
has low environmental impact because using water as a solvent during distillation process [10]. In 2014, scientist Sibel Karakaya experimented on extracting rosemary essential oil by HD and MAD methods. The results showed that specific gravity, primary component, and antimicrobial activity of oil extracted from those methods are the same. But antimicrobial activity against S. aureus 6538P from MAD is more significant than the HD method [11]. In 2018, Sara Moradi compared the quantity and quality of rosemary essential oil extracted by MAD with traditional distillation. The results show that MAD has many more outstanding advantages: shorter distillation time, reduced costs, and energy consumption. The MAD method is green technology cause less CO$_2$ ejected in the atmosphere [12].

Some researches show that the loss of certain volatile compounds due to long extraction times and degradation of unsaturated or esterified compounds. An example, monoterpenes may be susceptible to chemical changes under stream distillation conditions and even the conventional solvent extraction during the removal of the solvent by distillation. Besides, many of these methods are time-consuming and energy-intensive [10, 13].

However, to reduce the extraction time and improve the quality of essential oils, new extraction techniques have been developed such as microwave-assisted hydrodistillation, solvent extraction under vacuum pressure, and supercritical fluid extraction as CO$_2$, and ultrasound-assisted extraction [14, 15]. Microwave-assisted hydrodistillation was designed and developed to replace traditional distillation. The essential oil extraction based on this technique, which was successfully tested for the extraction of essential oil from rosemary, is an attractive alternative method not only to standard procedures of essential oil steam distillation. This process is based on microwave heating [16].

In this paper, the essential oil from rosemary obtained by microwave-assisted hydrodistillation has been compared with those obtained by hydrodistillation. We make appropriate comparisons in terms of quality and quantity of essential oil. We have also proposed a mechanism for the MAD technique. This study was supplemented by scanning electron micrographs to shed light on the extraction mechanism.

2. Material and methods

2.1 Plant material and reagents

Fresh rosemary leaves were collected in August 2019 from the Seed Garden of Lam Ha district, Lam Dong province, and air-dried at a temperature of 50 °C until the humidity reach 8-10%. Sodium sulfate was purchased from Merck, Germany. India’s water distillation unit distilled water used in the research with 4 L water capacity in an hour.

2.2 Microwave-assisted hydrodistillation process

This process bases on the interaction between radiation waves with non-polar volatile compounds. During distillation, rising temperatures increase the pressure in plant organs containing essential oils. When the pressure increases above a certain level, the cell walls are broken down, and the essential oil is released from the plant material’s outer surface, the rest diffusing from the inside of the plant material to their outer surface. The steam then takes away the essential oils from the outer surface of the plant material.

Rosemary leaves after harvest underwent air-drying, then stored in the cold chamber. Material is weighted, grinding to a specific size, and measured moisture content. After that, rosemary powder and water were added into the flask. The distillation unit is then heated to a fixed temperature. The distillation time is beginning when the first liquid is dropping into the receiver. The mixture of essential oil and distilled water after passing through the condenser is carried away to recover the essential oil. The essential oil obtained with a little water should be dehydrated with pure anhydrous sodium sulfate and passed through a filter to get absolute essentials. Figure 1 showed how to procedure rosemary essential oil by the MAD method.
Factors affecting the extraction of rosemary essential oil, including the ratio of material to water, extraction time, power of the microwave, and material size, were examined in this study. First of all, grinding rosemary to various length sizes ranging from 0.1 to 0.3 cm—raw leaves, which 1.5 and 3 cm length was investigated in this research. Then, the sample was measured humidity by moisture analyzer. After that, the raw material ratio to water (1/0.5, 1/1, 1/2, 1/3, and 1/4) and extraction time (5-50 mins) was studied. The microwave power was changed from 100 W, 230 W, 380 W, 520 W, and 700 W. Single-factor method was used to optimize all processes conditions. Each experiment was repeated three times, and the average value was recorded.

![Figure 1. Extraction process](image)

2.3 Analytical method

2.3.1 Specific gravity. Specific gravity is among the important criterion of the quality and purity of essential oil. Values for essential oils vary between the limits of 0.696 and 1.188 at atmospheric. In general, the specific gravity is less than 1.0 [17]. Hence essential oil can be collected over (floating on) water. The density of the oil was determined by using a density bottle. A clean and dry, empty bottle of 25ml capacity was weighed (w0), and then the bottle was filled with the oil, stopper inserted, and reweighed to give (w1). The oil was substituted with water after washing and drying the bottle and weighed to give (w2). The expression for specific gravity (D oil) is:

\[
D_{oil} = \frac{w_1 - w_0}{w_2 - w_0}
\]

2.3.2 Gas Chromatography-Mass Spectroscopy. The GC-MS analysis achieved the component identification using HP 5890 series GC equipped with the mass selective detector (MSD), HP 5972 series (German). Helium was used as carrier gas at a constant flow of 1 mL/min, and an injection volume of 1μl was employed, injector temperature 250°C and Ion-source temperature 280°C. The oven temperature was programmed from 50°C (isothermal for 4min.), with an increase of 30°C /min, 280°C, and held for 10 min isothermal at 280°C. THE total GC running time was 90.67 min [18].
2.3.3 The essential oil yield. According to the dry vegetal matter, the essential oil yield was estimated by using the following equation [16]:

\[ Y(\%) = \frac{\text{Vol}_\text{oil} \times \text{Doil}}{m_s} \times 100 \]  

Where:
- \( \text{Vol}_\text{oil} \): Volume essential oil (ml);
- \( \text{Doil} \): Specific gavity of essential oil (g/ml);
- \( m_s \): dry material mass (g);
- \( Y \): essential oil yield (%).

3. Results and discussions

3.1. Optimizing essential extraction oil from rosemary leaves by microwave-assisted hydrodistillation

3.1.1. Investigation material size. Rosemary leaves were cut and ground to various lengths from 0.1 to 0.3 cm. The material after the ground was placed into a flask containing water. This flask is attached with a microwave oven and heated at fixed power during extraction. Based on Figure 2a, the size does not affect much on the extraction yield. The powder has a small surface area that leads to increasing the contact area with water, so steam passes quickly through the material. The size that gave the highest performance is the grinding size of 0.3 cm.

3.1.2. Investigation distillation time. Each extraction process was carried out under similar conditions in a previous investigation with 0.3 cm of material size. Extraction time changed from 5 to 50 minutes. Figure 2b shows the effect of time distillation on extraction yield. It can be seen from the graph; the more extended time occurs, the more amount of essential oil obtains. It can be explained that when extraction time is short, the interaction between water and material is not long enough for steam attraction to occur. However, from the period of 30 minutes, the amount of essential oil no longer changes. So, the appropriate time for the extraction of essential oil in this method is 30 minutes.

3.1.3 Investigation microwave power. Figure 2c shows the effect of microwave power on the yield of the distillation process. The graph’s trend shows that the higher the microwave’s heat capacity is, the more essential oils are produced. But over time, 380 W of power produces more essential oil at the end. It can break the essential oil bag in the material with a small force, but it does not have enough energy to evaporate the mixture. The essential oil remained in the water for a long time will form emulsions with water. With the high power from 540 W to 700 W, the essential oil containing in the material will decompose. In this experiment, it is shown that 380 W of capacity is optimal for extracting rosemary essential oil.

3.1.4 Investigation ratio of material to water. The effect of the ratio of material to water on distillation efficiency is shown in Figure 2d. From the graph, the higher rate is used, the much essential oil is obtained. The extraction yield goes up by 0.5% from the chart when increasing the rate from 1:0.5 to 1:1. However, adding more water to enhance water and material diffusion causes falling down the extraction yield due to forming more emulsions. Thus, the ratio of material to water 1:1 is chosen for the best optimum in this investigation.
3.2 Chemical Composition of Essential Oils

The results relating to the chemical compositions of the essential oil of *rosemary* extracted by MAD and steam distillation (SD) methods are summarized in Table 1. There are around 27 compounds in rosemary essential oil. The results show that the chemical compositions of the essential oil obtained by the two methods are slight differences. Indeed, the 1,8-cineole is a significant component with a slightly higher rate in MAD than SD, at 16.256% and 15.349%, respectively. However, the percentage of camphene in MAD is lower than SD, at 3.254% and 3.507%, respectively. It is similar to the rate of α-Pinene. These results are consistent with those of Bousbia [13], Karakaya [19], and Moradi [20], which confirm that the contents of oxygenated compounds in the oil obtained by MAD are higher than those of the oil obtained by SD. This is because of the system’s low water content and the heating process’s speed compared with conventional hydrodistillation. Thus, the thermal and hydrolytic degradations of oxygenated compounds are limited [21, 22]. Oxygen compounds have a high dipole moment and will interact more vigorously with microwaves and can be extracted more easily, unlike monoterpene hydrocarbons with a weak dipole moment [23].

4. Conclusions

The essential oils extracted by MAD are quantitatively (yield) and qualitatively (compositions) similar to those obtained by steam distillation, although the treatment time has been significantly reduced in the case of MAD (20 min) by relative to SD (120 min). Therefore, the MAD method is a good alternative for extracting essential oils of rosemary. Besides, The significant components of rosemary essential oil extracted from MAD are α-pinene (22.277 %), verbenole (16.488%), 1,8-cineole (16.256%), and geraniol (5.705%). These compounds are potential for the cosmetic and pharmaceutical industry with their health benefit activities such as antioxidant, antibacterial, antiseptic, and reduced anxiety.
Table 1. Chemical composition of rosemary essential oils obtained by MAD, SD.

| No | Compound                                      | Composition (%) |
|----|-----------------------------------------------|-----------------|
| 1  | α-Pinene                                      | 22.277          |
| 2  | Camphene                                      | 3.254           |
| 3  | 2-Thujene                                     | 0.909           |
| 4  | Sabinene                                      | 1.25            |
| 5  | Bicyclo[2.1.1]hexane, 5,5-dimethyl-1-vinyl    | 1.011           |
| 6  | m-Mentha-1,8-diene                            | 0.791           |
| 7  | 1,3,5-Cycloheptatriene, 3,7,7-trimethyl        | 0.721           |
| 8  | D-Limonene                                    | 3.109           |
| 9  | 1,8-cineole                                   | 16.256          |
| 10 | Terpinene                                     | 1.278           |
| 11 | p-Month-1-ene                                 | 1.087           |
| 12 | Linalool                                      | 3.097           |
| 13 | 3,5-Heptadien-2-ol, 2,6-dimethyl              | 0.656           |
| 14 | Camphor                                      | 3.935           |
| 15 | Pinocarvone                                   | 0.465           |
| 16 | Camphol                                      | 4.248           |
| 17 | Bicyclo[3.1.1]heptan-3-one                    | 0.664           |
| 18 | Terpinen-4-ol                                 | 1.107           |
| 19 | Terpineol                                    | 3.004           |
| 20 | (−)-Myrtenol                                  | 0.764           |
| 21 | endo-Borneol                                  | 1.167           |
| 22 | Verbenol                                      | 16.488          |
| 23 | trans-Shisool                                 | 0.446           |
| 24 | Geraniol                                      | 5.705           |
| 25 | Bornyl acetate                                | 2.784           |
| 26 | Caryophyllene                                 | 3.048           |
| 27 | Humulene                                      | 0.48            |

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