The effects of influencing parameters on the *Eucalyptus globulus* leaves essential oil extraction by hydrodistillation method

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Abstract. This study examines the effects of influencing parameters to *Eucalyptus globulus* essential oil (EOs) in the hydrodistillation process including extraction temperature, time, and raw material to water ratio. The study also investigated the chemical composition of *Eucalyptus globulus* EOs by Gas chromatography-Mass Spectrometry (GC-MS). Based on the single factor investigation method, the yield of the EOs for 2.2% in the conditions including the leaves size about 0.5 mm, with the ratio of the leaves to water was 1:4 (g/mL), at 75 minutes in the extracting temperature of 120°C. The analytical results showed that the composition of Eucalyptol (35.034%), followed by α-Pinene (17.860%), β-Pinene (11.022%), α-Terpenol (4.756%), caryophyllene oxide (4.554%), camphol (2.123%), and other ingredients with content less than 1%.

1. Introduction

Nowadays, essential oils (EOs) have been viewed as a trend of research and applications in cosmetics, pharmaceuticals, and food [1-3]. EOs have formed the basis of many applications in today's life. *Eucalyptus* (*Eucalyptus globulus*) Oil is a complicated combination of many types of monoterpenes, sesquiterpene, and like other essential oils including esters, aromatic phenols, aldehydes, alcohols, and ethers. However, the structure and proportion of the extracts differ according to species [4]. On the other hand, it often differs by season, the position of the dealer, environment and soil quality, age of the plants, reproductive regimen and method of oil extraction. *Eucalyptus* essential oil belongs to the Myrtaceae family, which is used most in the pharmaceutical field, in Vietnam, they are used as "medicated oil” as a natural insect repellent to protect against mosquitoes and other pests. *Eucalyptus* oil contains many compounds with antimicrobial and antioxidant activities which have been proven through many previous studies thanks to the high content of Eucalyptol (1,8-cineole) and ingredients such as β-Pinene, α-Pinene α-Terpenol, caryophyllene oxide, and camphol [5–10].

There are many methods used to extract essential oils from classical (mechanical: cold- pressed, hydrodistillation, and steam hydrodistillation) [11-16] to the modern (using microwave, ultrasonic, supercritical CO₂, and Soxhlet) [17-23]. However, classical methods such as hydrodistillation and steam hydrodistillation extraction are most commonly used in industry. On top of that, the hydrodistillation
method is identified and used on an industrial scale because of the high EOs yield, the device is inexpensive and easy to implement. In addition, during distillation, it is possible to divide the constituents in the mixture by partial condensation over time. Besides, the parameters that make the process easy to set correctly by modern distillation equipment. EOs will be strongly applied in the pharmaceutical industry and especially cosmetics, in personal or family care products and some areas in the food industry. However, this method also has some disadvantages such as producing low-quality essential oil due to decomposition at high temperatures. As the size of raw materials, water to materials ratio, as well as extraction time and temperature play an essential role in determining the extraction efficiency, their effects demand evaluation to ensure that the resulted oil is of high yield and maintain their phytochemical content. However, such evaluation using Eucalyptus globulus leaf EOs remain lacking.

Therefore, the aim of this study is to examine the factors affecting the extraction process of Eucalyptus globulus EOs from leaf material by hydrodistillation method including material size, material and water ratio, time, and temperature. The resulting oil is dried and the content of volatile compounds in the oil is assessed.

2. Material and method

2.1. Plant samples
Eucalyptus globulus (E.G) leaves is collected in Tra Vinh, Vietnam. Then, it was washed with water several times to remove dirt on the leaves’ surface. Next, the E.G leaves is retained and kept in a non-hygroscopic bag in Alaska cooler (Vietnam). Lastly, 100 g of the E.G leaves are grinded by grinding equipment to the size of 1-3 mm (Sunhouse SHD5322, 220W, Vietnam).

2.2 Extraction Method
The Clevenger type apparatus (Bach Khoa Ltd, Ho Chi Minh city, Vietnam) is used to extract Eucalyptus globulus essential oil by hydrodistillation method. This system includes a heater equipped, which is equipped temperature regulator, a round-bottom flask with volume of 1.0L used for containing raw materials and solvent extraction, condensing equipment with cool water to condense the essential oil, and separator used to separate essential oils and water after condensation is presented in Figure 1. A 1.0L round bottom flask is filled with 100 g of pre-treated Eucalyptus globulus leaves and gives a quantity of water according to the survey ratios ranging from 2:1 to 6:1. The cooling system has been turned on to condense volatile liquids. Other influential parameters of this extraction, including material size (e.g. original size of the leaves, fiber (5 cm x 1 cm) and grinded particles (1-3 mm), extraction time (30-135 min) and temperature (100-150 °C) were investigated and the EOs was dehydrated by anhydrous sodium sulfate (Na₂SO₄, Sigma Aldrich) before component testing by GC-MS.

The yield (%) of the collected Eucalyptus globulus oil is determined by following formula (1):

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\text{Yield} (\%) = \frac{\text{the volume of Eucalyptus globulus oil obtained (mL)}}{\text{the amount of Eucalyptus globulus originally used (g)}}
\]  

(1)
Figure 1. The process of the *Eucalyptus globulus* leaves EOs extraction

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) analyses of *Eucalyptus globulus* oil

Chemical composition of *Eucalyptus globulus* 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 EOs was added with n-hexane and dehydrated with Na$_2$SO$_4$. 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 Results of the single-factor experiments

The size of the material, time, temperature, and the ratio of solvent to material extraction is considered the factors affecting the yield of *Eucalyptus globulus* leaves oil during the extraction process, which was investigated in this study and displayed in Figure 2.

Firstly, when accessing the effect of different size of raw materials (original leaf size, fiber and grinded size), the other 3 parameters would be fixed as 3:1 mL/g of water:material ratio, 60 min and 110°C. As seen in Figure 2A, the yield of EOs increased as the sample size decreased. The yield of EOs increases from 1.0 to 1.65% when changing from the natural size of the leaves to the grinded size. The substance is made thinner, so the oil-bearing cells disintegrate in greater amounts. It thus makes the water diffuse more quickly into the oil bags of the leaves. This forces the essential oils out easily under heat control and contributes to better efficiency.

After selecting the suitable size of raw materials as the grinded size, the water-material ratio factor was then studied. The extraction time and temperature was kept constant at 60 min and 110°C. From Figure 2B, when increasing the ratio from 2: 1 to 4: 1 (mL/g), the extraction yield increased from 1.55 to 1.9%. This encourages the absorption of the substance by water, thereby allowing a greater diffusion of essential oils into the water, resulting in improved yield of EOs. We omitted the ratio of 1:1 (mL/g)
because the amount of water with this ratio is not enough to cover the leaves volume, does not make the water absorb into the leaves well and can cause the burning of materials leading to essential oils be strongly smell. Conversely, increasing this ratio from 4:1 to 6:1 (mL/g) makes the yield of EOs drop from 1.9 to 1.65% because excess water can dissolve or emulsify oil. Therefore, for better performance, the ratio of water to raw materials 4:1 (mL / g) is chosen, and for corresponding tests obtained a yield of 1.9%.

The raw materials at grinded size and water:raw materials ration of 4:1 mL/g were applied for the next investigation of optimal extraction time. The extraction temperature was held constant at 110 °C. The extraction effect over time for the yield of eucalyptus leaf oil is examined as indicated in Figure 2C. When the extraction time increased from 30 to 75 minutes, the yield of the extracted EOs increased gradually from 0.8 to 2.15%, but it decreased to 1.7% after 135 minutes due to the denaturation of some substances in the essential oil exposure to high temperatures for a long time. Therefore, the extraction time was selected as 75 min.

Finally, with the raw materials at grinded size, water:raw materials ration of 4:1 mL/g and 75 min of extraction time, the effect of extraction temperature was evaluated. Figure 2D describes that high temperatures lead to better extraction efficiency, but only to a certain extent, when the temperature surpasses the optimal point, the yields are decreased later. Regarding the effect of temperature, it can be considered the most important factor because it affects all three parameters. High temperatures make heat transfer and mass transfer from outside into materials faster thanks to the weakening of surface tension and water viscosity. On the other hand, at very high temperatures, some limitations occur, some components of the EOs which are sensitive to decomposition temperatures, adversely affecting extraction yield, oil quality and production cost due to increased energy consumption. Thus, the chosen temperature for that phase is 120°C.

Figure 2. The impact of these influences on Eucalyptus globulus leaves the extraction of oil.
3.2 GC-MS analysis
Using the optimized conditions, the hydrodistillation process was carried out to extract essential oil from Eucalyptus leaves, resulting in 2.2% of oil yield. The obtained Eucalyptus oil was subjected to phytochemical screening using GC-MS analysis. Results from GC-MS analysis have revealed 32 components present in Eucalyptus leaves oil, as shown in Figure 3 and Table 1. The composition of Eucalyptol found has high content (35.034%), this is the main ingredient in the essential oil of Eucalyptus family, then α-Pinene (17.860%), β-Pinene (11.022%), α-Terpenol (4.756%), caryophyllene oxide (4.554%) and camphol (2.123%). The other components in Table 1 accounted for almost no high content. As mentioned above, in Eucalyptus globulus EOs, Eucalyptol has antibacterial properties like Aspergillus parasiticus Speare and Aspergillus flavus Link. Therefore, Eucalyptus globulus EOs is used in the area of medicine.

![Gas chromatogram of the EOs of Eucalyptus globulus leaves by the hydrodistillation](image)

Figure 3. Gas chromatogram of the EOs of Eucalyptus globulus leaves by the hydrodistillation

| R.T. (min) | Constituent    | %       | R.T. (min) | Constituent    | %       |
|-----------|----------------|---------|------------|----------------|---------|
| 7.397     | α-Pinene       | 17.860  | 21.211     | Myrtenal       | 0.304   |
| 7.889     | α-Fenchene     | 0.222   | 21.274     | Myrtenol       | 1.036   |
| 7.951     | Camphene       | 0.913   | 22.309     | Fenchyl acetate| 0.286   |
| 9.227     | β-Pinene       | 11.022  | 22.664     | Cyclohexanol   | 0.386   |
| 11.716    | o-Cymene       | 2.000   | 27.025     | α-Terpiny acetate| 1.366   |
| 12.082    | Eucalyptol     | 35.034  | 29.137     | β-Caryophyllene| 1.431   |
| 13.650    | γ-Terpinene    | 0.555   | 29.712     | β-Diploalbicine| 1.739   |
| 16.871    | Fenchol        | 1.708   | 31.333     | Elixene        | 0.849   |
| 18.241    | Norinone       | 0.148   | 32.818     | Epiglobulol    | 0.477   |
| 18.314    | Pinen-3-ol     | 2.496   | 32.985     | Maaliol        | 0.338   |
| 18.795    | Camphene hydrate| 0.268  | 33.205     | Spathulenol    | 1.874   |
| 19.590    | Pinen-3-one    | 0.847   | 33.330     | Caryophyllene  |        |
| 19.768    | Camphol        | 2.123   | 33.487     | Himbaocol      | 0.550   |
| 20.374    | Terpene-4-ol   | 1.458   | 33.508     | Globulol       | 0.228   |
| 20.918    | menth-8-dien-2-ol| 0.637 | 33.696     | β-Selinenol    | 0.475   |
| 21.054    | α-Terpenol     | 4.756   | 36.258     | Ylangenal      | 0.305   |

Table 1. Results of GC-MS analysis of ingredients in essential oils
4. Conclusion
From the results and discussion above, it is shown that Eucalyptus essential oil was necessary for research and application in all areas of life. Factors selected for evaluation include material size, material and water ratios, extraction time and temperature. Results from the present study suggested using material size of 0.5 mm with the following conditions: 4:1 mL/g of water to raw materials ratio, 120°C of temperature and 75-90 min of extraction time. The oil yield obtained was 2.2%. A total of 32 components were identified in the obtained Eucalyptus leaves oil, with main components being α-Pinene, β-Pinene, α-Terpenol, caryophyllene oxide and camphol. This study has helped to find the right conditions for the highest Eucalyptus oil yield and investigated the highest content of Eucalyptol to serve the application of insecticidal products.

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