Essential oil from fresh and dried Rosemary cultivated in Lam Dong province, Vietnam

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Abstract. Rosemary essential oil has been widely used in folk medicine for treatment of anxiety, memory deficit and cancer thanks to its high antioxidant activity and antibacterial properties. Composition of rosemary essential oil largely depends on the geographical position of the cultivated plant and conditions of the extraction process. In this study, fresh and dried rosemary leaves were used for extraction of essential oil using hydrodistillation method. The extraction performance, composition and antioxidation activity of the essential oil were measured. The oil yield for dried leaves (1.2 ml/g) is significantly lower than that for fresh leaves (3.16 ml/g). However, the difference on chemical profile and antioxidation activity of the two oil samples was indistinguishable. The most remarkable finding was the presence of Levoverbenone at very high concentration in rosemary essential oil, accounting for 10.87% and 12.12% of the oil sample extracted from fresh and dried leaves respectively.

1. Introduction
Nowadays, traditional medicines make use of natural products (such as plants, animals, microorganisms, and marine organisms), which are of great importance [1-4]. Rosemary(R. officinalis) is the plant that is cultivated mainly in Mediterranean region. Rosemary essential oil was also found to have the capability to relieve the symptoms caused by respiratory disorders, to stimulate hair growth, to reduce stress and mental alertness, and to treat Rheumatoid disease[5-7]. The benefits and application of rosemary essential oil are diverse [8-10] The essential oil could be extracted from both flower and leaf organs of the plant, oils extracted from leaves often display higher quality. The chemical composition of the essential oil largely depends on the extraction conditions. Methods for extraction of rosemary essential oil range from mechanical pressing to hydrodistillation distillation and microwave assisted distillation[11,12]. Among such methods, microwave-assisted distillation, albeit not economically feasible for large-scale production, was found to give higher extraction yield [13,14]. In this research, the essential oil from rosemary was obtained by conventional hydrodistillation to find out the optimal parameters with regards to storage duration, and drying temperature. In addition oil samples obtained from fresh and dried Rosemary leaves were compared in terms of extraction performance and antioxidation activity. The chemical profiles of essential oil from Rosemary cultivated in Lam Dong province were also bestudied to compare with other research.

2. Material and methods
2.1. Sample preparation
Rosemary plants were harvested from The Seed Garden located Lam Dong Province, Vietnam. It was cleaned and split into 3 parts. In order to investigate the impacts of different storage conditions on the
compositions of distilled oils, the material samples were subjected to different storage temperatures such as fresh, refrigerator (4°C), different drying temperatures (40°C to 65°C and sample to be dried until constant weight) and at room temperature (30°C). The oils analysis of all storage treatments performed daily.

2.2. Extraction of rosemary essential oil by Hydrodistillation method

Before extracting, the moisture for each sample moisture was measured by Electronic moisture driers Ohaus MB25.100g sample was transferred into the 1L fit round bottom flask containing 500mL of water. Depending on the requirements of the experiments, parameters may vary. To be specific, water/material ratio varies from 1:2 to 1:5, distillation time varies from 1 hour to 4 hours and material sample alternates from fresh to dry. The collected essential oil was anhydrous with Na2SO4 and stored in small glass bottles sealed with teflon sealed caps and covered with foil to prevent oxidation. Bottles are stored in dry and cool condition with the absence of direct sunlight and extraction yield (Y):

\[ Y(\text{ml/100g}) = \frac{\text{V_{essential oil}}}{m(1-\alpha)} \times 100 \]

Where V (mL) is the volume of oil, m (g) is the mass of water and \( \alpha \) (%) is the moisture content of the material.

2.3. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

Two samples of essential oils extracted from fresh and dry leaves were analyzed for radical scavenging activity at Academy of Science and Technology, Ho Chi Minh City, Vietnam. 50 µL of DPPH (OD 520 nm = 0.0403 ± 0.013) was introduced into 150µL of sample solution. The mixture was then mixed at room temperature in the dark for 30 min till stable state. The optical measurement of the mixture was performed by UV/VIS - PowerWave HT Microplate Spectrophotometer at 517 nm. Blank sample, but 150 µL solution replaced EtOH 99.7%. Standard sample: Vitamin C 400ppm.

\[ \% \text{DPPH} = \frac{A_b - A_s}{A_b} \times 100 \]

where: \( A_b \) is the optical density in the blank sample; \( A_s \) is the optical density of sample solution

%DPPH is percentage of free radical DPPH.

2.4. Gas chromatography – mass spectroscopy (GC-MS)

The gas chromatography-mass spectroscopy (GC-MS) was employed to analyze the chemical composition of the essential oil samples. GC-456 SQ with SCION performance RESTEK Rxi-5ms (30 m x 0.25 mm (for example), 0.25 µm df), bring the gas Helium constant flow rate: 1 mL/min. Injector temperature is 250°C the rate of Division: 30

3. Result and discussion

3.1. Effects of storage duration, distillation time, material/water ratio and drying temperature on extraction yield

The distillation yield of rosemary essential oil was examined with respect to three variables including storage duration, distillation time, material to water ratio, and drying temperature. From Figure 1, it is observed that the preservation time of raw materials shows an inverse relationship with the essential oil production. This is possibly due to the low temperature which increases moisture, and in turn, incites decomposition of the plant. In addition, prolonged storage duration also creates favorable environments for microorganisms to flourish, causing oil degradation in terms of quality and composition. Regarding influence of extraction, Figure 2 showed that the oil yield was proportional with increased extraction time, peaking at 3.43% at 3 hours. This indicates that almost essential oil in the materials has been fully extracted after 3 hours. Therefore, to save energy and time, the appropriate time period is 3 hours.

Figure 3 expresses the influence of material/water ratio on production Rosemary essential oil. Accordingly, the highest yield was achieved at 1:5 ratio. Under effect of heating, the penetration of water through cell membrane was enhanced, rupturing oil sacs and releasing the essential oils out in the form of evaporation. On the contrary, insufficient water impairs the viscosity of the membrane, reducing the permeability of water vapor and quantity of essential oils. The drying temperature is one of the important factors in the process of distillation of rosemary oil as it affects the color, smell and yield of essential oil. The experimental results obtained from Figure 4 shows that, at 40-45°C, the
materials gave the highest yield of essential oil. Minimum oil yield is reached at temperature of 65°C. Therefore, the suitable temperature range is with 40-55°C.

Figure 1. Effect of storage duration on extraction yield from fresh leaves

Figure 2. Effect of distillation time on extraction yield

Figure 3. Effect of material/water ratio on extraction yield

Figure 4. Effect of drying temperature on extraction yield

3.2. The extraction yield, antioxidant activity and GC-MS of fresh and dried leaves

Two samples, fresh leaves and dried leaves (at 45°C), were used in this analysis. After pre-treatment, the sample was distilled in 500 ml of water in 4 hours. Figure 5 shows the oil yield of fresh material (4.818%), dried material (4.334%). In addition, the two samples also exhibited similar antioxidant activities where antioxidant activity of the fresh sample is recorded at %DPPH 49.6% and that for dried sample was % DPPH 52.73%. The essential oil of fresh rosemary leaves obtained using hydrodistillation was analyzed by gas chromatography-mass spectroscopy (GC-MS). 20 components were identified in Table 1. The major components were 1R-α-Pinene (26.252%), followed by Eucalyptol (Cineole) (14.490%), Levoverbenone(12.121%), Geraniol(6.361%), Bornyl acetate (4.938%), and Camphol(4.235%). For dry leaves, table 2 shows 17 components, in which the major components were 1R-α-Pinene (23.001%), Eucalyptol (Cineole) (11.858%), Levoverbenone(10.866%), Geraniol (4.024%), Camphor (3.177%), and Bornyl acetate (3.046%). From the results, we found that the essential oil extracted from rosemary leaves from Lam Dong province is characterized by the abundance of L-Verbenone, which has been proved to be a health beneficial and active substance used in the treatment of respiratory diseases. In comparison to results of other studies [15, 16] where L-Verbenone content was either nonexistent or very low, it is suggested that the oil is suitable to support the treatment of allergic rhinitis [17] and that the substance found in the fresh material is higher than that found in the dried raw material. Regarding other components, it is implied that trans-Verbenol (0.795%), Levomenthol (2.622%), and L-Borneol (1.013%) were lost in the drying process.
Table 1. Chemical compound of Rosmarinus officinalis essential oils obtained using fresh ingredients.

| S/N | Rt   | Compounds         | Concentration (%) | Mass | Homology mass spectrometry |
|-----|------|-------------------|-------------------|------|---------------------------|
| 1   | 5.090| 1R-α-Pinene       | 26.252            | 136  | 947                       |
| 2   | 5.349| Camphene          | 3.189             | 136  | 951                       |
| 3   | 5.832| β-Pinene          | 2.159             | 136  | 933                       |
| 4   | 5.976| β-Myrcene         | 1.140             | 136  | 938                       |
| 5   | 6.781| D-Limonene        | 3.016             | 136  | 934                       |
| 6   | 6.851| Eucalyptol (Cineole) | 14.490          | 154  | 954                       |
| 7   | 7.392| γ-Terpinene       | 1.400             | 136  | 944                       |
| 8   | 8.054| Terpinolene       | 1.129             | 136  | 941                       |
| 9   | 8.228| Linalool          | 3.045             | 154  | 929                       |
| 10  | 9.377| trans-Verbenol    | 0.795             | 152  | 937                       |
| 11  | 9.415| (-)-Camphor       | 2.627             | 152  | 937                       |
| 12  | 9.913| Camphol           | 4.235             | 154  | 931                       |
| 13  | 10.030| Levomenthol      | 2.622             | 156  | 957                       |
| 14  | 10.175| Terpinen-4-ol    | 1.641             | 154  | 914                       |
| 15  | 10.482| Terpineol         | 2.665             | 154  | 928                       |
| 16  | 10.759| L-Borneol        | 1.013             | 154  | 797                       |
| 17  | 10.982| Levoverbenone    | 12.121            | 150  | 959                       |
| 18  | 11.973| Geraniol         | 6.361             | 154  | 941                       |
| 19  | 12.874| Bornyl acetate   | 4.938             | 196  | 934                       |
| 20  | 16.327| Caryophyllene    | 2.616             | 204  | 942                       |

Table 2. Chemical compound of Rosmarinus officinalis essential oils obtained using material is dried.

| S/N | Rt   | Compounds         | Concentration (%) | Mass | Homology mass spectrometry |
|-----|------|-------------------|-------------------|------|---------------------------|
| 1   | 5.087| 1R-α-Pinene       | 23.001            | 136  | 946                       |
| 2   | 5.346| Camphene          | 2.521             | 136  | 948                       |
| 3   | 5.829| β-Pinene          | 1.440             | 136  | 938                       |
| 4   | 5.973| β-Myrcene         | 0.803             | 136  | 937                       |
| 5   | 6.778| D-Limonene        | 2.178             | 136  | 937                       |
| 6   | 6.847| Eucalyptol (Cineole) | 11.858          | 154  | 953                       |
| 7   | 7.389| γ-Terpinene       | 1.056             | 136  | 942                       |
| 8   | 8.051| Terpinolene       | 0.736             | 136  | 945                       |
| 9   | 8.224| Linalool          | 1.988             | 154  | 935                       |
| 10  | 9.412| (-)-Camphor       | 2.517             | 152  | 938                       |
| 11  | 9.909| Camphol           | 3.177             | 154  | 937                       |
| 12  | 10.171| Terpinen-4-ol    | 0.814             | 154  | 914                       |
| 13  | 10.478| Terpineol        | 1.834             | 154  | 935                       |
| 14  | 10.978| Levoverbenone    | 10.866            | 150  | 961                       |
| 15  | 11.966| Geraniol         | 4.024             | 154  | 936                       |
| 16  | 12.870| Bornyl acetate   | 3.046             | 196  | 938                       |
| 17  | 16.324| Caryophyllene    | 1.000             | 204  | 939                       |

4. Conclusion
We attempted the extraction and compared composition and antioxidant activity of the essential oils extracted from dried and fresh rosemary leaves, harvested in Lam Dong province, Vietnam. It is found that some components in the essential oil of Rosemary were lost due to the drying process affect the material. In addition, the oil sample from dried leaves exhibited higher antioxidant activity compared to the sample from fresh leaves. Storage time and drying temperature were found to be inversely proportional to the yield of the essential oil. We also found high concentration of Levoverbenone in the oil, accounting for 10.87% and 12.12% of the oils extracted from fresh and dry leaves respectively. This remarkable result suggested that Rosemary planted at Lam Dong Provine may act a good source for alternative medicine for respiratory diseases and also support treatment methods for allergic rhinitis.

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