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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 antioxidant 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 antioxidant 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 of 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 today. 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):

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

Where V (mL) is the volume of oil, m (g) is the mass of water and α (%) 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 \pm 0.013) was introduced into 150 μL 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 4.33% 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.

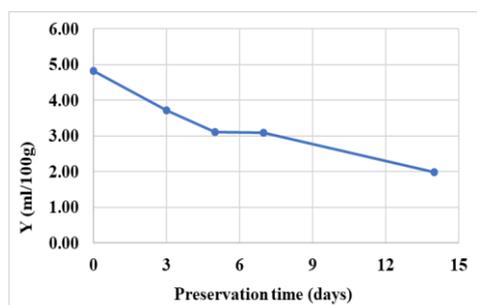


Figure 1. Effect of storage duration extraction yield from fresh leaves

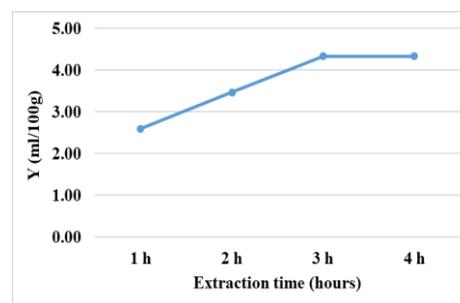


Figure 2. Effect of distillation time on extraction yield

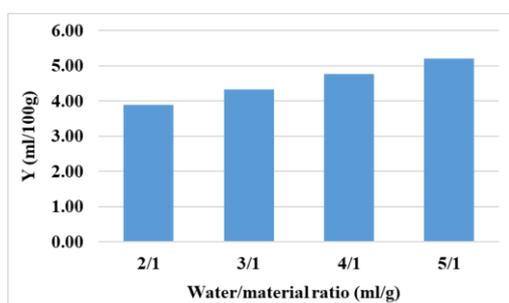


Figure 3. Effect of material/water ratio on extraction yield

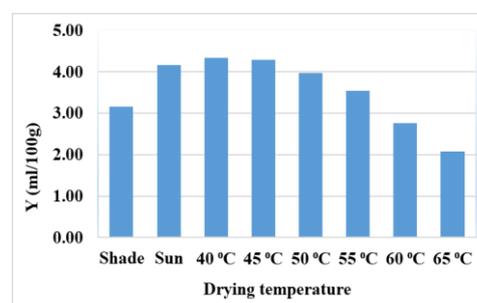


Figure 4. Effect of drying temperature on extraction yield

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 hydro-distillation 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.

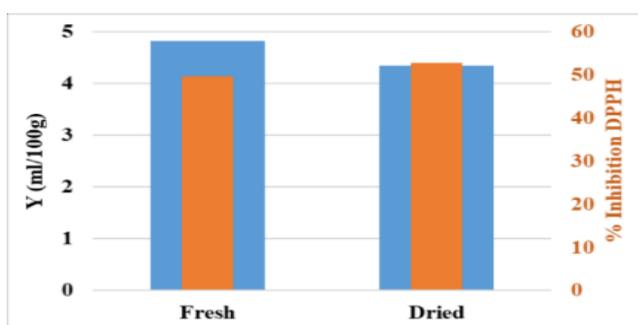


Figure 5. Changing in extraction yield and antioxidant activity of fresh and dried leaves

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 Province may act a good source for alternative medicine for respiratory diseases and also support treatment methods for allergic rhinitis. **Acknowledgments:** This work was supported by grants from Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam.

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