



Supercritical Fluid Extraction as a Technique to Obtain Essential Oil from *Rosmarinus officinalis* L.

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ABSTRACT

The aim of this study was the comparison of essential oil of one herb from lamiaceae family namely Rosemary, via supercritical fluid extraction (SFE) and hydrodistillation (HD). The essential oils were analyzed by gas chromatography–quadrupole mass spectrometry (GC-MS). The compounds identified in higher relative abundance were, α -pinene (HD: 30.42%, SFE: 0.47%), 1,8-cineole (HD: 15.46%, SFE: 25.05%), Verbenone (HD: 13.09%, SFE: 5.17%), Camphor (HD: 8.97%, SFE: 15.69%), Borneol (HD: 7.16%, SFE: 9.15%) and β - Caryophyllene (SFE: 6.85%).

Keywords: Rosemary, Supercritical fluid extraction, Hydrodistillation, Essential oil

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a Long-lasting evergreen herb that belongs to the Lamiaceae family¹. Rosemary is native to the Mediterranean region. It possesses needle-like leaves which contain a powerful fragrance². It is used as a food flavoring agent, and well known medicinally for its powerful antitumor, antibacterial, antifungal and antioxidant activity³⁻⁷. Rosemary essential oil composition and the ratio between different oil constituents plays a role in its bioactivity⁸. Rosemary oil composition depends on various environmental and genetic factors, as well as on postharvest processing factors⁹. Supercritical fluid extraction showed extremely higher selectivity compared to the conventional techniques

tested. Supercritical fluid extraction is an innovative, clean and environmental friendly technology with particular interest for the extraction of essential oil from plants and herbs. Furthermore, supercritical extracts were often recognized of superior quality when compared with those produced by hydrodistillation¹⁰⁻¹¹. The advances that supercritical technology is reaching in the past years are related to the continuous increase of activities connected to scientific research and technological development, which focus on inserting novel processes in the food, pharmaceutical, chemical and cosmetics industries¹¹. In this paper, we present a comparative study of the ability of two different techniques to extract the essential oils from *Rosmarinus* leaves to find the most advantageous in term of essential oil quantity and quality.

MATERIALS AND METHODS

Materials

HPLC grade n-hexane and analytical grade anhydrous sodium sulfate were purchased from Merck (Darmstadt, Germany). Dichloromethane was purchased from Aldrich. Carbon dioxide (99.8% purity) contained in a cylinder was purchased from Sabalan Co. (Tehran, Iran). The Rosemary (*Rosmarinus officinalis* L.) leaves used was collected from the north of Tehran, Iran in August 2016.

GC-MS analysis

The Analyses were performed using a GC-MS system (Hewlett-Packard 6890 gas chromatograph coupled to a 5973A mass spectrometer), equipped with split/splitless injector in the splitless mode and at 250°C during the chromatographic run. The volatile compounds were separated in a capillary column (30 m × 0.25 mm, 0.25 µm film thickness, HP-5MS) using N₂ (99.99 %) as a carrier gas at a 1 ml/min flow rate. The oven temperature was varied as follows: 60°C (5 min), then warmed to 250°C at 5°C/min. then held 10 min. at 250°C. The mass detector conditions were: transfer line temperature of 220°C, ion source temperature of 220°C and ionization mode with electron impact at 70 eV. The Kovats retention index (RI) values were calculated using a homologous series of n-alkanes (C₈- C₂₅) and values were compared with values reported in the literature and available Adams data¹². All mass spectra were also compared with the data system library (Wiley 275). Identification of volatile compounds was achieved by matching mass spectra and retention indices (RI) with those obtained from Wiley library as well as from literature data.

Hydrodistillation (HD)

Rosemary leaves were shade dried at room temperature for 72 hours. The dried rosemary leaves were loaded to hydrodistillation using a Clevenger-type apparatus, according to European Pharmacopeia, and extracted with 1 l of water for 240 min. (until no more essential oil was reused).

Supercritical fluid extraction (SFE)

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode was used for all the extractions.

The extraction vessel was a 10 ml stainless steel vessel. Supercritical fluid extractions were conducted at pressure 30 MPa and temperature 40°C for a duration of 20 min. static, followed by 35 min. dynamic. These optimized conditions were obtained from literature¹³. A Duraflow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted essential oil. In order to prevent sample plugging, the restrict point was warmed electrically. The supercritical CO₂ flow rate through the Duraflow restrictor was approximately 0.3-0.4 ml/min. (compressed). Plant powder (3.0 g) was well mixed with 2 mm diameter glass beads, and was then charged into the 10 ml extraction vessel. The essential oil was extracted from the plant using supercritical CO₂. The extracted essential oil was collected in dichloromethane in a 5.0 ml volumetric flask. The final volume of the extract was adjusted to 5.0 ml with dichloromethane at the end of the extraction. Four milliliters of solution were poured into a 20 ml beaker. The solvent was evaporated by Bubbling argon gas through the solution. Then the weight of essential oil was measured and the extraction yield was calculated.

RESULT AND DISCUSSION

The identified constituents in the extracted essential oils from both methods are present in Table 1. For hydrodistillation and supercritical fluid extraction 29 and 16 compounds, respectively were characterized, according to 99.91% and 91.75% of the essential oils. In the hydrodistillation technique, the essential oil consists of oxygenated compounds 56.33% and 42.39% monoterpene hydrocarbons with, α -pinene (30.42%), 1,8-cineole (15.46%), Verbenone (13.09%), Camphor (8.97%) and Borneol (7.16%) as the major components in the oil.

Comparatively, the main constituents of the oil in supercritical fluid extraction technique were α -pinene (0.47%), 1,8-cineole (20.05%), Verbenone (5.17%), Camphor (15.69%), Borneol (9.15%) and β -Caryophyllene (6.85 %). Furthermore, the oil consists of 76% oxygenated compounds and 1.39% monoterpene hydrocarbons.

Fig.1 shows the major compounds in *Rosmarinus officinalis* l. essential oil extracted by hydrodistillation and supercritical fluid extraction, it

was noticed that a large amount of α -Pinene was present in the essential oil via hydrodistillation.

Additionally, higher levels of oxygenated compounds and lower levels of monoterpene

hydrocarbons were found in the essential oil extracted by supercritical fluid extraction (Fig. 2). The selectivity of supercritical fluid extraction technique allowed to increase the quantities of oxygenated compounds.

Table.1 :Chemical composition of the essential oil isolated from Iranian *Rosmarinus officinalis* via hydrodistillation (HD) and supercritical fluid extraction (SFE)

Compounds	Formula	RI	HD(%)	SFE(%)
α -Pinene	C ₁₀ H ₁₆	939	30.42	0.47
Camphene	C ₁₀ H ₁₆	954	5.29	0.31
β -Pinene	C ₁₀ H ₁₆	979	1.66	0.12
3-Octanone	C ₈ H ₁₆ O	984	2.48	-
β -Myrcene	C ₁₀ H ₁₆	991	2.37	0.11
α -Terpinene	C ₁₀ H ₁₆	1017	0.50	-
p-Cymene	C ₁₀ H ₁₄	1026	0.93	0.15
1,8-Cineole	C ₁₀ H ₁₈ O	1031	15.46	25.05
α -Terpinene	C ₁₀ H ₁₆	1060	0.60	-
Terpinolene	C ₁₀ H ₁₆	1089	0.62	0.23
Linalool	C ₁₀ H ₁₈ O	1097	2.08	1.67
Chrysanthenone	C ₁₀ H ₁₄ O	1128	0.32	-
Unknown	-	-	-	0.15
Camphor	C ₁₀ H ₁₆ O	1146	8.97	15.69
Pinocarvone	C ₁₀ H ₁₄ O	1165	0.11	-
Borneol	C ₁₀ H ₁₈ O	1169	7.16	9.15
Terpinen-4-ol	C ₁₀ H ₁₈ O	1177	0.60	1.79
α -Terpineol	C ₁₀ H ₁₈ O	1189	1.72	-
Verbenone	C ₁₀ H ₁₄ O	1205	13.09	5.17
Geraniol	C ₁₀ H ₁₈ O	1253	0.55	1.32
Unknown	-	-	-	1.45
Unknown	-	-	-	1.13
Bornyl acetate	C ₁₂ H ₂₀ O ₂	1289	2.07	1.29
Thymol	C ₁₀ H ₁₄ O	1290	-	1.71
Carvacrol	C ₁₀ H ₁₄ O	1299	0.21	2.07
Unknown	-	-	-	0.12
Piperitenone	C ₁₀ H ₁₄ O	1343	-	1.85
α -Copaene	C ₁₅ H ₂₄	1377	0.10	-
Geranyl acetate	C ₁₂ H ₂₀ O ₂	1381	0.11	2.49
β - Caryophyllene	C ₁₅ H ₂₄	1419	0.36	6.85
α -Humulene	C ₁₅ H ₂₄	1455	0.12	3.49
Germacrene D	C ₁₅ H ₂₄	1485	0.10	-
β -Selinene	C ₁₅ H ₂₄	1490	-	1.86
Valencene	C ₁₅ H ₂₄	1496	-	0.81
β -Bisabolene	C ₁₅ H ₂₄	1506	0.11	1.35
δ -Cadinene	C ₁₅ H ₂₄	1523	0.40	-
Caryophyllene oxide	C ₁₅ H ₂₄ O	1583	1.40	2.87
Globulol	C ₁₅ H ₂₆ O	1585	-	1.71
Caryophylla-4 (12), 8 (13) -dien-5- β -ol	C ₁₅ H ₂₄ O	1641	-	2.17
Unknown	-	-	-	1.67
Grouped components				

Table 1. continued

Compounds	Formula	RI	HD(%)	SFE(%)
Hydrocarbon compounds			43.58	15.75
Oxygenated compounds			56.33	76.00
Monoterpene hydrocarbons			42.39	1.39
Oxygenated monoterpenes			50.27	65.47
Sesquiterpene hydrocarbons			1.19	14.36
Oxygenated sesquiterpenes			1.40	6.75
Others			4.66	3.78
Total identified (%)			99.91	91.75
Extraction time (min)			240	35
Oil yield (ml /100 g dry wt.)			1.14	0.75

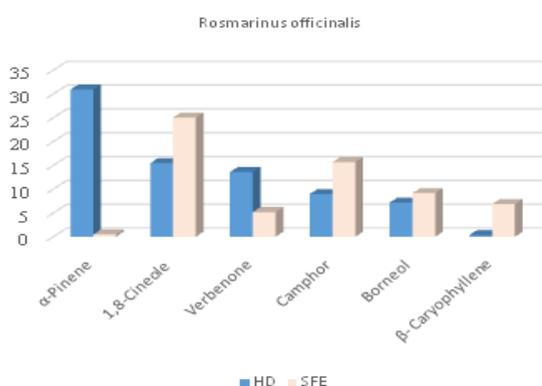


Fig. 1. Comparison the major compounds in Rosmarinus officinalis L.essential oil extracted via hydrodistillation (HD) and supercritical fluid extraction (SFE).

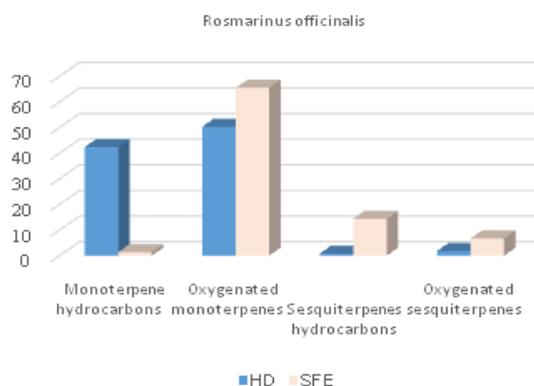


Fig. 2. Comparison hydrocarbon compounds and oxygenated compounds of essential oil extracted via hydrodistillation and supercritical fluid extraction

CONCLUSION

The supercritical fluid extraction of *Rosmarinus officinalis* L. was investigated, and the results, were compared with essential oil composition obtained via hydrodistillation. The SFE technique suggests well-known advantages over hydrodistillation. SFE needs shorter extraction times and less energy consumption. The possibility of influencing the composition of the oil, by altering the parameters of the extraction (pressure, temperature, modifier volume and dynamic extraction time) is more manageable in SFE. We

obtained a higher selectivity in SFE than by the subsequent hydrodistillation technique. Besides, the use of polar co-solvent is not necessary and the subsequent processing for solvent elimination is not required.

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REFERENCES

- Bousbia, N.; Vian, M.A.; Ferhat, M.A.; Petitcolas, E.; Meklati, B. Y.; Chemat, F. *Food Chem.* **2009**, *114*, 355–362.
- Jacotet-Navarro, M.; Rombaut, N.; Fabiano-Tixier, A.S.; Danguien, M.; Bily, A.; Chemat, F. *Ultrason. Sonochem.* **2015**, *27*, 102-105.
- Jarrar, N.; Abu-Hijleh, A.; Adwan, K. *Asian Pac. J. Trop. Med.* **2010**, *3*, 121-123.
- Okoh, O.O.; Sadimenko, A.P.; Afolayan, A.J. *Food Chem.* **2010**, *120*, 308-312.
- Wang, W.; Wu, N.; Zu, Y.G.; Fu, Y.J. *Food Chem.* **2008**, *108*, 1019-1022.

6. González-Vallinas, M.; Molina, S.; Vicente, G. *Pharmacol. Res.* **2013**, *72*, 61-68.
7. Hernández, M.D.; Sotomayor, J.A.; Hernández, Á.; Jordán, M.J. Chapter 77 - Rosemary (*Rosmarinus officinalis* L.) Oils," in *Essential Oils in Food Preservation, Flavor and Safety.* **2016**, 677-688.
8. Lemos, M.F.; Lemos, M.F.; Pacheco, H.P.; Endringer, D.C.; Scherer, R. *Ind. Crops Prod.* **2015**, *70*, 41-47.
9. Li, G.; Cervelli, C.; Ruffoni, B.; Shachter, A.; Dudai, N. *Ind. Crops Prod.* **2016**, *84*, 381-390.
10. Fornari, T.; Vicente, G.; Vázquez, E.; García-Risco, M. R.; Reglero, G. *J. Chromatogr. A.* **2012**, *1250*, 34-48.
11. Zabet, G. L.; Meireles, M. A. A. *J. Supercrit. Fluids.* **2014**, *94*, 234-244.
12. R. P. Adams, R. P. *Identification of essential oil components by gas chromatography/mass spectrometry*, no. Ed. 4. Allured publishing co. **2007**.
13. Carvalho Jr., R. N.; Moura, L. S.; Rosa, P.T.V.; M. A. A. Meireles, M. A. A. *J. Supercrit. Fluids.* **2005**, *35*, 197-204.