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Chemical Composition of the Essential Oil of Rosemary (*Rosmarinus officinalis* L.) of Tunisian Origin

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The hydrodistilled essential oils of three samples of aerial parts of *Rosmarinus officinals* L. growing in Tunisia were analyzed by GC-MS. Capillary GC-MS analysis of the essential oils permitted the detection of 38 components. Among them, four were tentatively identified. The oils were found to be rich in 1,8-cineol (33.08-37.75 %), camphor (13.55-18.13 %), α-pinene (8.58-9.32 %), α-terpineol (6.79-8.17 %), camphene (5.07-5.58 %), borneol (4.08-5.48 %), limonene (3.19-3.04 %) and *p*-cymene (2.42-3.11 %).

Key Words: *Rosmarinus officinals* L, Essential oil, Chemical composition, GC-MS.

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. It is an important medicinal and aromatic plant, which belongs to the Lamiaceae family and has been cultivated for a long time. Anthropologists and archaeologists have found evidence that rosemary herbs were used as medicinal, culinary and cosmetic virtues in the ancient Egypt, Mesopotamia, China and India[1]. Rosemary is a perspective plant culture in the world; it is in the middle of interest of plant breeders[2-4]. Rosemary is cultivated for the valuable oil, which can be extracted from the harvested plants when flowers are in buds[1]. Rosemary leaves are a very common spice and its oil is used in fragrance flavour industry aromatherapy[5], antioxidant activity[6,7], antimiccrobial and antitumour properties[8,9]. It is used for flavouring food, in cosmetic and in traditional medicine for choretics, hepatoprotective and antimorigenic activity[10].

The essential oil enhances the blood-circulation of the limbs, has antirheumatic effect and relieves the neuralgic pains. Besides the therapeutical application, the essential oil is widely applied in the cosmetic industry producing various Cologne waters, bathing essences, hair lotions and shampoons. The leaf of rosemary is an indispensable spice of the French, Italian and Spanish cuisine[1]. The rosemary essential oil composition has been investigated and reported in literature[11]. The studies include countries mainly from the Mediterranean region, Balkans, South-Eastern regions[12]. Chalchat *et al.*[2] reported comparison Spain, Morocco and France Rosemary oils composition. Oils of the other origins have also been studied[13, 14]. At present, demand for *R. officinalis* is increasing for its use in traditional medicine, pharmaceutical industries and agribusiness[13]. However, most of the material used comes from natural Rosemary populations growing in sites characterized by low rainfall (<300 mm/year), repeated drought, low soil quality and overgrazing[15]. These factors compounded with an increasing harvest have led to continuous degradation of populations[16].

The aim of the present work is to evaluate the chemical composition of rosemary essential oil originated from Tunisia.

EXPERIMENTAL

Aerial parts plants of *Rosmarinus officinalis* L. were randomly collected from three geographic origins (Beja, Sidi Bouzid and Gabes) in Tunisia. Fresh aerial parts of plants were dried in a forced-air drier at 35 ºC for 48 h, until it reached a constant weight. Stem and leaves of *Rosmarinus officinalis* L were collected at the flowering stage from different localities and identified by Dr. Sotomayor, botanist at the IMIDA Institution. Voucher specimens of the species are deposited at the Herbarium of the Laboratory of vegetable Biotechnology and morphogenesis at the faculty of sciences of Tunis under the numbers ROB 2008-117, ROSB 2008-118 and ROG 2008-119), respectively, for the (Beja, Sidi Bouzid and Gabes) sites.

Essential oil extraction: Fresh aerial parts of plants were dried in a forced-air drier at 35 ºC for 48 h, until it reached a constant weight. Aerial parts of individual plants were distilled for 3 h using a Clevenger-type system. The oil volume was measured directly in the extraction buret. Samples were dried

with anhydrous sodium sulfate and kept in amber vials at 4 ºC until chromatographic analysis[17]. Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter.

Gas chromatography: Samples of 0.1 mL were subjected to analysis by capillary gas chromatography, using a Hewlett-Packard 5890 gas chromatograph (GC) (Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm HP-5 (cross-linked phenyl-methyl siloxane) column with 0.25 mm film thickness (Hewlett-Packard, Palo Alto, CA, USA) was used for this study according to the method developed by Jordán *et al*.[17]. The FID and the injector were maintained at 280 and 250 ºC, respectively. Helium was used as carrier gas, the flow through the column was 1 mL/min and the split ratio was set to 100:1. The column was maintained at 60 ºC for 4 min, increased to 64 ºC at a rate of 1 ºC/min, then increased to 155 ºC at a rate of 2.5 ºC/min and finally raised from 155 to 250 ºC at a rate of 5 ºC/min. For the identification of the compounds, retention times and retention index were confirmed with commercially available standard compounds (Acros, Fisher Scientific S.A. and Sigma Aldrich Qui´mica S.A.).

Mass spectrometry analysis: Gas chromatography-mass spectrometry was used for the identification of components in rosemary essential oil. For that, a Hewlett-Packard 5890 Series II Plus gas chromatograph, equipped with a 30 m × 0.25 mm HP-5 column with 0.25 mm film thickness was used. The GC was linked to a Hewlett-Packard Model 5972 mass spectrometry detector. The chromatographic conditions were identical to those used for the gas chromatography analysis[18].

Qualitative and quantitative analysis: The individual peaks were identified by retention times and retention indices, compared with those of known compounds and by comparison of mass spectra of their corresponding commercially available standard components, using the NBS75K library (United States, National Bureau of Standards, 2002). Authentic standard was obtained from Sigma-Aldrich (Madrid, Spain). Percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current.

RESULTS AND DISCUSSION

The essential oils isolated by water distillation were obtained in yield 1.8 % based on dry weight of sample for Beja, 1.6 % for Sidi Bouzid and 1.4 % for Gabes. Essential oil yields obtained from three populations did not show statistically significant differences among percentages calculated on a dry matter basis. Thirty eight volatile components were identified on the basis of their mass spectra characteristics, retention indices. Qualitative and quantitative analysis of the essential oils of rosemary of three populations are given in Table-1. The main components of the Rosemary oils were 1,8-cineole (33.08, 37.75 and 36.75 %), camphor (18.13, 13.55 and 15.57), α-pinene (9.23, 9.32 and 8.58 %), α-terpineol (8.17, 6.79 and 6.98 %), borneol (5.48, 4.08 and 4.49 %), camphene (5.07, 4.18 and 5.58 %) and *p*-cymene (2.42, 3.11 and 2.67) for Beja, Sidi Bouzid and Gabes, respectively.

The present study revealed that rosemary from Tunisia includes 1,8-cineole and camphor chemotypes. The 1,8-cineole chemotype is similar to that previously reported for the species from other Mediterranean countries[19, 20,12]. There were some reports of the presence of α-pinene, 1,8-cineole, camphor, verbenone and borneol, constituting about 80% of the total *R. officinalis* oil[21]. The major components, α-pinene, borneol, camphene, camphor, verbenone and bornyl-acetate, were also reported to be present in Sardinian *R. officinalis* L. oil[22]. Cineole, borneol, pinene and camphor are the major constituents of rosemary oil, comprising about 28, 18, 12 and 10 % of the oil[23]. Compared with other rosemary oils, Brazilian oils were more similar to those of French origin due to their 1,8-cineole and camphor contents[24,2,20].

The variability of the qualitative and quantitative composition of the essential oil is due to intrinsic features (*e.g.* genetics, plant age) and also to extrinsic factors such as climate, cultivation conditions, extraction methods, *etc*.,[25].

Significant variations in the chemical composition of oil have been reported with relation to the geographic origin[24,5,2,20].

TABLE-1
CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *Rosmarinus officinalis* L GROWING IN TUNISIA

Nº	Compounds	RI	Aire (Beja) (%)	Aire (Sidi Bouzid) (%)	Aire (Gabes) (%)
1	Tricyclene*	1 068	0.18	0.14	0.11
2	α-Pinene	1 075	9.23	9.32	8.58
3	Fenchene	1 097	0.14	0.16	0.14
4	Camphene	1 102	5.07	4.18	3.58
5	α-Pinene	1 132	1.86	1.28	1.59
6	Δ^3-Carene	1 164	0.04	0.20	0.17
7	Myrcene	1 174	1.11	1.06	1.13
8	α-Phellandrene	1 178	0.22	0.21	0.27
9	α-Terpinene	1 191	0.65	0.62	0.86
10	Limonene	1 210	3.19	2.99	3.04
11	1,8-Cineole	1 221	33.08	37.75	36.75
12	γ-Terpinene	1 256	0.61	0.46	0.79
13	*p*-Cymene	1 284	2.42	3.11	2.67
14	Terpinolene	1 296	0.37	0.22	0.39
15	Styrene*	1 465	0.09	0.13	0.13
16	1-Octen-3-ol	1 475	0.31	0.22	0.20
17	α-Cubenene*	1 515	0.09	0.20	0.09
18	Camphor	1 547	18.13	13.55	15.57
19	Linalool	1 580	0.89	1.14	1.13
20	Bornyl acetate	1 612	0.77	0.87	0.50
21	Fenchole	1 618	0.15	0.18	0.16
22	(E) caryophyllene	1 627	0.90	1.65	1.22
23	Terpinen-4-ol	1 637	1.23	1.40	1.43
24	E-pinocarveol	1 692	0.06	0.10	0.09
25	α-Humulene	1 702	0.28	0.36	0.21
26	Isoborneol	1 710	0.89	1.14	1.18
27	γ-Muurolene*	1 723	0.11	0.24	0.11
28	α-Terpineol	1 736	5.48	6.79	6.98
29	Borneol	1 741	8.17	4.08	4.49
30	Δ-Cadinene	1 796	0.24	0.54	0.22
31	Citronellol	1 808	0.02	0.04	0.06
32	*p*-Cymen-8-ol	1 902	0.11	0.14	0.12
33	Caryophyllene	2 047	0.26	0.20	0.23
34	Eugenol methyl ether	2 095	0.16	0.47	0.44
35	Eugenol	2 301	0.21	0.29	0.22
36	Thymol	2 305	0.12	0.18	0.17
37	Thymol acetate	2 324	0.04	0.04	0.12
38	Carvacrol	2 370	0.17	0.16	0.79

*Tentative identification

Times of harvest, condition of the twigs and leaves, distillation equipment and management was also reported to have an important role in the overall quality of the oil[24].

Conclusion

The analysis of different of essential oils of rosemary that grow in Tunisia province identified the components and the percent of components are slightly different. The soil, climate and altitude may be affected on the components and percent component therefore for different use of essential oils of rosemary different geographic for grown is necessary. The essential oil composition is a very complex one also because it is influenced by different factors such as climate, extraction methods, *etc*. In order to obtain a high fidelity fingerprint of the essential oil it is necessary an optimization of the separation method but also of the extraction technique.

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