

SEASONAL VARIATION OF THE ESSENTIAL OIL OF *ROSMARINUS OFFICINALIS* L.

F. Bannour^{a*}, I. Chéraïf^b, M. El Gazzah^c, F. Farhat^d, M. Hammami^b, R. Chemli^a

^a *Faculté de Pharmacie de Monastir, Laboratoire de Pharmacognosie,
5000 Monastir, Tunisie*

^b *Faculté de Médecine, USCR de Spectrométrie de Masse, Laboratoire de Biochimie,
5019 Monastir, Tunisie*

^c *Faculté des Sciences de Tunis, Laboratoire de Génétique et de Biométrie,
1020 Tunis, Tunisie*

^d *Faculté de Pharmacie de Monastir, Laboratoire de Chimie Analytique,
5000 Monastir, Tunisie*

(Reçu le 20 Septembre 2005, accepté le 4 Avril 2006)

RESUME: On analyse les huiles essentielles, obtenues par hydrodistillation, de jeunes pousses de *Rosmarinus officinalis* L. (Lamiaceae) récoltées dans quatre populations naturelles différentes de Tunisie (El Houdh, Chambi, Oueslatia et Zaghouan) durant trois périodes différentes de l'année 2000 (février, avril, juillet). Pour cela on utilise la chromatographie en phase gazeuse et on identifie les composés par spectrométrie de masse. 36 composés ont été isolés avec des différences quantitatives entre les différentes populations étudiées. Le 1,8-cinéole est le constituant majoritaire de l'huile essentielle de romarin (31.1-55.4%). Les composés monoterpéniques (1,8-cinéole, α -pinène, camphène, p-cymène, limonène, α -terpinéol, camphre, bornéol et l'acétate de bornyle) forment la fraction la plus importante (91.3-98.8%). Dans toutes les populations, les échantillons récoltés au mois d'avril ont montré la teneur la plus élevée en huile essentielle.

Mots clés : *Rosmarinus officinalis*, Lamiaceae, Huile essentielle, 1,8-cinéole

ABSTRACT: The essential oil isolated by hydrodistillation from young sprouts of *Rosmarinus officinalis* L. (Lamiaceae) collected from four natural populations of Tunisia (El Houdh, Chambi, Oueslatia and Zaghouan) at three periods of the year 2000 (February, April, July) have been analyzed by gas chromatograph and gas chromatograph coupled with mass spectrometer. 36 compounds have been isolated with quantitative variations between the various populations examined. 1,8-cineole (31.1-55.4%) was found to be the main component of this oil. Monoterpenes were shown to be the major fraction (91.3-98.8%) and mainly composed of 1,8-cineole, α -pinene, camphene, p-cymene, limonene, α -terpineol, camphor, borneol and bornyl acetate. For all populations, the highest yield of oil was obtained from samples collected in April.

Key words: *Rosmarinus officinalis*, Lamiaceae, Essential oil, 1,8-Cineole

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a very important medicinal and aromatic plant, which belongs to the Lamiaceae family. It is well represented in the Mediterranean countries under different climatic conditions. In Tunisia, natural rosemary populations, growing mainly in the semi-dry stage are exploited for their essential oil [1]. The plant produces pale yellow oil known for many therapeutic indications (analgesic, antidepressant, antispasmodic, antirheumatic, diuretic, hepatic, restorative, stimulant, and tonic.) [2,3].

* Corresponding author, tel: 216 97 128 485, e-mail: banftama@yahoo.fr



Rosemary oil has a strong fresh, clear woody aroma. It is used in the perfume industry as a flavor agent; in addition, it possesses carminative properties [4]. It is also used in liniment as rubefacient. Several compounds found in this oil have been reported to be inhibitor of microorganisms [5,6]. The main components of the leaf oil from *Rosmarinus officinalis* collected from the University of Fort Hare Research farm in Alice (South Africa) were 1,8-cineole (31.12%), camphor (30.12%), α -pinene (18.18%), camphene (6.08%), β -pinene (2.58%), bornyl acetate (3.17%), and unknown components (3.88%) [7]. The oil of Italian *Rosmarinus officinalis* was shown to be mainly composed of 1,8-cineole (44%), camphor (12%), α -pinene (10%), and borneol (6%) [8]. The major components of *R. officinalis* growing in Spain were camphor (38%), 1,8-cineole (16%), α -pinene (8%) and borneol (7%). Generally, the yield and composition of the oil varies depends upon the origin and collection period of the plant. The yield of the oil of *Artemisia compestris* collected from different areas in Tunisia was found to reach its maximum (1.2%) in the flowering period [9]. The oil yield of *Tanacetum annuum* decreased from July to September (1.0-0.8%) and the composition varied depending upon the growth stage [10].

In view of the increasing use of essential oils in the food, cosmetic and pharmaceutical industries, it is important to examine the oils from indigenous plants such as rosemary. This paper reports the first study of the seasonal variation of the young sprout essential oil of *R. officinalis* collected from various areas in Tunisia.

MATERIALS AND METHODS

Plant material:

Aerial parts of *R. officinalis* (Lamiaceae) were collected randomly from four natural populations of Tunisia (El Houdh, Chambi, Oueslatia and Zaghouan) at three periods of the year 2000 according to the growing stage of this plant (February = young sprouts before the flowering period, April = flowering period, July = production of seeds). For each population, we have harvested one individual sample consisted of the young sprout of the plant. About 2 kg materials of the plant's young sprouts were separated from the lignified part and dried for 15 days. These young sprouts were then used for oil isolation. Voucher specimens of each sample have been deposited in the Herbarium of the Faculty of Pharmacy in Monastir (Tunisia) with numbers O2/00, O4/00, O7/00, Ch2/00, Ch4/00, Ch7/00, E12/00, E14/00, E17/00, Za2/00, Za4/00 and Za7/00, respectively. The climatically and geographical parameters of the studied areas are presented in Table I.

Table I: Geographical and climatic data of the studied Tunisian areas

	Oueslatia	Chambi	El Houdh	Zaghouan
Mean of yearly maximum temperature (°C)	25.2	24.4	26.3	25.1
Mean of yearly minimum temperature (°C)	11.8	10.6	12.8	12.2
Mean of yearly rainfall (mm)	340	365	330	430
Altitude (m)	150	300	220	250

Oil isolation:

A 100-g amount of the air dried young sprouts mixed with 600 ml distilled water were subject to hydrodistillation for 3 h using the apparatus described in the IXth edition of French Pharmacopoeia (similar to a Clevenger-type apparatus) [11].

Oil analyses:

The composition of the oil was investigated by GC and GC/MS. The analytical GC was carried out on an HP 5890 gas chromatograph equipped with a flame ionization detector (FID), under the following conditions: the fused silica capillary column HP5 (30 m x 0.25 mm, film thickness 0.25 μ m) was temperature programmed from 50-280°C at 5°/mn; and the polar column HP-20M (30 m x 0.25 mm, film thickness 0.25 μ m) was temperature programmed from 50-250°C at 5°/mn.

carrier gas: nitrogen at a flow rate 0.8ml/mn; injector and detector temperature: 250°C and 250°C, respectively; the volume injected: 0.1 µl of oils diluted in hexane (10%). The percentage composition of oils was computed from the GC peak areas without using any correction factors.

The GC analyses were performed using Hewlett-Packard GC-MS system (GC: 5890 series II; MSD 5972 A). 2 columns have been used: the fused silica capillary column HP5 (30 m x 0.25 mm, film thickness 0.25µm) was temperature programmed from 50-280°C at 5°/mn; and the polar column HP-20M (30 m x 0.25 mm, film thickness 0.25µm) was temperature programmed from 50-250°C at 5°/mn and coupled to the MS (HP5.25 m x 0.2 mm, film thickness 0.25µm), recording at 70 eV.

The GC was operated under the following operating conditions: injector temperature: 250°C; oven temperature programmed from 60°-200°C at 3°C/min; carrier gas: He 20 psi; injector volume: 1µL of 1/100 diluted solution in hexane. The oil components were identified by comparison of their retention indices (relatives to C9-C28 alkenes on the HP5 column) and mass spectra with those of authentic standards of library search routines based on fit and purity of mass spectra [12]. Retention times of individual components were compared with those of authentic samples.

RESULTS AND DISCUSSION

For all areas, the highest oil yield (v/w) was obtained from samples collected in April (flowering period). During this period, the yield of oil in samples collected from Oueslatia (2.5%) was higher than that in the samples from Chambi (2.2%), El Houdh (2%) and Zaghuan (1.9%). Investigating samples collected in February (before the flowering stage) and July (seed production stage), we noticed that the highest amount of oil was obtained in Oueslatia (1.95%) young sprouts (Table II).

Figure.1 shows a gas chromatogram of the essential oil of *Rosmarinus officinalis* and table II lists the components identified in the whole distilled oil. 1,8-Cineole was the most abundant component for all samples (31.1-55.4%). For all areas, samples collected in April contained the highest amount of this compound (40.7-55.4%).

Table II: The yield and the percentage composition of the essential oil of *Rosmarinus officinalis* collected from different Tunisian areas at different periods

N°	Compound	Retention indices		February 2000				April 2000				July 2000				
		HP-20M	HP 5	O	Ch	El	Za	O	Ch	El	Za	O	Ch	El	Za	
1	Tricycene	1014	926	0.1	0.1	tr	0.1	tr	0.1	tr	tr	tr	tr	tr	tr	tr
2	α-Pinene	1033	939	6.3	6.4	5.9	4.1	3.9	7.3	1.8	4.7	5.0	6.9	3.5	3.1	
3	α-Fenchene	1059	950	2.5	2.4	1.9	1.8	1.8	2.6	1.0	2.1	1.6	2.3	1.5	1.3	
4	Camphene	1068	950	0.9	tr	1.9	0.1	1.1	1.2	1.3	3.7	3.3	2.3	0.1	2.8	
5	β-Pinene	1115	976	tr	3.1	tr	1.4	tr	tr	tr	tr	tr	tr	2.5	tr	
6	β-Myrcene	1152	991	0.1	0.9	0.8	0.7	0.8	1.1	0.5	0.7	0.9	0.8	0.5	0.6	
7	α-Phellandrene	1160	1007	0.1	0.1	0.1	0.1	0.1	0.1	tr	tr	tr	0.2	t	0.1	
8	α-Terpinene	1176	1009	0.2	0.4	0.3	0.4	0.2	0.3	0.1	0.2	0.1	0.5	0.3	0.3	
9	Limonene	1198	1033	1.7	1.9	1.8	1.6	1.5	1.9	1.1	2.3	1.4	1.9	1.3	1.1	
10	1,8-Cineole	1208	1021	52.6	41.9	39.9	43.8	55.4	53.1	40.7	43.9	51.6	38.7	31.1	39.3	
11	p-Cymene	1258	1026	1.4	0.8	0.9	1.2	1.6	1.3	1.1	1.1	0.9	0.8	0.9	0.5	
12	α-Terpinolene	1280	1088	0.1	0.3	0.4	0.3	0.2	0.2	0.2	0.2	0.3	0.4	0.3	0.3	
13	p-Cymenene	1459	1071	tr	tr	tr	tr	tr	1.4	tr	tr	tr	tr	0.1	tr	
14	trans-Linalool oxide	1469	1073	0.3	0.5	0.1	0.4	tr	0.3	0.4	tr	0.2	tr	0.3	0.5	
15	cis-Linalool oxide	1477	1085	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.4	0.1	0.3	0.2	
16	α-Campholenal	1496	1126	tr	0.2	0.1	0.2	0.2	tr	0.1	tr	0.2	tr	0.4	0.3	
17	Camphor	1509	1145	15.3	17.0	22.4	17.1	15.9	13.7	22.8	17.4	13.6	19.1	7.7	13.9	



N°	Compound	Retention indices		February 2000				April 2000				July 2000			
		HP-20M	HP 5	O	Ch	El	Za	O	Ch	El	Za	O	Ch	El	Za
18	Linalool	1547	1098	0.3	0.9	0.8	0.7	0.4	0.2	1.1	1.4	1.0	0.7	2.5	1.9
19	Pinocarvone	1561	1140	0.1	0.2	tr	0.2	tr	0.1	0.2	0.2	0.1	0.1	0.2	tr
20	Terpinen-4-ol	1571	1179	1.3	1.4	1.4	2.6	1.5	1.2	2.2	1.8	1.7	1.2	1.4	1.7
21	Bornyl acetate	1573	1278	0.6	1.6	3.4	1.9	2.0	0.6	2.2	0.7	1.4	2.3	5.2	5.3
22	β -Caryophyllene	1587	1418	tr	0.1	tr	tr	tr	tr	tr	tr	0.1	tr	0.1	0.1
23	<i>iso</i> -Borneol	1642	1145	0.1	0.2	0.1	0.2	tr	0.1	0.2	tr	0.1	tr	0.1	tr
24	<i>cis</i> -Verbenol	1655	1128	1.1	0.9	0.9	1.1	0.9	0.7	1.5	0.8	0.8	1.1	0.9	1.4
25	α -Humulene	1660	1370	0.1	0.2	0.1	tr	tr	0.1	0.1	tr	0.1	tr	0.2	0.2
26	<i>trans</i> -Verbenol	1669	1130	4.9	4.7	0.4	5.8	0.3	3.5	0.3	tr	tr	0.3	4.5	0.9
27	α -Terpineol	1673	1198	tr	0.3	5.2	1.1	4.4	tr	7.3	6.6	5.0	4.7	2.5	5.9
28	Borneol	1691	1149	5.7	5.5	6.7	7.0	4.3	7.7	8.1	4.8	4.4	6.2	19.7	7.6
29	Verbenone	1701	1183	0.1	0.1	tr	tr	tr	tr	tr	tr	0.1	0.2	0.1	0.3
30	Carvone	1713	1242	0.1	0.3	0.2	0.3	0.2	0.1	0.1	tr	0.3	0.2	0.7	0.6
31	Myrtenol	1782	1181	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.4	0.4
32	<i>p</i> -Cymen-8-ol	1820	1184	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1
33	<i>trans</i> -carveol	1825	1198	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.1
34	Methyl-eugenol	1839	1369	0.2	0.4	0.4	0.5	0.2	0.2	0.6	0.8	0.3	0.4	2.1	1.2
35	Carvacrol	1850	1504	tr	tr	tr	0.1	tr	tr	tr	tr	tr	0.1	0.1	0.2
36	β -Caryophyllene oxide	2008	1576	0.1	0.2	0.5	0.4	0.1	0.2	0.4	0.3	0.2	0.5	0.9	0.6
Identified compound				97.5	94.2	97	95.7	97.3	99.1	96	94	96.1	92.3	92.8	92.2
Grouped components															
Monoterpenes				97.1	93.7	96.4	95.3	97.2	98.8	95.5	93.7	95.7	91.8	91.6	91.3
Sesquiterpenes				0.2	0.5	0.6	0.4	0.1	0.3	0.5	0.3	0.4	0.5	1.2	0.9
Yield (ml/100 g)				1.95	1.70	1.60	1.80	2.50	2.20	2.00	1.90	1.95	1.40	1.20	1.30

tr= amount <0.10%

O: Oueslatia, Ch: Chambi, El: El Houdh, Za: Zaghouan

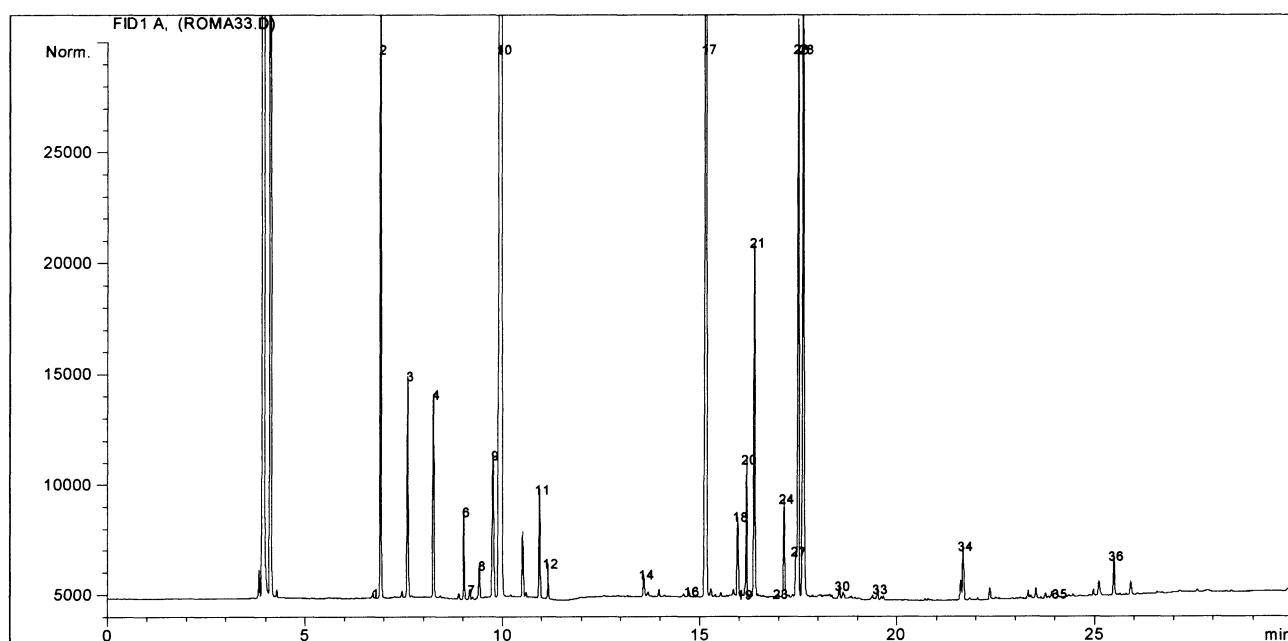


Fig.1: Gas chromatogram of the essential oil of Tunisian *Rosmarinus officinalis*



The monoterpene fraction was present in relatively high amounts (>91%) and mainly composed of 1,8-cineole, camphor, α -pinene, bornyl acetate camphene, p-cymene, limonene, α -terpineol, borneol and bornyl acetate representing more than 70% of the total oil. Young sprouts from rosemary collected in the flowering period contained the highest percentage of 1,8-cineole (55.4%), α -pinene (7.3%), camphene (3.7%), α -fenchene (2.6%), limonene (2.3%), p-cymene (1.6%), α -terpineol (7.3%) and camphor (22.8%). Samples collected in February was found to relatively contain the highest amount of β -pinene (3.1%), terpinen-4ol (2.6%), and *trans*-verbenol (5.8%) while samples collected in July contained the highest percentage of borneol (19.7%), bornyl acetate (5.3%) and linalool (2.5%).

For the three periods, the highest amount of 1,8-cineole existed in Oueslatia samples but the highest percentage of α -pinene, was found in samples collected from Chambi population where the altitude reach its maximum (300m).

Among sesquiterpenes, β -carophyllene oxide was the main compound identified in this oil, its amount ranged from 0.1 to 0.9%. Samples collected from El Houdh presented the highest amount of this component (0.4-0.9%). However, for all periods, young sprout from Oueslatia contained the lowest percentage of this sesquiterpene (0.1-0.2%).

The difference of the oil chemical composition among areas, which can affect the biological activities and pharmaceutical uses, could be due to differences in the climatic and geographical parameters (altitude, temperature, rainfall, wind direction, number of sunshine hours, etc.). The difference of the oil composition between the periods of the collect (growth stage) is very meaningful. The use of this plant in the flowering period in the traditional medicine could be due to the high oil content and or to the chemical composition that is characterized by the presence of high amount of monoterpenes. Rosemary oil, because of its content of 1,8-cineole, camphor, borneol and bornyl acetate is used for circulation stimulation [13]. The oil composition of Tunisian *Rosmarinus officinalis* was found to be different from the oil of different countries [14,15]. All samples analyzed in this study belonged to the same chemical species (with 1,8-cineole).

The study of the biological activities of this oil is very important to see if there is any correlation between biological activities and chemical composition.

ACKNOWLEDGEMENTS

This study was funded by the research program "Valorization of Rosemary in Tunisia" financed by the Institute of the Research and the Agriculture High Study. Thanks are due to the Genetic and Biometry Laboratory from the Faculty of Science of Tunis. Mr. Mohamed BEN SALAH is acknowledged for his help with distillation facilities.

REFERENCES

- [1] E. Le Floc'h, *Contribution à une étude ethnobotanique de la flore tunisienne*, Ministère de l'enseignement supérieur et de la recherche scientifique, Tunis, 1983, 208.
- [2] E. S. Banyai, M. H. Tulko, A. Hegdusi, C. Renner, I. S. Varga, *Acta Biologica Szegediensis*, 2003, 47(4), 111.
- [3] I. Taddei, D. Giagheti, E. Taddei, P. Mantovani, *Fitoterapia*, 1988, 9(6), 144.
- [4] G. Roulier, *Les huiles essentielles pour votre santé. Traité botanique d'aromathérapie: propriétés et indications thérapeutiques des essences de plantes*, Dangles, 1990, 45.
- [5] G. Garnier, L. Bezanger, G. Debraux, *Ressources médicinales de la flore Française*, 1961, Vigot Frère, Paris, 1211.
- [6] V. E. Tyler, L. R. Brady, J. E. Robber, *Pharmacognosy*, LEA and FIBIGER, Philaolelphia, 1976, 134.
- [7] T. Mangena, N. Y. O. Muyima, *Letters in Applied Microbiology*, 1999, 28, 291.
- [8] R. Granger, J. Passet, G. Arbousset, *Parf. Cos. Sav. France* 1973, 3(6), 307.



- [9] A. Akrouf, R. Chemli, M. Simmonds, M. Hammami, I. Chreif, *J. Essent. Oil Res.*, **2003**, 15, 333.
- [10] H. Greche, M. I. Aloui, N. Ajjaji, S. Zrira, A. Bellanger, B. Benjilani, *Rendement et composition chimique de l'huile essentielle de Tanacetum annuum L*, Actes éditions, **1997**.
- [11] H. Moyse, R. R. Paris, *Matière médicale*, Masson, France, **1965**, 98.
- [12] R. P. Adams, *Identification of essential oil components by Gas chromatography/Mass Spectrometry*, Allured Publ, corp., Carol Stream, IL, **1995**.
- [13] A. Baerheim Sevensen, J. J. C. Scheffer, *Essential oil and aromatic plants*, Martinus Nijhoff, New York, **1984**, 217.
- [14] P. Mailhebiau, *La nouvelle aromathérapie: Caractérogies des essences et tempérament humain*, Jakin, Paris, **1994**, 215.
- [15] K. H. Baser, *Lamiales Newsletter*, **1994**, 3, 6.