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Volatile Variability and Antioxidant Activity of *Rosmarinus officinalis* Essential Oil as Affected by Elevation Gradient and Vegetal Associations

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The essential oil yield, volatile profile and antioxidant activity, of 37 samples collected from the *Rosmarinus officinalis* shrubs growing wild in the different ecosystems of the provinces of Taourirt and Jerada (Oriental region in the east of Morocco), were studied in order to determine the effect of the habitat and how the vegetal associations are influencing the volatile variability of their essential oil. The essential oil compositions were determined by gas chromatography-mass spectrometry (GC-MS). The yield of the essential oil extraction ranged from 1.09 to 2.81 %. The major components found were 1.8-cineol (37.71-65.02 %), camphor (6.09-27.49 %), α-pinene (3.08-9.98 %) and β-pinene (2.83-11.7 %). The interpretation of these results revealed that the variability is attributed to varieties and genetic background rather than the ecosystems. The antioxidant activity was determined by 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay which revealed a strong radical scavenging capacity for all the samples with some variability which can be explained by the variability of the content of monoterpenes oxygenated.

Keywords: ***Rosmarinus officinalis*, Essential oil, Antioxidant activity, Gas chromatography, Oriental, Morocco.**

INTRODUCTION

Morocco is a Mediterranean country with wide ranging ecological conditions. As a result its land offers a rich biodiversity with more than 4200 species of plants. About 800 of these flora have an aromatic and therapeutic virtues and known as medicinal and aromatic plants [1]. Rosemary, *Rosmarinus officinalis*, which belongs to the Lamiaceae family, is deemed the most used aromatic and medicinal plant worldwide [2]. It's an evergreen shrub with needle like leaves and grows spontaneously especially in the western part of the Mediterranean basin [3,4]. In Morocco, rosemary is found in various regions. It grows wild, especially in the eastern parts of the country [1,5]. It's used in folk medicine and as a culinary additive since antiquity [6,7]. Those benefits are especially due to its essential oil, which are volatile compounds synthesized by aromatic plants as secondary metabolism [8].

The essential oil volatile composition of the *Rosmarinus officinalis* has been the focus of numerous researches in the recent years. It contains mainly monoterpenes and monoterpene derivatives (95-98 %), the rest is sesquiterpenes [9,10]. The principal volatile compounds in rosemary essential oil are camphor, 1,8-cineol, borneol, verbenone, α-pinene and camphene [11-13].

Those diverse components of the essential oil, are the responsible of various antimicrobial, antiviral and antioxidant activities [14-16].

The latest research over rosemary essential oil has mainly put the emphasis on its antibacterial [17-19], antifungal [9,20], insecticidal [21], anticancer [22], anti-inflammatory [23], anticorrosion of steel [24] and antioxidant properties [25,26]. This last one is considered very crucial against oxidant molecules because antioxidants act as free radical scavengers and minimize the impact of oxidative damage; as a result, they can alleviate oxidative stress, which causes a considerable damage to biological molecules [27,28]. Furthermore, these antioxidants are used as food preservator [26].

However, essential oil from wild populations of rosemary shows high variation concerning its composition, antimicrobial and antioxidant activities [12,14]. Various factors are influencing the composition and the effectiveness of those activities, likewise the place of origin and genetics [29], bioclimatic conditions [30], the phenological stage [31], the method of extraction [32] and the drying time and method [10,11]. This variability has pushed many authors to classify the essential oil composition to different chemotypes. For instance, Napoli *et al.* [33] demonstrate that Sicilian rosemary essential oil can be classified into three chemotypes: cineoliferum (high content in 1,8-cineol); camphoriferum (camphor > 20 %); and verbenoniferum (verbenone > 15 %).

Oriental region in Morocco (Fig. 1), which is our study area; is a large region with an area of 82820 Km^2, located in the northeast of the country, it's bordered at the east and the south with Algeria, the north the Mediterranean coastline; and by the desert in the south. The Mediterranean climate in the north of the region becomes more arid and continental to the south. Those ecological conditions impact the vegetal cover and offer the adequate area to rosemary to grow spontaneously. Thus, *Rosmarinus officinalis* covers 450 000 ha of shrubs and it is spread along the mountains of the horst in the provinces of Jerada, Taourirt and Figuig, growing from sea level to 2000 meters of altitude through different ecosystems. Rosemary take an important role for the local economy since it's harvested and exploited by local cooperatives. Nevertheless, this plant is subject to excessive human exploitation and traditional valorization [34].

Knowing the importance of this plant in medical and food industry and the impact of this plant in the local economy of Oriental region and to understand the factors impacting the chemical composition, this work aims to study the relationship between the vegetal association and the chemical composition and the antioxidant activity of rosemary's essential oil. Another objective is to study the variability of rosemary's essential oil in detailed scale to have a clear vision on how the chemical composition varies through the different factors. Furthermore, this study opts for the characterization of the essential oil chemical profiling of *Rosmarinus officinalis* in the region which will represent an added value to the rosemary essential oil of the region as a commercial product.

EXPERIMENTAL

Total of 37 samples of *Rosmarinus officinalis* were collected from 37 wild populations belonging to different ecosystems of the provinces of Jerada and Taourirt in the Oriental (Eastern region of Morocco) (Fig. 1). All the samples are identified by the Forest Management and Studies Service of the Oriental region. The collection was processed in the beginning of the May 2017 at blooming stage. The collection was processed in five forests in the two provinces: Ben Yâala (Province of Jerada); and El Ayat, Nerguechoum, Lamkam, Debdou (Province of Taourirt). The sampling was based upon the altitude, longitude and the vegetal associations (the main tree or the vegetal specie) that characterize the ecosystem where rosemary shrub grows, which are: *Tetraclinisarticulata*, *Quercusrotundifolia* and *Steppatenacissima*. Before essential oil extraction, the plant

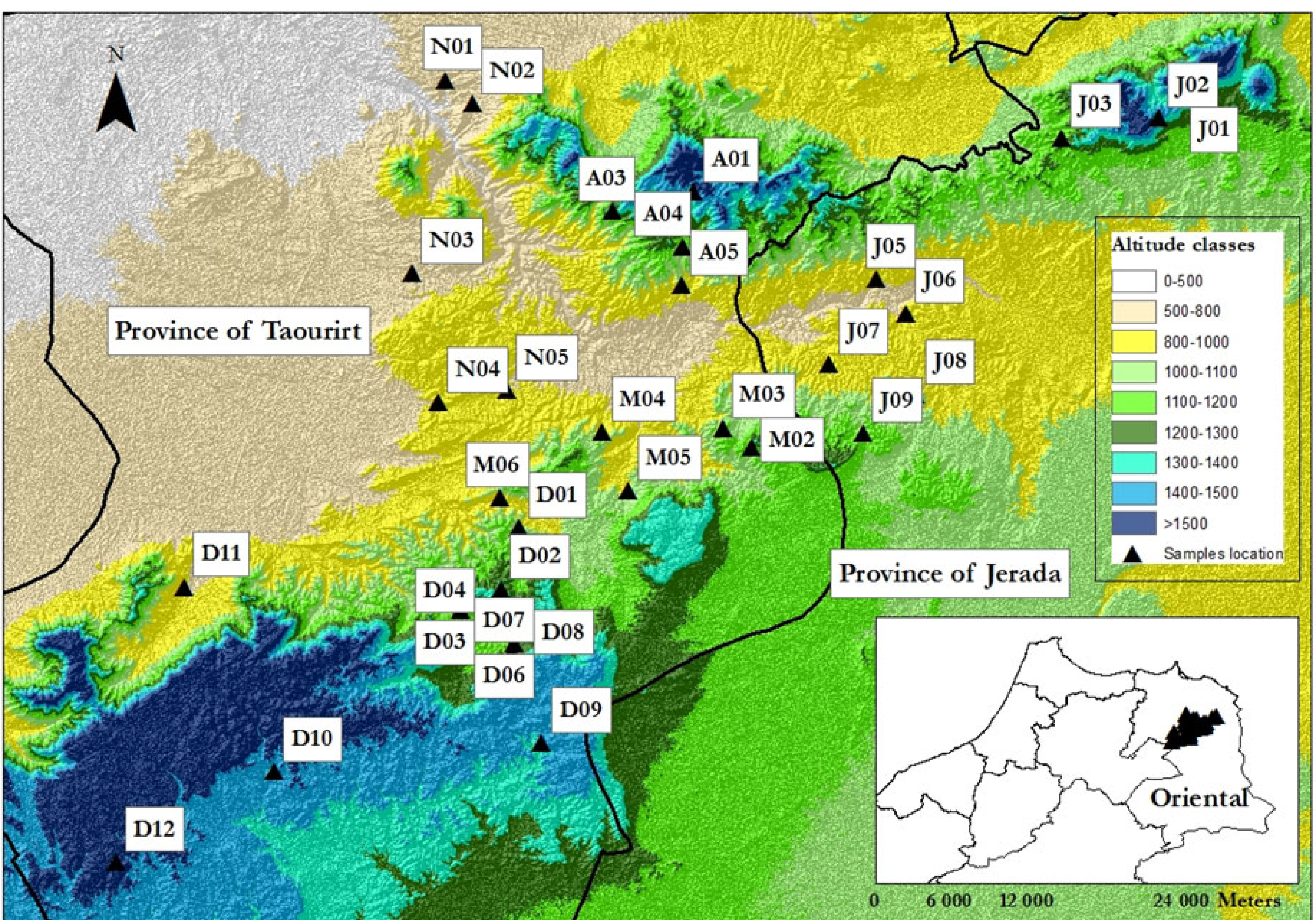

Fig. 1. Map of samples' location in the Oriental region (Provinces of Taourirt and Jerada) according to altitude classes

material was dried at room condition (25 °C) for 7 days. All information concerning the 37 samples are summarized in Table-1.

Essential oil extraction: The leaves of rosemary samples were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia (1996). The oil obtained was separated from water and dried over anhydrous sodium sulphate and kept in amber vials at 4 °C until chromatographic analysis. Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter.

Chromatography-mass spectrometry analysis: The oil was analyzed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph equipped with a 30 m × 0.25 mm HP-5 (cross-linked phynel-methyl siloxane) column with 0.25 µm film thickness (Agilent). The injection was done manually using splitless mode, helium was used as carrier gas and the flow through the column was 1.4 mL min^{-1}. The column was of 10 °C min^{-1} and finally raised from 230 to 280 at rate of 30 °C min^{-1}. The mass spectrometry (MS) operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200 °C, resolution, 1000. Mass unit were monitored from 30 to 450 *m/z*. Identification of components in the oil was based on matching the retention time and kovat's index relatives to *n*-alkanes, with the WILEY 275 Library as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature [35].

DPPH radical scavenging capacity (RSC): The scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Kumar *et al*. [36] with some modifications. The method is based on the reduction of the stable free radical DPPH in the presence of a hydrogen-donating antioxidant and the formation of the non-radical form DPPH-H as a result of the reaction. This

TABLE-1
LAMBERT COORDINATES, ALTITUDE, LOCATION NAME, THE FOREST NAME AND VEGETAL SPECIES CHARACTERIZING THE ECOSYSTEM RELATED TO SAMPLES OF WILD ROSEMARY POPULATIONS COLLECTED IN 2017 FROM THE FORESTS OF THE PROVINCES OF TAOURIRT AND JERADA, MOROCCO

Sample	Location	Forest	UTM coordinates		Altitude (m)	Vegetal species[a]
			X	Y		
A01	Tnezart	Ayate	761024	415381	1434	Quercus R.
A02	Ayate	Ayate	758360	413355	1217	Quercus R.
A03	Sidi Belkacemazaroual	Ayate	754707	413898	1228	Quercus R.
A04	Dadda Ali	Ayate	760157	411155	1038	Tetraclinis A.
A05	Baouess	Ayate	760057	408187	920	Tetraclinis A.
N01	Talmest1	Nerguechoum	741652	424062	526	Tetraclinis A.
N02	Talmest	Nerguechoum	743750	422253	615	Tetraclinis A.
N03	Ousraf	Nerguechoum	739033	409099	735	Steppa T.
N04	Beni Chbel	Nerguechoum	741035	399103	819	Pure
N05	Beni Chbel 2	Nerguechoum	746425	400038	822	Pure
M01	Sidi Smail	Mkam	768789	395261	1193	Steppa T.
M02	Taida 2	Mkam	765518	395628	1133	Tetraclinis A.
M03	Taida	Mkam	763260	397112	1126	Tetraclinis A.
M04	Ioussidene	Mkam	753886	396803	950	Tetraclinis A.
M05	Mkam	Mkam	755927	392275	1040	Steppa T.
M06	Sfia	Mkam	745886	391707	935	Tetraclinis A.
D01	Lamsadak	Debdou	747379	389388	1012	Quercus R.
D02	Zamtat	Debdou	745986	384610	1313	Quercus R.
D03	Beni Mâala	Debdou	742855	382979	1123	Tetraclinis A.
D04	Wizaght	Debdou	744108	382000	1111	Quercus R.
D05	Garage	Debdou	749950	380628	1404	Quercus R.
D06	Zoubia 2	Debdou	747050	380381	1205	Quercus R.
D07	Zoubia	Debdou	746808	379670	1261	Steppa T.
D08	Boukraker	Debdou	747802	378767	1487	Quercus R.
D09	AiounDehaguna	Debdou	749143	372720	1429	Quercus R.
D10	LallaMimouna	Debdou	728294	370460	1476	Quercus R.
D11	Flouch	Debdou	721221	384799	827	Tetraclinis A.
D12	El Ateuf	Debdou	715974	363462	1567	Quercus R.
J01	JbelKeltoum	Béni Yaala	799023	422724	1451	Quercus R.
J02	Sidi Belkacem	Béni Yaala	797289	421185	1400	Quercus R.
J03	Khtitila	Béni Yaala	789698	419616	1182	Steppa T.
J04	Gafait 2	Béni Yaala	779778	408971	804	Pure
J05	Gafait	Béni Yaala	775274	408674	825	Pure
J06	Wizghad	Béni Yaala	777527	406002	820	Steppa T.
J07	Tifirassine	Béni Yaala	771589	402061	905	Tetraclinis A.
J08	TiziGuezmane	Béni Yaala	778345	399708	1017	Quercus R.
J09	Loukto	Béni Yaala	774231	396717	1039	Steppa T.

[a]Vegetal species associated to the rosemary ecosystem: Quercus R. (*Quercus rotundifolia*); Tetraclinis A (*Tetraclinis articulata*); Steppa T. (*Steppa tenacissima*); pure (there is just rosemary).

reduction can be monitored at 517 nm by measuring the bleaching of DPPH (violet) to DPPH-H (yellow). 0.6 mL of various concentration (50, 100, 150 and 200 µL/mL) of the sample were mixed with 2.4 mL of DPPH in methanol diluted at 0.004 %, incubated afterwards 30 min in dark at room temperature. Finally, we measured the absorbance at 517 nm. Methanol and acid ascorbic were used as negative control and as standard antioxidant of the assay, respectively. The radical scavenging capacity (RSC) (%) of DPPH radicals was calculated as:

$$\text{RSC}\,(\%) = \left(\frac{(A_0 - A_1)}{A_0}\right) \times 100$$

where A_0 is the absorbance of the negative control and A_1 is the absorbance of the extracts. The concentration of sample required to reduce 50 % of DPPH radicals (IC_{50}) is calculated from linear regression analysis. A low IC_{50} indicates a high radical scavenging capacity.

Statistical analyses: To assess the effect of the studied parameters (class of altitude, the characteristic tree of the ecosystem where the rosemary grows and the forest of origin) over the essential oil yield, composition and the antioxidant activity across the different localities, a one-way analysis of variance (ANOVA) was carried out. Noting that we used for the ANOVA analysis the altitude classes figured on Fig. 1. Results were considered statistically significant when $P < 0.05$.

To study the affinity between the essential oil belonging to different localities, a hierarchical cluster analysis (HCA) was conducted with the Euclidean distance as a measure of dissimilarity noting that we considered in those calculations the 20 major compounds, which has a mean value of at least 0.15 % of the total composition. All the analyses were conducted using SPSS software, version 19 (IBM SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Yield of the essential oils: As listed in Table-2, the essential oil yields of the tested samples ranged between 1.09 and 2.81 %. The lowest oil yields was found in Lalla Mimouna (Debdou forest, Province of Taourirt) while the highest in the sample harvested from Flouch (Debdou forest, Province of Taourirt). Statistical analysis showed no significant variation according to the altitude or the associated vegetation (the dominant tree where the rosemary shrub grows). Thus, this variability could be attributed mainly to genetic factors and geographical origin that might stimulate the essential oil production by the rosemary plants. The age of the plant can also be responsible of this variability [37,38].

TABLE-2a
CHEMICAL COMPOSITIONS PERCENTAGE OF THE ESSENTIAL OIL OF ROSEMARY LEAVES FROM THE ORIENTAL REGION (MOROCCO)

	Samples																RT[a]	IK[b]
Samples	A01	A02	A03	A04	A05	N01	N02	N03	N04	N05	M01	M02	M03	M04	M05	M06		
Yield (%)	2.31	1.70	1.81	1.60	1.94	2.44	1.70	2.03	2.34	1.92	1.55	1.60	1.92	1.95	1.58	2.27		
Compounds (%)																		
Monoterpenes hydrocarbons	23.68	22.94	20.64	24.38	22.46	23.39	22.62	27.49	21.11	26.01	21.02	24.95	24.19	16.42	20.32	22.29		
α-Thujene	0.44	0.39	0.34	0.26	0.42	-	-	0.68	0.34	0.49	-	-	-	-	-	-	4.45	931
α-Pinene	7.49	7.75	6.41	8	6.55	9.25	7.04	8.59	7.83	7.35	4.72	8.19	9.98	6.03	6	9.46	4.63	932
Camphene	2.78	1.87	1.81	1.78	2.58	2.88	3.57	3.84	1.65	3.75	2.3	3.56	3.15	1.46	1.68	2.74	5.06	953
Sabinene	-	-	-	-	-	-	0.57	0.59	-	-	-	-	-	-	-	-	5.49	976
β-Pinene	7.68	7.34	6.77	7.3	6.85	6.19	5.02	6.66	6.12	8.49	6.47	8.85	6.83	6.09	6.9	7.36	5.613	986
β-Myrcene	1.24	1.36	1.36	0.92	1.64	1.24	1.05	1.2	1.33	1.47	0.82	1.22	1.36	0	1.22	2.73	5.695	991
α-Phellandrene	-	-	-	-	-	-	-	0.26	-	-	-	-	-	-	-	-	6.11	1005
α-Terpinene	0.56	0.52	0.53	0.41	0.73	0.53	0.76	0.87	0.6	0.7	0.45	-	-	-	0.49	-	6.49	1018
D-Limonene	1.89	1.56	1.31	3.69	1.71	1.82	3.11	1.9	1.81	1.73	4.7	1.7	1.96	2.84	2.46	-	6.49	1021
β-Terpinene	0.45	0.37	0.46	0.27	0.63	-	-	0.42	0.42	0.64	0.32	-	-	-	0.58	-	6.64	1027
β-Cymene	-	0.87	0.65	0.76	-	0.48	-	0.83	-	-	-	-	-	-	-	-	6.74	1028
δ-Terpinene	1.15	0.91	1	0.99	1.35	1	1.5	1.65	1.01	1.39	1.24	1.43	0.91	-	0.99	-	7.10	1066
Monoterpenes oxygenated	73.76	74.66	75.14	73.87	75.05	75.58	69.72	70.35	76.59	72.76	76.77	73.53	74.85	82.1	77.52	77.03		
(+)-2-Carene	0.67	0.57	0.53	0.41	0.69	-	-	0.78	0.68	0.4	0.77	-	-	-	-	-	6.27	1010
1,8-Cineol	54.32	48.59	48.32	55.15	49.62	43.63	37.93	37.71	44.98	43.62	53.5	53.14	51.35	59.65	54.03	62.39	6.81	1033
Linalool	-	0.75	0.79	-	-	-	-	0.98	0.48	1.02	0.81	-	-	-	0.95	-	7.99	1098
Camphor	8.79	13.14	13.91	10.93	18.46	27.24	27.49	24.74	23.29	21.12	10.23	12.45	15.51	14.63	15.48	13.25	9.62	1143
Borneol	3.85	4.28	4.88	2.6	-	-	-	-	-	-	3.67	3.03	3.52	3.36	-	1.39	9.66	1165
Pinocarvone	-	-	-	-	0.3	-	-	0.18	0.25	0.24	-	-	-	-	0.53	-	9.90	1185
α-Terpineol	4.82	6.43	5.47	4.37	3.54	4.19	3.53	4.63	3.41	4.17	3.87	4.91	3.41	4.46	5.18	-	10.00	1189
3-Pinanone	-	-	-	-	0.94	-	-	-	1.79	1.06	-	-	-	-	-	-	10.07	1190
Verbenone	-	0.41	0.8	-	0.9	-	-	0.27	1.36	0.73	1.47	-	-	-	0.64	-	10.91	1211
Bornylacetate	1.31	0.49	0.44	0.41	0.6	0.52	0.77	0.66	0.35	0.4	2.45	-	1.06	-	0.71	-	11.18	1295
α-Terpineolacetate	-	-	-	-	-	-	-	0.22	-	-	-	-	-	-	-	-	12.1	1353
cis-Sabinenehydrate	-	-	-	-	-	-	-	0.18	-	-	-	-	-	-	-	-	8.23	1120
5-Isopropyl-2-methyl-bicyclo[3.1.10]hexan-2-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.56	1179
Sesquiterpenes	2.56	2.4	2.16	1.75	2.49	1.03	0.84	1.82	2.3	1.23	2.21	1.52	0.96	1.48	2.16	2.56		
Careophyllene	2.03	1.47	1.27	1.26	1.64	1.03	0.84	1.09	1.95	0.84	1.73	1.52	0.96	1.48	1.67	-	12.771	1418
δ-Cadinene	0.53	0.46	0.89	-	0.46	-	-	0.29	0.35	0.39	0.48	-	-	-	-	-	14.266	1528
Guaiene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.21	1613
Caryophyllene oxyde	-	0.47	-	0.49	0.39	-	-	0.44	-	-	-	-	-	-	0.49	-	15.70	1581
Others	-	-	1.06	-	-	-	6.38	-	-	-	-	-	-	-	-	-		
Spinacene (squalène = triterpenes)	-	-	-	-	-	-	6.38	-	-	-	-	-	-	-	-	-	20.39	1846
4,7,7-Trimethyl-bicyclo heptan-3-ol (ester)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Diethyl phtalate	-	-	1.06	-	-	-	-	-	-	-	-	-	-	-	-	-	16.6	1640
Diisbutyl phtalate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19.4	1760
Total identified	100	100	99	100	100	100	99.56	99.66	100	100	100	100	100	100	100	99.32		

[a]RT = Retention time. [b]IK = kovats index

TABLE-2b
CHEMICAL COMPOSITIONS PERCENTAGE OF THE ESSENTIAL OIL OF ROSEMARY LEAVES FROM THE ORIENTAL REGION (MOROCCO)

	Samples												RT[a]	IK[b]
	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12		
Yield (%)	1.90	1.66	1.49	1.62	1.58	2.63	1.83	2.32	1.33	1.09	2.81	1.69		
Compounds (%)														
Monoterpenes hydrocarbons	19.44	14.75	22.45	9.35	27.52	23.07	20.86	21.84	25.27	30.45	20.36	21.18		
α-Thujene	-	-	-	-	0.42	-	-	0.33	0.48	0.56	-	-	4.45	931
α-Pinene	6.25	5.5	7.53	3.08	9.2	7.48	6.03	7.4	7.49	8.88	7.03	7.64	4.63	932
Camphene	1.65	-	1.85	0.84	2.63	2.6	2.17	2.37	3.18	3.16	2.42	2.7	5.06	953
Sabinene	-	-	-	-	-	-	-	-	-	0.16	-	-	5.49	976
β-Pinene	7.58	4.4	8.4	2.83	9.49	7.58	8.63	6.64	7.75	11.17	5.98	7.17	5.613	986
β-Myrcene	1.23	0	1.48	0.47	1.61	0.79	1.43	1.32	1.52	1.81	0.91	1.18	5.695	991
α-Phellandrene	-	-	-	-	-	-	-	-	-	-	-	-	6.11	1005
α-Terpinene	-	-	-	-	0.58	-	-	0.49	0.61	0.7	-	-	6.49	1018
D-Limonene	1.44	4.85	1.56	1.31	1.92	3.65	1.37	1.94	1.82	2.21	3.05	1.56	6.49	1021
β-Terpinene	-	-	-	-	0.61	-	-	0.45	0.51	0.57	-	-	6.64	1027
β-Cymene	-	-	0.67	0.54	-	-	-	-	0.8	-	-	-	6.74	1028
δ-Terpinene	1.29	-	0.96	0.28	1.06	0.97	1.23	0.9	1.11	1.23	0.97	0.93	7.10	1066
Monoterpenes oxygenated	79.72	71.87	76.55	88.33	70.67	74.88	76.97	76.49	72.63	67.25	78.86	77.43		
(+)-2-Carene	-	-	-	-	0.72	-	-	0.67	0.64	0.81	-	-	6.27	1010
1,8-Cineol	57.43	39.84	60.58	43.98	38.57	62.04	65.02	44.78	49.12	42.16	58.61	59.04	6.81	1033
Linalool	-	-	-	1.71	3.54	-	-	1.86	0.76	0.75	-	0.97	7.99	1098
Camphor	16.39	24.85	11.07	19.51	20.95	6.09	6.76	23.26	13.44	15.02	13.95	8.42	9.62	1143
Borneol	-	-	-	8.84	-	1.76	-	-	3.57	-	1.97	3.53	9.66	1165
Pinocarvone	-	-	-	-	-	-	-	-	0.31	0.25	-	-	9.90	1185
α-Terpineol	5.09	5.14	4.9	11.28	6.15	4.27	5.19	5.37	3.35	5.2	3.6	4.44	10.00	1189
3-Pinanone	-	-	-	-	-	-	-	-	0.71	1.5	-	-	10.07	1190
Verbenone	-	-	-	-	-	-	-	-	-	0.35	-	-	10.91	1211
Bornyl acetate	0.81	2.04	-	2.26	0.74	0.72	-	0.55	0.73	0.96	0.73	1.03	11.18	1295
α-Terpineolacetate	-	-	-	-	-	-	-	-	-	-	-	-	12.1	1353
cis-Sabinenehydrate	-	-	-	-	-	-	-	-	-	0.25	-	-	8.23	1120
5-isopropyl-2-methylbicyclo[3.1.10]hexan-2-ol (carène)	-	-	-	0.75	-	-	-	-	-	-	-	-	7.56	1179
Sesquiterpenes	0.84	1.38	1	2.32	1.81	2.05	2.17	1.67	1.24	2.3	0.78	1.39		
Careophyllene	0.84	1.38	1	1.86	1.22	2.05	2.17	1.22	1.24	1.49	0.78	1.39	12.771	1418
δ-Cadinene	-	-	-	-	0.36	-	-	0.22	-	0.26	-	-	14.266	1528
Guaiene	-	-	-	-	-	-	-	-	-	-	-	-	16.21	1613
Caryophyllene oxide	-	-	-	0.46	0.23	-	-	0.23	-	0.55	-	-	15.70	1581
Others	-	12	-	-	-	-	-	-	0.86	-	-	-		
Spinacene (squalene = triterpenes)	-	-	-	-	-	-	-	-	-	-	-	-	20.39	1846
4.7.7-Trimethyl-bicycloheptan-3-ol (ester)	-	7.65	-	-	-	-	-	-	-	-	-	-		
Diethyl phtalate	-	4.35	-	-	-	-	-	-	0.86	-	-	-	16.6	1640
Diisbutyl phtalate	-	-	-	-	-	-	-	-	-	-	-	-	19.4	1760
Total identified	100	100	100	100	100	100	100	100	100	100	100	100		

[a]RT = retention time, [b]IK = Kovats index

TABLE-2c
CHEMICAL COMPOSITIONS PERCENTAGE OF THE ESSENTIAL OIL OF ROSEMARY LEAVES FROM THE ORIENTAL REGION (MOROCCO)

	Samples									Mean value of all the 37 samples	Standard deviation of all 37 samples	RT[a]	IK[b]
	J01	J02	J03	J04	J05	J06	J07	J08	J09				
Yield (%)	1.87	2.42	1.82	1.89	1.89	1.96	2.03	1.70	2.37	1.91	0.28		
Compounds (%)													
Monoterpenes hydrocarbons	23.75	26.55	26.46	23.08	22.24	24.87	23.28	26.9	20.66	22.66	2.58		
α-Thujene	-	-	0.42	-	-	0.45	-	1.04	-	0.19	0.23	4.45	931
α-Pinene	7.82	9.18	7.91	8.74	7.41	8.64	7.65	7.33	7.02	7.46	0.98	4.63	932
Camphene	2.32	2.71	2.58	3.43	2.04	2.87	1.34	3.65	1.85	2.43	0.66	5.06	953
Sabinene	-	-	-	-	-	-	-	1.22	-	0.07	0.12	5.49	976
β-Pinene	6.81	8.12	9.34	9.16	8.02	8.83	8.06	6.64	7.65	7.33	1.09	5.613	986
β-Myrcene	0.87	1.25	1.39	-	1.17	0.69	1	0.75	1.14	1.14	0.35	5.695	991
α-Phellandrene	-	-	-	-	-	-	-	-	-	0.01	0.01	6.11	1005
α-Terpinene	0.7	0.67	0.57	-	0.54	0.5	-	1.19	-	0.37	0.30	6.49	1018
D-Limonene	1.77	1.78	1.78	1.75	1.66	1.59	4.26	1.5	1.61	2.12	0.73	6.49	1021
β-Terpinene	0.41	-	0.64	-	0.39	-	-	-	-	0.22	0.24	6.64	1027
β-Cymene	1.18	1.1	0.59	-	-	-	-	1.01	-	0.26	0.35	6.74	1028
δ-Terpinene	1.87	1.74	1.24	-	1.01	1.3	0.97	2.57	1.39	1.07	0.35	7.10	1066
Monoterpenes oxygenated	72.85	71.83	65.25	75.56	75.61	74.57	73.88	71.33	68.87	74.61	2.89		
(+)-2-Carene	0.32	0.52	0.61	-	0.73	0.42	-	0.63	-	0.31	0.30	6.27	1010
1,8-Cineol	46.71	46.51	46.1	52.89	51.39	50.21	61.98	49.61	53.25	50.75	6.07	6.81	1033
Linalool	0.44	0.83	0.57	-	0.69	-	-	-	-	0.48	0.53	7.99	1098
Camphor	20.57	19.28	12.38	17.04	17.23	20.64	8.59	16.28	15.62	16.16	4.52	9.62	1143
Borneol	-	-	-	-	-	-	-	-	-	1.36	1.69	9.66	1165
Pinocarvone	-	-	-	-	-	-	-	-	-	0.06	0.09	9.90	1185

α-Terpineol	4.3	3.99	4.49	4.09	4.58	3.3	3.31	4.03	-	4.39	1.01	10.00	1189
3-Pinanone	-	-	-	-	-	-	-	-	-	0.16	0.28	10.07	1190
Verbenone	-	-	-	-	0.38	-	-	-	-	0.20	0.29	10.91	1211
Bornyl acetate	0.51	0.7	1.1	1.54	0.61	-	-	0.78	-	0.70	0.42	11.18	1295
α-Terpineolacetate	-	-	-	-	-	-	-	-	-	0.01	0.01	12.1	1353
cis-Sabinene hydrate	-	-	-	-	-	-	-	-	-	0.01	0.02	8.23	1120
5-Isopropyl-2-methylbicyclo-[3.1.10]hexan-2-ol	-	-	-	-	-	-	-	-	-	0.02	0.04	7.56	1179
Sesquiterpenes	3.4	1.62	4.61	1.36	2.15	0.56	2.84	1.77	4.27	1.85	0.69		
Careophyllene	2.26	1.62	3.4	1.36	1.53	0.56	1.97	1.77	1.91	1.45	0.43	12.771	1418
δ-Cadinene	0.66	-	0.66	-	0.62	-	0.87	-	1.12	0.23	0.27	14.266	1528
Guaiene	-	-	-	-	-	-	-	-	1.24	0.03	0.07	16.21	1613
Caryophyllene oxide	0.48	-	0.55	-	-	-	-	-	-	0.13	0.18	15.70	1581
Others	0	0	0	0	0	0	0	0	4.2	0.66	1.15		
Spinacene (squalène = triterpenes)	-	-	-	-	-	-	-	-	-	0.17	0.34	20.39	1846
4.7.7-Trimethyl-bicyclo heptan-3-ol (ester)	-	-	-	-	-	-	-	-	-	0.21	0.40		
Diethyl phtalate	-	-	-	-	-	-	-	-	3.06	0.25	0.45	16.6	1640
Diisobutyl phtalate	-	-	-	-	-	-	-	-	1.14	0.03	0.06	19.4	1760
Total identified	100	100	96.32	100	100	100	100	100	98				

[a]RT = retention time, [b]IK = Kovats index

Different reports can be found in literature concerning rosemary essential oil yield and how it varies depending on the plant location and ecosystem conditions. Khia *et al.* [39] studied Moroccan *Rosmarinus officinalis* in the region of Taza, which yielded from 1.2 % in Aknoul (North of Taza) to 2.21 % in Rchida (South of Taza); however, they didn't attribute this difference to the ecological conditions. Also, in the region of Hammamat in Tunisia, *Rosmarinis officinalis* yielded from 1.6 to 2.29 % without registering a strong link between ecological conditions and the yield [40]. Angioni *et al*. [9] studied wild Sardinian *Rosmarinus officinalis,* from different locations, reported differences at the essential oil yield, however, they didn't relate these difference to climatic or edaphic condition. Concerning Spanish Rosemary, Varela *et al.* [41] found a yield ranging from 0.82 to 2.99. While Jordan *et al.* [30] reported that the essential oil yield varies from 1.74 to 2.58 %. Based on results, it is suggested that rosemary essential oil is influenced by the habitat in which the plants grow. By contrast, the influence of ecological factor that modifies this parameter remains not clear.

Composition of the essential oils: Concerning the essential oil volatile profile, chromatographic analysis through the total ion current (TIC) chromatograms of the individual samples allowed the identification of 34 major components, which represent between 96.32 and 100 % of the volatile components identified in rosemary essential oil (Table-2). However, quantitatively speaking, the chemical composition of essential oil submits to variations over the different locations studied (Fig. 2). The relative concentration of 1,8-cineol, camphor and α-pinene, the three components which determine the rosemary essential oil chemotype, range from 37.71 %, 6.09 %, 3.08-65.02 %, 27.49 %, 9.98 % respectively mentioning that the highest content on 1,8-cineol is registered in Zoubia (Debdou Forest). In addition, we point out that β-pinene (2.83-11.17 %) and camphene (0.84-3.93 %) are present, with less importance, in the majority of the harvested samples. The other components as β-myrcene, borneol, α-terpineol and bornyl acetate show low concentrations (excepted for Wizaght where we found 11.18 and 8.84 % of α-terpineol and borneol, respectively). These major components were also reported to be present in different regions around the world with different concentrations. Whereas α-pinene is the major component in *Rosmarinus officinalis* oil from Poland [11], camphor and 1,8-cineol in Spain [42], α-pinene and 1,8-cineol in Iran [43] and myrcene for the Brazilian Rosemary [23]. Concerning, the Moroccan *Rosmarinus officinalis*, our finding were close to the results of Ait-ouazzou *et al.* [44] and Ghadraoui *et al.* [45] who reported a content of 1,8-cineol of 43 and 42 %, respectively.

Nevertheless, the concentration of essential oil component didn't show statistically significant difference between the zones studied depending to the altitude or the vegetal association. However, there is a significant variation according to the forest where samples were harvested. Table-3 illustrates the mean value of the percentage of the two major compounds (1,8-cineol and camphor) contained in rosemary essential oil sorted by forest. Thus, we report that the mean value related to 1,8-cineol for the forest of Mkam are the highest (55.67 ± 3.56 %) followed by the forest of Debdou (51.76 ± 8.68 %). Right after, we find Ayate (50.20 ± 2.82 %) and Beni Yaala forest (50.96 ± 3.48 %). While the lowest content is in Nerguechoum forest (41.57 ± 3.00 %). But, we have to mention that the standard deviation remains relatively high especially in Debdou forest and this could be related to its much expanded area. Those results lead us to conclude that the essential oil composition is influenced by the habitat and the origin of the plant. Those results are in agreement with those published by Zaouali *et al.* [46] who affirmed that variations in the chemical composition of rosemary essential oil from Tunisia should be attributed to varieties and location rather than ecologic conditions.

TABLE-3
MEAN VALUE THE PERCENTAGE OF 1,8-CINEOL AND CAMPHOR CONTAINED IN ROSEMARY ESSENTIAL OIL, SORTED BY FOREST

Forest	Ayat	Nerguechoum	Mkam	Debdou	Beni Yaala
1,8-Cineol (%)	50.20 ± 2.82	41.57 ± 3.00	55.67 ± 3.56	51.76 ± 8.68	50.96 ± 3.48
Camphor (%)	13.04 ± 2.54	24.77 ± 2.77	13.59 ± 1.61	14.97 ± 5.02	16.40 ± 2.83

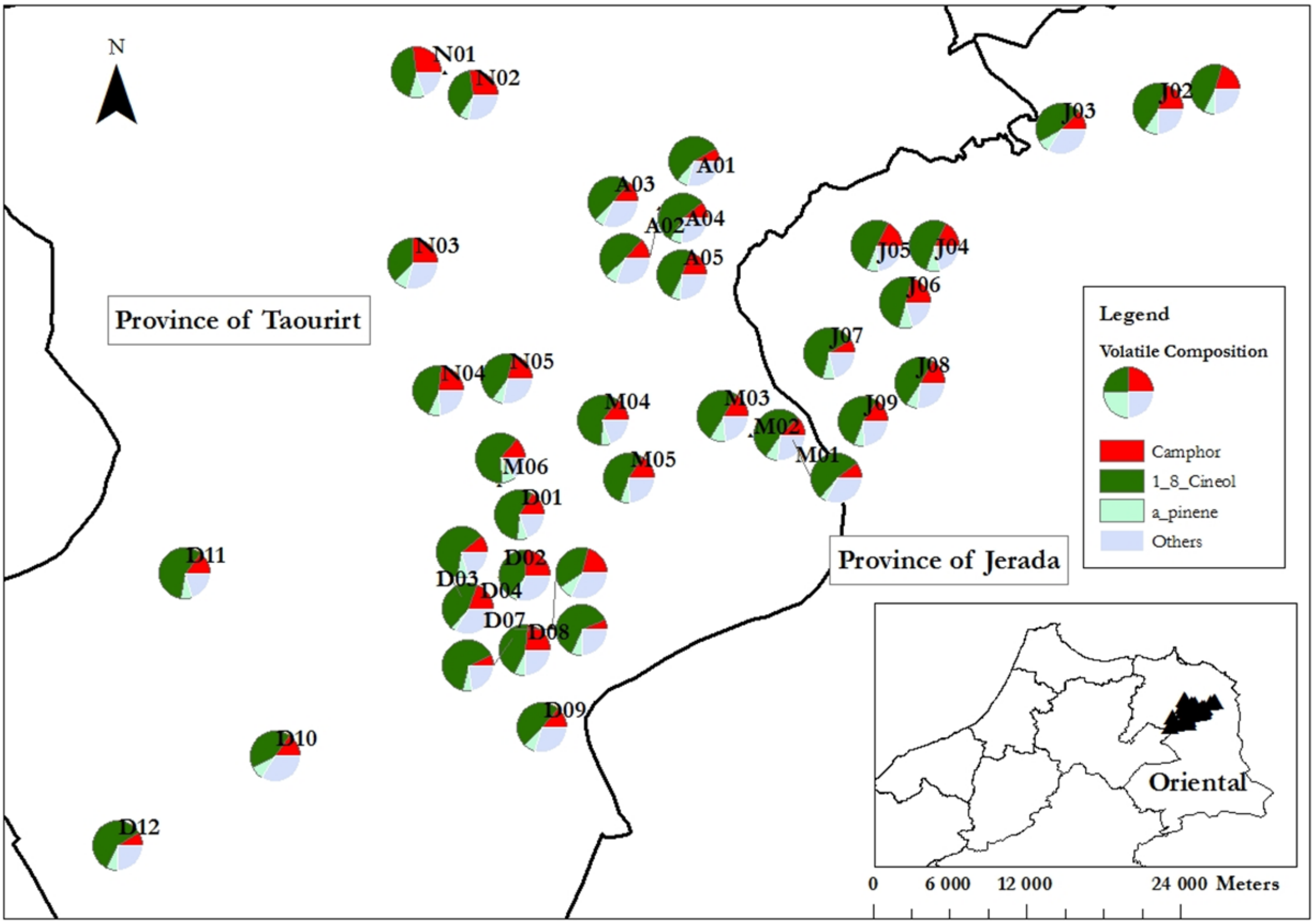

Fig. 2. Volatile variability of rosemary essential oil of the provinces of Jerada and Taourirt, Oriental region (Morocco)

Regarding to molecule groups as shown in Table-2, the monotepenes hydrocarbons range from 9.35 % in Wizghad (Debdou) to 30.45 % in Lalla Mimouna (Debdou) respectively with a mean value of 22.65 ± 2.58 %. They are represented mainly by α-pinene, camphene, β-pinene, myrcene. Mean while monoterpenes oxygenated, are represented by 1,8-cineol, camphor, α-terpineol and borneol and they constitute the main part of the essential oil with a proportion of 65.25 % (Khtitila) to 88.33 % (Wizaght) with a mean value of 74.61 ± 2.88. While the other groups such as sesquiterpenes represent 1.84 ± 0.69 % on average. Those results are in accordance with the previous finding [19,25].

The cluster analysis carried out on the essential oil composition is represented in the dendrogram reported in Fig. 3, which reflects the large compositional complexity of rosemary essential oils. The analysis of this graph allowed subdividing the samples in 2 main groups: Group I can be divided into 4 subgroups: Group A where there is a content of 1,8-cineol varying between 48.32 and 52.89 % and a camphor content varying between 13.14 and 20.64 % and this is mainly found in the samples harvested in Beni Yaala and Ayat forest. Group B where 1,8-cineol varies from 53.14 to 59.55 % and camphor from 8.79 to 16.39 % found in Mkam and Ayat forest. Group C which encompasses samples with (42.16-46.1 %) of 1,8-cineol and (12.38-15.02 %) of camphor. Group D is characterized by the highest content of 1,8-cineol (59.04-65.02 %) and the least of camphor (6.09-13.25 %) and it's found for instance in the Zoubia (center of Debdou Forest). Group II which contain 2 sub-groups: Group E with the lowest content of 1,8-cineol (37.71-43.63 %), while the camphor content varies from 24.74 to 27.49 %; this subgroup is represented by the northern samples of the forest of Nerguechoum. Group F where there is just one sample of Wizaght with 43.98 % of 1,8-cineol and 19.51 % of camphor. This sample is characterized by a relative high content of α-terpineol and borneol (respectively 11.18 and 8.84 %).

DPPH radical scavenging capacity: We studied the radical scavenging capacity (RSC) of 37 essential oils from rosemary leaves by the original DPPH test of Kumar *et al.* [36]. All the analyzed samples showed an increase in radical scavenging capacity, in agreement with the oil concentration (Table-4). The essential oil from Lalla Mimouna (Debdou forest) was the most active with (29.02 ± 1.04 μL/mL) and the less active was Wizaght (Debdou forest) sample with (43.95 ± 1.11 μL/mL). The one-way ANOVA showed a no significant variability among the samples according to the forest, altitude or the associated vegetal species. Compared with ascorbic acid (Table-4), which is a well-known potent antioxidant (IC_{50} = 22.65 ± 0.61 μL/mL with r^2 = 0.97), the essential oils were less active. Nevertheless, it still could be considered as a strong antioxidant. Present findings are in accordance with other publications [25,47,48].

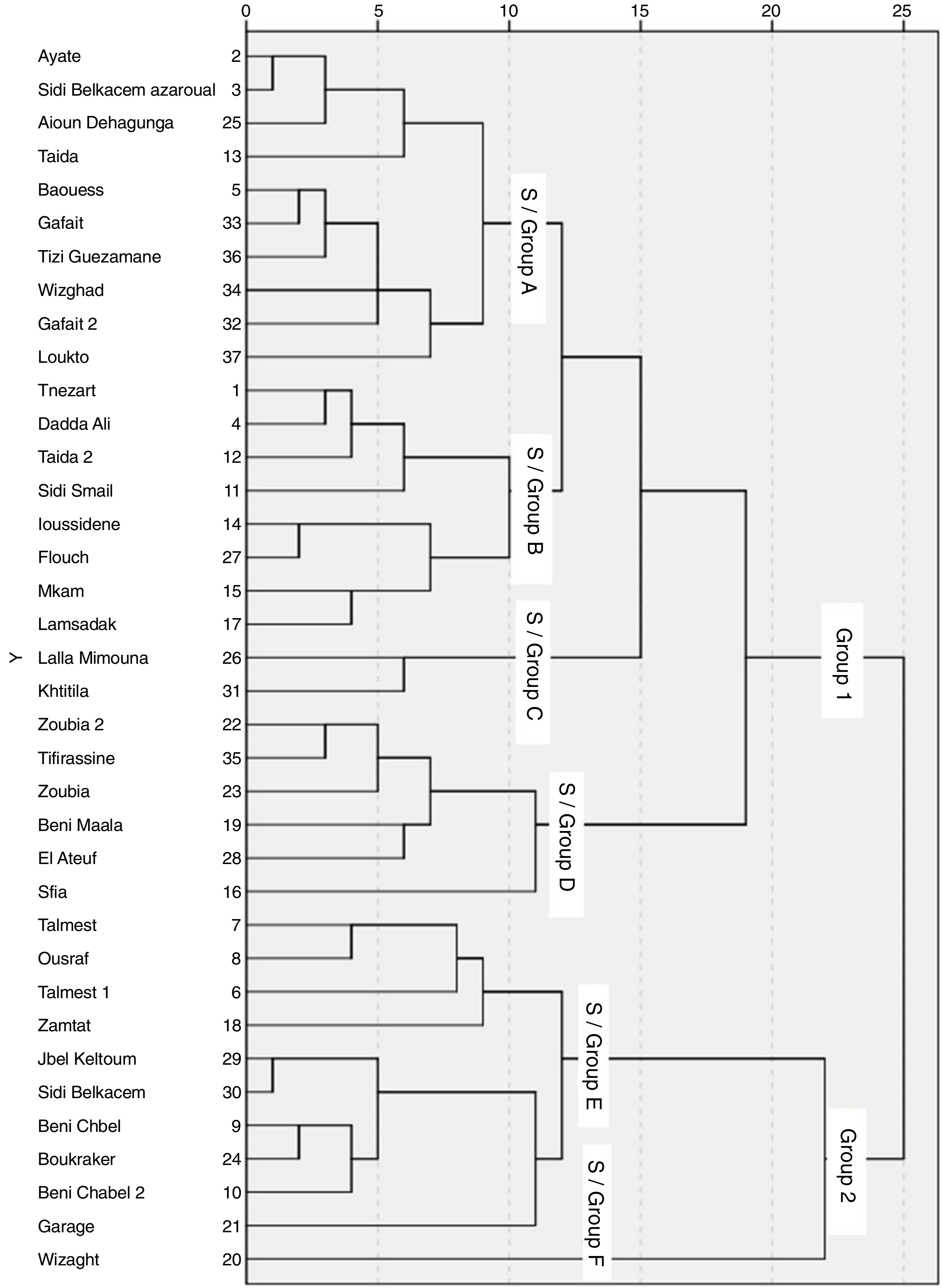

Fig. 3. Cluster dendrogram using the main 20 constituents (and with an average content more than 0.15 %) of the 37 populations of rosemary of Oriental Region (Taourirt and Jerada provinces)

TABLE-4
FREE RADICAL-SCAVENGING CAPACITY OF ROSEMARY ESSENTIAL OIL OF THE ORIENTAL REGION IN MOROCCO LAND THE TOTAL OF MONOTERPENES HYDROCARBONS (MH) AND MONOTERPENES OXIGINATED (MO)

Sample	Location name	Concentration of essential oil (µL/mL)				R^{2a}	DPPH IC_{50}^{b} (µL/mL)	Total MH	Total MO
		20	40	60	80				
A01	Tnezart	40.16	57.83	63.65	74.20	0.95	33.52	23.68	73.76
A02	Ayate	43.85	52.69	65.26	74.65	0.96	32.69	22.94	74.66
A03	Sidi Belkacemazaroual	45.83	51.45	62.77	76.03	0.97	32.33	20.64	75.14
A04	Dadda Ali	42.16	58.34	62.11	73.05	0.94	31.53	24.38	73.87
A05	Baouess	46.14	51.03	63.16	75.66	0.96	32.16	22.46	75.05
N01	Talmest1	42.90	55.26	61.15	73.22	0.98	33.22	23.39	75.58
N02	Talmest	42.69	52.09	62.78	78.63	0.98	34.76	22.62	69.72
N03	Ousraf	42.68	56.89	64.98	71.36	0.96	30.97	27.49	70.35
N04	Beni Chbel	43.97	53.09	65.16	74.25	0.99	32.31	21.11	76.59
N05	Beni Chbel 2	43.20	56.20	64.2	72.90	0.98	31.23	26.01	72.76
M01	Sidi Smail	44.96	51.68	63.98	71.98	0.98	32.59	21.02	76.77
M02	Taida 2	41.26	53.69	66.12	76.50	0.99	34.15	24.95	73.53
M03	Taida	42.12	57.30	64.05	72.29	0.96	31.64	24.19	74.85
M04	Ioussidene	40.05	55.18	67.12	75.62	0.98	34.01	16.42	82.1
M05	Mkam	44.50	53.26	64.80	70.15	0.98	31.56	20.32	77.52
M06	Sfia	42.58	54.78	66.67	72.25	0.97	32.06	22.29	77.03
D01	Lamsadak	40.15	57.22	64.10	72.22	0.95	33.68	19.44	79.72
D02	Zamtat	38.15	55.14	64.20	73.25	0.97	36.63	14.75	71.87
D03	Beni Mâala	43.15	54.69	66.89	74.58	0.99	31.57	22.45	76.55
D04	Wizaght	34.20	52.20	60.11	69.15	0.96	43.45	9.35	88.33
D05	Garage	44.20	57.12	63.11	73.80	0.98	29.83	27.52	70.67
D06	Zoubia 2	42.56	54.88	66.12	75.65	0.99	32.3	23.07	74.88
D07	Zoubia	41.78	55.25	66.12	73.25	0.98	32.73	20.86	76.97
D08	Boukraker	41.25	57.48	66.78	73.98	0.96	31.65	21.84	76.49
D09	Aioun Dehaguna	41.62	58.20	66.34	76.02	0.97	31.09	25.27	72.63
D10	Lalla Mimouna	42.69	59.66	66.26	75.75	0.96	29.09	30.45	67.25
D11	Flouch	40.69	56.56	64.23	72.12	0.96	33.57	20.36	78.86
D12	El Ateuf	42.87	54.65	64.10	73.45	0.99	32.66	21.18	77.43
J01	Jbel Keltoum	41.12	56.89	64.13	76.12	0.98	32.97	23.75	72.85
J02	Sidi Belkacem	42.56	58.21	64.89	75.69	0.97	30.52	26.55	71.83
J03	Khtitila	44.15	57.20	62.20	75.32	0.97	30.3	26.46	65.25
J04	Gafait 2	40.14	58.64	67.05	74.22	0.94	31.91	23.08	75.56
J05	Gafait	43.20	54.76	63.95	70.17	0.98	32.24	22.24	75.61
J06	Wizghad	42.88	55.43	65.67	72.19	0.98	31.63	24.87	74.57
J07	Tifirassine	41.98	55.06	68.35	76.14	0.98	32.11	23.28	73.88
J08	Tizi Guezmane	42.62	58.76	63.26	76.18	0.96	30.64	26.9	71.33
J09	Loukto	40.12	57.11	62.72	72.20	0.95	34.24	20.66	68.87
Acid ascorbic[c]	–	46.61	63.34	71.16	82.77	0.97	22.65	–	–

[a]R^2, the regression coefficient used to measure the linear dependence between the essential oil concentration and the percentage of radical scavenging capacity; [b]Essential oil concentration required to scavenge 50 % of DPPH solution; [c]Acid ascorbic used as a reference antioxidant.

It is noticed in present study that this activity is negatively in line with the content of monoterpenes hydrocarbons (R^2 = 74 %). Many studies pointed out that the capacity of an antioxidant component to scavenge DPPH mainly relate to its hydrogen donating ability, which is directly depends to the presence of the abundance of monoterpenes hydrocarbons which are rich of functional groups [25].

Conclusion

The present research shown that *Rosmarinus officinalis* of the Oriental is characterized by 1,8-cineol as a major component for all the locations studied. We also reported that the camphor is present with relatively high concentrations. Nevertheless, we highlight the considerable volatile variability, concerning the content of those major compounds, among the population, which can be explained mainly by the genetic and the geographic factor rather than the altitude or the associated vegetation (which defines the ecosystem). Besides, the antioxidant activity of their essential oils is deemed very important which could be used as potent antioxidant in food and pharmaceutical industry.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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