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Chemical Variability of *Rosmarinus officinalis* Essential Oil According to the Geographical Origin

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ABSTRACT

Rosemary (Rosmarinus officinalis L.) is a plant widely used in Morocco for these remedies in traditional medicine and for these essential oils. The objective of this work is to compare the yield and chemical composition of wild and cultivated rosemary essential oils from different regions of Morocco that differ in their geographical positions and climatic conditions. Essential oils of R. officinalis obtained by hydrodistillation were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The yield of wild rosemary essential oils according to the regions studied; Boulmane, Berkane, Bouiblane and Errachidia vary between 1.57% and 2.90% and for rosemary grown in the region of Fez; it is 2.42%. The GC-MS analysis identified 41 compounds whose the major were 1,8-Cineole (26.2% to 48.2%), Camphor (7.8% to 10.4%), Borneol (6.4% to 9.4%) and α-pinene (4.4% to 9.8%). The chemical composition of the essential oil of various spontaneous and cultivated rosemary samples is qualitatively similar, but there are quantitative differences between some compounds because of several intrinsic and extrinsic factors.

Keywords: *Rosmarinus officinalis*, Essential oils, CPG-SM analysis, Yield, Chemical composition.

INTRODUCTION

Rosmarinus officinalis L is a medicinal and aromatic plant native to the Mediterranean basin. It is cultivated all over the world for medicinal, pharmaceutical, cosmetic and food purposes. Rosemary contains several active components among which; flavonoids [1], diterpenes such as carnosolic acid [2], tannins [3], rosmarinic acid [4,5]. These compounds are responsible for a wide variety of activities; including anti-cancer activity [2], antioxidants for food preservation [6], anti-inflammatory and analgesic effect [4-7].

Rosemary is also a source of an essential oil whose majority compounds are generally 1.8 cineole, camphor, camphene and α-pinene [8-10]. Several studies have focused on the biological activities of rosemary essential oil (REO). Thus, the REO has a great antioxidant effect [11], anticancer effect [12] and antileishmanial activity [13], in addition to the antimicrobial effects including antibacterial and antifungal activity [10,14,15]. Other studies have shown ovicidal and repellent effect of ROE [16] and also larvicidal properties [17].

In Morocco, *R. officinalis L* is intensively exploited in the form of dried leaves and essential oils. In 2014, Morocco exported nearly 8,000 tonnes of dry matter rosemary more to the production of essential oils [18]. However, the majority of the essential oils of Moroccan rosemary are intensively extracted from spontaneous plants [19-21].

This work focuses on the qualitative and quantitative analysis of the essential oils of wild rosemary from several regions of Morocco in comparison with the rosemary cultivated in the region of Fez in the aim to determine the influence of the origin and the climate of the growth zone rosemary on the yield and the chemical composition of the essential oils.

The purpose of this analysis is to encourage the production of essential oils from cultivated rosemary under suitable conditions in order to increase the quantitative yield of essential oils and to contribute to the conservation of the species for sustainable development in the regions threatened.

MATERIALS AND METHODS

Regions of study and plant materials

For cultivated rosemary, the study area is an open space Fez which is part of northern Morocco Saïss basin characterized by the semi-arid

bioclimatic stage with temperate winter.
For wild rosemary, the samples were collected from different Moroccan regions. Geographic and climatic characteristics of each region are:
1. Tafoughalt (Berkane) (34° 48′ 20″ N, 2° 24′ 41″ W); this region belongs to the Rif mountain range and it has as climax Ras Foughal (1532 m). The region is dominated by a semi-arid Mediterranean climate except on the massif of Beni Snassen where a subhumid climate with a cold and wet winter is altered.
2. Taferdouste - Skoura M'daz (Boulemane) (33°28'25.8"N 4°39'05.8"W); it is part of the mountainous area of the middle atlas which is located in the center north of Morocco. This region is a part of the high hills of Tafrdouste with altitudes ranging from 700 m to 1400 m; it belongs to the semi-arid to sub-humid bioclimatic stage.
3. Talzemt (Bouiblane); (33°36'51.3"N 4°11'29.7"W); it belongs to the subhumid meso-mediterranean bioclimatic stage with an average temperature of 3 to 20°C and an average rainfall of 450 to 800 mm/year.
4. Ait Ben Akki (Errachidia) (32°05'15.5"N 4°43'58.1"W); it is located in the High Atlas with an altitude of 1515 m. The bioclimatic stage is arid to semi-arid with a definite moderation between summer and winter.

Figure 1 shows the geographical location of harvest areas of wild and cultivated rosemary.

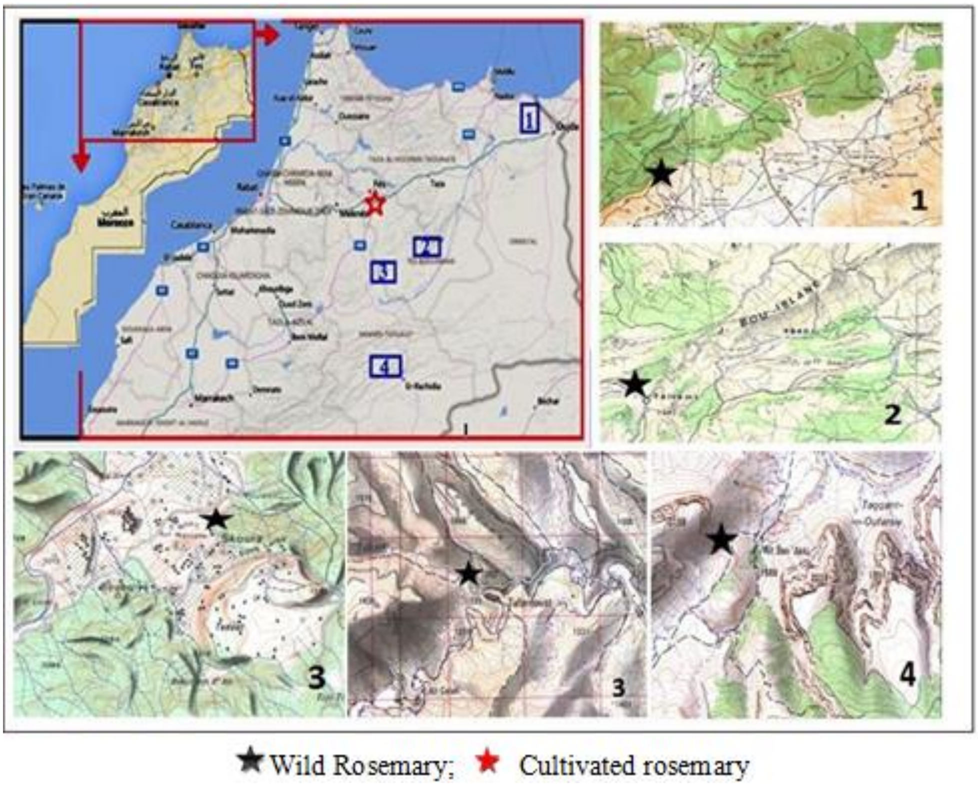

Figure 1: Location of sampling areas of wild rosemary and cultivated rosemary

1- Topographical extract from the region of Berkane - Tafoughalt in the 1/50000.
2- Topographical extract from the region of Boulemane - Talzemt in the 1/100000.
3- Topographical extract from the region of Boulmane - Skoura and Taferdouste in the 1/50000.
4- Topographical extract from the region of Errachidia - Aït Ben Akki) in the 1/50000.

Sampling and extraction of essential oils

Leaves, flowers and flowering tops of cultivated or wild rosemary were freshly harvested and dried in the shade at room temperature (25°C) for 3 days. The drying time of the plant has been studied previously in order to get maximum yield in essential oils.

Essential oils extraction was carried out by hydrodistillation in a Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia [22].

All parameters such as quantity of plant, water volume and also time of distillation have been previously optimized. The extraction was performed for 2 h by boiling the samples of 100 g of rosemary in 500 ml of distilled water at a constant temperature (100°C) in a heating mantle. The distillation was carried out in a system consisting of a flask of tow liter surmounted by a column of 60 cm length connected to a condenser. After training with water vapor, a mixture of essential oil with the water was condensed and then recovered, after cooling, the water and the essential oil are separated by difference in density. The essential oil was stored at 4°C in the dark before their use. The yield of essential oil was determined relative to the dry matter.

Physico-chemical characteristics

Two parameters were determined; the refractive index and the density. The refractive index was measured using an Erma 1537 type refractometer at a temperature of 25°C. The density was determined using a Sartorius TP scale.

Gas chromatography-mass spectrometry analysis

The essential oils of *R. officinalis* samples were analyzed using gas chromatography coupled to mass spectrometry (GC-MS). The essential oil was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness of 0.25 µm; Agilent-Technologies, Little Falls, CA, USA). The carrier gas was helium with a flow rate of the 1.0 ml/min. The essential oils samples were diluted 1: 100 in n-hexane, and 0.1 µl were injected into the GC systems. The injector and detector temperatures were set at 250°C and 280°C, respectively. The column temperature was programmed from 35°C to 250°C at a rate of 5°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. All quantifications were carried out using a built-in data-handling programme provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The constituents of the volatile oils were also identified by comparing their GC retention indices. A mixture of aliphatic hydrocarbons (C8-C24) in hexane (Sigma-Aldrich, St. Louis, USA) was injected as under the above-mentioned temperature programme to calculate the retention indices using the generalized equation of Van den Dool and Kratz [23].

RESULTS AND DISCUSSION

Yield and physico-chemical properties of essential oils rosemary

The yields of essential oils from rosemary samples are reported in Table 1.

Table 1: Yields and physicochemical properties of *Rosmarinus officinalis* essential oils of different Moroccan origins

Origin	Yield% (w/w)	Refractive index at 25°C	Density at 25°C
RCS	2.42	1.471	0.911
RBS	2.52	1.473	0.912
RTz	2.9	1.475	0.913
RTf	2.33	1.469	0.987
RSM	2.24	1.464	0.986
RABA	1.57	1.478	0.915

(RCS: Rosemary Cultivated of Saiss; RBS: Rosemary of Beni Snassen; RTz: Rosemary of Talzemt; RTf: Rosemary of Tafrdoust; RSM: Rosemary of Skoura M'daz; RABA: Rosemary of Ait Ben Akki)

The results obtained show that the average yields of rosemary essential oils vary according to the geographical location of the plant. Thus, the better yield of essential oils obtained is 2.90% from Bouiblane-Talzemt region (RTz) followed by 2.52% and 2.42 % from Berkane-Beni Snassen (RBS) and Rosemary cultivated of Fez-Saïss (RCS) respectively, then Tafrdoust (RTf) and Skoura M'daz (RSM) have yields of 2.33% and 2.24% respectively, and the lowest yield is 1.57% from Errachidia-RABA region.

If we compare these results with other work carried out in Morocco; the yield of essential oil of Rosemary of Errachidia found by Khia *et al* [24] is 2.21%; which is a much higher yield in comparison with the yield found by our study in the same region with 1.57%. The study performed by Megzari *et al* [10] found that the yield of rosemary EO from the region of Sefrou is equal to 2.4%, a value similar to that obtained in Skoura M'daz (RSM) and Tafrdoust (RTf) in our study. Sefrou is a region away from Skoura M'daz (RSM) and Tafrdoust (RTf) by 62.7 km and 91.6 respectively, but with a higher altitude than Sefrou. However, Derwich et al. [25] found that the productivity of Rosemary Taferdoust (Skoura) is only 0.5%. The yield of EO rosemary varies according to geographical origin. The rosemary harvesting areas are very diverse in terms of climate, with annual precipitation varying with altitude. In fact, the best EO yield is found in Bouiblane-Talzemt region.

In the other hand, the density and refractive index of all samples of the rosemary essential oils are approximately similar. Density values are between 1.478 and 1.473 and refractive index values are between 0.911 and 0.987, these parameters are in agreement with those authenticated by ONIPPAM [26].

The determination of the chemical properties is a necessary step but insufficient to characterize the quality of essential oils. It is therefore necessary to complete it by chromatographic analyzes. The coupling of gas chromatography to mass spectrometry (GC-MS) is an analytical method to identify different constituents of rosemary after their separation.

Chemical composition of the essential oils

The results obtained by GC-MS analyses of the essential oils of *R. officinalis* from different regions of Morocco are presented in Table 2.

Table 2: Chemical composition of the essential oils of wild and cultivated *Rosmarinus officinalis* of different Moroccan origins

No	Compound	RCS	RBS	RTz	RTf	RSM	RABA
1	Tricyclene	≤ 0.1	≤ 0.1	---	≤ 0.1	≤ 0.1	0.2
2	Camphene	3.2	4.1	2.8	3.4	3.2	4.3
3	α-Pinene	8,5	9.1	4.4	6.4	6.1	9.8
4	β-Pinene	1.6	≤ 0.1	0.6	0.8	0.8	2.2
5	Thuja-2,4(10)-diène	≤ 0.1	---	---	---	≤ 0.1	0.2
6	δ-3-carene	0.8	0.4	0.3	0.3	0.4	0.9
7	Myrcene	2.2	1.5	0.6	0.8	0.8	2.2
8	O-cymene	≤ 0.1	---	---	---	---	≤ 0.1
9	α-terpinene	0.2	≤ 0.1	---	---	---	0.2
10	Dehydro-1,8-cineole	≤ 0.1	≤ 0.1	---	---	---	0.2

11	Limonene	1.8	0.4	0.2	0.8	0.8	2.2
12	1,8-cineole	44.8	34.8	26.2	24.8	30.6	48.2
13	γ-terpinene	≤ 0.1	---	---	0.2	0.2	0.2
14	P-cymene	1.2	0.8	0.2	0.2	0.2	1.4
15	Terpinolene	≤ 0.1	≤ 0.1	≤ 0.1	0.2	0.2	0.4
16	Trans-Linalooloxide (Furanoid)	---	0.2	0.2	0.2	0.2	0.8
17	1-octen-3-ol	0.2	0.4	0.8	0.8	0.8	0.2
18	Cis-Linalooloxide (Furanoid)	0.4	≤ 0.1	≤ 0.1	---	---	0.8
19	Camphor	8.4	8.6	7.8	7.8	7.8	10.4
20	Linalool	4.2	3.8	4.2	4.2	4.6	4.4
21	Terpinen-4-ol	0.6	0.6	0.4	0.4	0.4	0.8
22	β-caryophyllene	0.1	0.2	0.2	0.3	0.3	0.4
23	Myrtenal	≤ 0.1	≤ 0.1	---	---	---	≤ 0.1
24	Phenylacetaldehyde	≤ 0.1	≤ 0.1	1.4	1.4	1.6	0.2
25	Trans-Pinocarveol	≤ 0.1	≤ 0.1	≤ 0.1	---	---	≤ 0.1
26	δ-terpineol	0.4	0.3	0.2	0.2	0.2	0.1
27	Trans-verbenol	0.2	0.2	0.1	0.1	0.1	0.1
28	α-humulene	≤ 0.1	≤ 0.1	≤ 0.1	---	---	0.2
29	P-Mentha-1,8-dien-4-ol	---	---	---	≤ 0.1	≤ 0.1	0.2
30	α-terpineol	1.7	1.6	1.6	1.6	1.6	1.8
31	Borneol	6.4	8.4	8.6	8.4	8	9.4
32	Verbenone	1.2	1.3	1.4	1	1.2	2.1
33	P-Mentha-1,5-dien-8-ol	1	≤ 0.1	4.6	2.1	2.1	1.6
34	Cis-calamenene	≤ 0.1	≤ 0.1	---	---	---	0.4
35	m-cymen-8-ol	---	---	1	1	1	1
36	p-cymen-8-ol	1.2	1.3	1.2	1.2	1.4	1.2
37	α-calacorene	≤ 0.1	≤ 0.1	1.2	1.2	1.2	1.4
38	Piperitenone	1.2	1.4	0.8	0.8	0.9	1.4
39	caryophyllene oxide	4.4	3.4	2.4	5.4	4.6	6.8
40	Methyl eugenol	0.3	0.2	0.2	0.1	0.1	0.4
41	Eugenol	≤ 0.1	≤ 0.1	---	---	---	0.1
42	Clovenol	0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	0.2
	Total	≈ 95%	≈ 85%	≈ 74%	≈ 78%	≈ 69 %	≈ 98 %

RCS: Rosemary cultivated of Saiss; RBS: Rosemary of Beni Snassen; RTz: Rosemary of Talzemt; RTf: Rosemary of Tafrdoust; RSM: Rosemary of Skoura M'daz; RABA: Rosemary of Ait Ben Akki;
(---): Not detected

GC-MS analysis indicates the identification of divers chemical compounds in the essential oils of *R. officinalis*. They are divided in 33 compounds with a total of 69% for Rosemary from Skoura M'daz, 32 compounds with a total of 74% and 78% for Rosemary from Talzemt and Tafrdoust respectively, 37 compounds with a total of 85% for Rosemary from Beni Snassen, 39 compounds with a total of 95% for Saïss Rosemary and 42 compounds with a total of 98% for Rosemary from Ait Ben Akki.

This difference between the chemical compounds only affects the minority compounds; however the major compounds are roughly similar for all the samples. We notice that the 1.8-Cineole is the major compound in all samples of essential oils, its concentration varies from 26.2% to 48.2%. Camphor (7.8% to 10.4%), Borneol (6.4% to 9.4%) and α-pinene (4.4% to 9.8%) are ranked second followed by caryophylleneoxide (2.4% to 6.8%), Linalool (3.8% to 4.6%), Camphene (3.2% to 4.3%), P-Mentha-1,5-dien-8-ol (0.1% to 4.6%), Myrcene (0.6% to 2.2%), Limonene (0.2% to 2.2%), Verbenone (1.0% à 2.1%), α-Terpineol (1.6% to 1.8%) and β-Pinene (0.1% to 2.2%).

The interpretation of these results shows that the essential oil of Moroccan rosemary is rich in 1.8-Cineol whatever its origin with some discord between the regions. Similarly, the essential oil of the cultivated rosemary (RCS) gave a good yield of 1.8-Cineole (44.8%) and which is classified better than that of the wild rosemary of all the other regions except RABA.

The same can be said for the other compounds that are Camphor (4.8%), Borneol (6.4%) and α-Pinene (8.5%), Caryophylleneoxide (4.4%), Linalool (3.8% to 4.6%), Camphene (3.2% to 4.3%), P-Mentha-1,5-dien-8-ol (0.1% to 4.6%), Myrcene (0.6% to 2.2%), Limonene (0.2% to 2.2%), Verbenone (1.0% to 2.1%), α-Terpineol (1.6% to 1.8%) and β-Pinene (0.1% to 2.2%).

In addition, the chemical composition of the essential oils of cultivated rosemary (Fez-Boulmane region) is very rich and similar to that of the essential oils of Errachidia rosemary (Ait Ben Akki).

If we compare our study to other regions of Morocco; the study carried out by Kamli et al. [27] shows that the major components of the EO of Skoura M'daz is 1.8-cineole (51.77%) followed by camphor (22.31%) and α-pinene (9.84%). Rosemary's EO of Sefrou gave the same quantity of Camphor (22.1%) and lower amount of 1.8- Cineole (18.35%) followed by α-Pinene (12.19%) and Camphene (6.81%) [10].

However, the major compound in the essential oil of *R. officinalis* obtained from Taza is Dimenthol (38.83%) followed by Campholene aldehyde (16.02%), pinene (11.05%) and Borneol (10%) [28].

There is a variation of chemical compounds of REO from one region to another and also in the same region but at different times. These results are consistent with international research work. The chemical composition of the essential oils extracted in this study was compared with those mainly from other Mediterranean regions and the world; Alger, Corsica, India, Iran, Sardinia, Portugal, Tunisia, Turkey and Uruguay (Table 3).

Table 3: Major components of the essential oils of rosemary in the world

Origin	Major components of the essential oils of rosemary in the world
Alger	- α-pinene (23.1%), camphor (15.3%), β-pinene (12.2%) [29]
Brazil	- 1.8 cineole (52.2%), camphor (15.2%) and a-pinene (12.4%) [7]
France (Corsica)	- verbenone (22.3%), α-pinene (17.4%), 1.8-cineole (6.4%) [30]
India	- camphor (26.4%), 1,8-cineole (23.40%), α-pinene (9.94%) [31]
	- camphor (22.00 %), α-pinene (16.33%), 1.8-cineole (14.32%), camphene (9.28%) [32]
Iran	- α-pinene (43.9%), 1.8-cineole (11.1%), camphene (8.6%), myrcene (3.9%) [9]
Italy (Sardinia)	- verbenone (20.3%), α-pinene (13.7%) ; 1.8-cineole (3,4%) [30]
Portugal	- verbenone (35.4%) ; camphor (5.5%) ; 1.8-cineole (3.1%) [33]
Tunisia	- 1.8-cineole (35.32%), trans-caryophyllene (14.47%), borneol (9.37%) camphor (8.97%) [34]
	- 1.8-cineole (34.82%), Camphor (12.91%) and α-pinene (11.87%) [35]
Turkey	- 1.8-cineole (60.9%), α-pinene (7.8%) and camphor (7.1%) [36]
	- para-cymene (44 %), linalol (21%), gamma-terpinene (17%) [37]
Uruguay	- α-pinene (40.55 to 45.10%), 1.8-cineole (17.40 to 19.35%), camphene (4.73 to 6.06%) [38]

Table 3 shows a significant variation in the chemical composition of its essential oils. The main component of the Tunisian, Turkish and Brazil oils is 1.8-cineole with over 35%. Whereas, Alger Iran, Uruguay REOs have α-pinene the major component. The major constituent of the REO from France (Corsica), Italy (Sardinia) Portugal is verbenone.

In general, the majority compounds of rosemary are 1.8-cineole, α-pinene, verbenone, camphor, camphene but with percentages that differ from one region to another.

The variability detected in the chemical composition and yield of *R. officinalis* essential oil in the world is related to several intrinsic and extrinsic parameters. The main intrinsic factors are; genetics, subspecies and age of planting [39,40].

The extrinsic factors are represented by geographical origin, climate effects, time of harvest, growing conditions [30,33,36,41] and also by the extraction method and the storage and transport processes that also influence the performance and composition of essential oils [41,42].

Also, other works indicate that the yield and chemical composition of REO is highly influenced by soil water content, harvest time and type of soil [43,44]. Gharib et al. [44] suggests that the EO yield of rosemary plant were consistent with corresponding changes in photosynthetic pigments.

Khorshidi et al. [41] collected and studied 87 spontaneous populations of *R. officinalis* in Spain. The results showed that; among all the samples, thirty-eight have a high content of 1,8 cineole (>24%) and an essential oil yield of greater than 2%, six populations have a high 1,8 Cineole/linalool ratio, three samples have high linalool content and only two samples have high levels of verbenone (≈ 5%). This comparison confirms that the chemical composition of essential oils is also variable within the same region.

The study and analysis of essential oils of Rosemary from different Moroccan regions show that the yields and the chemical composition as well as the major compounds are different and variable according to the regions of the harvest.

CONCLUSION

To conclude, the chemical composition of the essential oil of the cultivated rosemary is very rich in major components and it can compete in quantity as well as in quality and consequently in price the essential oils of the wild rosemary. This wealth can be improved by taking into account the extrinsic conditions that seem to influence the quantity and quality of REO. This comparative study is a good approach from an economic point of view; it makes it possible to better target the desired rosemary according to the region from which it comes in order to extract the major component sought in the REO according to its percentage. Our work can serve as a basis for developing the regional economy to generate financial income for rural and urban populations encouraging them to cultivate rosemary.

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