Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(12):9-22
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Provenance effect on the yield, chemical composition and antibacterial activity of Moroccan rosemary essential oils

Afaf Megzari[1,2*], Abdellah Farah[2], Mohammed Iraqui Houssaini[3] and El Mestafa EL Hadrami[1]

[1]Laboratory of Applied Organic Chemistry, Faculty of Science and Technology, Sidi Mohammed Ben Abdellah University, Fez, Morocco
[2]NationalAgency of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdallah University, Taounate principal, Morocco
[3]Laboratory of Microbial Biotechnology, Faculty of Sciences and Technology, Sidi Mohammed Ben Abdellah University, Fez, Morocco

---

ABSTRACT

To contribute to the valorization of Moroccan Rosemary, we focused on one hand on the study and comparison of the yields and chemical compositions of the essential oils of rosemary in four provenances (Wad Laou, Ayoun Charkiya, Sefrou and Agadir's Gardens Jacky).On the other hand, we evaluated their antimicrobial activities. Essential oils obtained by hydro-distillation of rosemary were analyzed by gas chromatography coupled to mass spectrometry. The average yields obtained and chemical composition vary according to the four provenances. The descriptive and comparative statistical study of chemical composition of Rosemary from different regions by the principal component analysis method showed a good classification of Rosemary essential oils from different regions according to the chemical composition. The study of antibacterial activity of essential oils of Rosemary in four regions showed an inhibitory power over all the strains studied. This activity differs statistically from one region to another. Indeed, the highest antibacterial activity was observed for the Rosemary from Sefrou region and the lowest activity was observed for Rosemary from Agadir region and this for three strains used.

Keywords: *Rosmarinus Officinalis, Prostratus,* Essential oil, Chemical composition, Antibacterial Activity, Provenance, ACP

INTRODUCTION

Historically**,** Man has used his environment and in particular plants for healing. Indeed, the plants contain a wide variety of chemical molecules of physico-chemical properties which aremuch diversified and have various biological activities (antitumor, antiviral, antimicrobial, antioxidant, healing ...) [1].

Situated in the Mediterranean basin with large climatic variations from north to south, Morocco has a favorable ground for the development of a rich and varied flora including significant potential often endemic MAP. Morocco occupies the first place among the countries of the Southern Mediterranean for its wealth of endemic plants [2]. In addition to this promising natural context, Morocco is one of the Mediterranean countries that have a long medical tradition and traditional expertise in herbal medicine[3, 4]. This ancestral medicinal knowledge would guide and develop several research works, especially in herbal medicine [5-8]. Moreover, faced with the growing emergence of the global phenomenon of bacterial resistance to antibiotics, the development of new antibacterial molecules is essential to fight infections caused by resistant bacterial strains.

To contribute to the promotion of medicinal and aromatic plants in Morocco and the Moroccan Rosemary precisely, we are interested firstly in studying the chemical composition of essential oils of *Rosemary*fromfour sources; It is about *RosmarinusOfficinalisvar. Prostratus* from the northern region ofMorocco (wadiLaou),*RosmarinusOfficinalis* from the East (AyouneCharkiya), *RosmarinusOfficinalis* from Sefrou and *RosmarinusOfficinalis* from Agadir(Jacky gardens). On the other hand, for the recovery of these essential oils, we have evaluated their antimicrobial activities. Generally, *Rosmarinus* (Rosemary) is one of the natural plants of Morocco, which is known for its medicinal and aromatic properties since ancient times. The essential oils of this plant in fact have been the subject of recent research in the pharmaceutical and food processing areas and possess excellent antimicrobial properties [9-11], anti-inflammatory [12-13], antispasmodic [14], and antioxidant [9,15, 16]. However few studies exist on essential oils chosen for this study except that no study has been done on the *RosmarinusOfficinalis var. Prostratus* is a rare variety that grows in Morocco in the north, in the region of wadiLaou.

Indeed, Morocco operates a potential of 1 million hectares of Rosemary producing an annual yield of 60 tons of essential oil for export. Improving the quality of essential oils exported allows Morocco to win the confidence of applicants and increasesthe standard of living of the local people and the national economy [17].

Our work identifies several components, namely:

- A qualitative study including evaluation of antibacterial essential oil of Rosemary of the four regions, a quantitative study thatpermits the MIC determination of these oils.

- An analysis and identification of constituents of essential oils from each region by the technique of gas chromatography coupled to mass spectrometry.

- A descriptive and comparative statistical study of the chemical composition of the Rosemary of different regions by the multidimensional data analysis method: Principal Component Analysis (PCA).

MATERIALS AND METHODS

1. Plant material

Samples of the aerial part (stems, leaves and flowers) of *RosmarinusOfficinalis var. prostratus* were collected in March 2012, when the plants were in full bloom.

These samples come from Sefrou, the southern region (Agadir's jacky gardens), Oriental (Ayoun Charkiya) and North (Wadi Laou) of Morocco. The latter species was identified by Prof. Abdelah Farah, of Nationel Agency of Medicinal and Aromatic Plants. It is characterized by a low size, creeping form, linear leavesmeasuring up to 5 cm in length, the top is dark green color and, and the lower is white color, and fluffy. Small tubular flowers 1 cm in length, spread on the branches, color clear blue with white.

2. Bacterial Strains

The essential oils antibacterial activity of different individuals was evaluated at all three strains:

- *Mycobacterium Smegmatis*(MC2 155): Nonpathogenic atypical mycobacterium, it presents a susceptibility to antituberculous agents similar to that of M. tuberculosis. [18]

- *Escherichia coli* (ATCC25922): Pathogen Gram-negative bacteria known for its strong antibiotic resistance and its toxic and invasive power in human beings. It causes intestinal diseases which vary in severity from benign to serious or even life threatening forms.

- *Bacillus Subtilis* (ATCC 23857): Non-pathogenic Gram-positive bacterium for human beings, but may contaminate food and may exceptionally cause food poisoning. It is regarded as an excellent model for the pathogenic bacteria study, such as *Staphylococcus Aureus*, *Streptococcus Pneumoniae*, and*Bacillus anthracis*.

These strains belong to the bacteria culture collection of the Microbial Biotechnology Laboratory at the Faculty of Sciences and Techniques (FST) in Fez, Morocco.

2.1. Méthods

2.3.1.Extraction of essential oils

Essential oils extraction was done by hydro-distillation by means of Clevenger type hydro distillation system. Two distillations have been made by boiling each individual for 2 h 30 of 150g of fresh plant material with one liter of water into a flask of tow liter of a column with 60 cm in length connected to a condenser. The essential oil yield was identified in relation with the dry matter, and was evaluated from three samples of 25 g dried in an oven at 100°C

within 24 hours. The essential oil was stored in the dark at 4°C. Every oil extraction was divided into two parts: The first was used for chemical analysis while the second was used for antibacterial in vitro tests. Oils, after extraction, are recovered and kept in small opaque flasks and stored at 4 ° C before their use.

2.3.2.Chromatographic analysis

GC: GC analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (FID), equipped with a 5% phenyl methyl silicone HP-5 capillary column (30 m x 0.25 mm x film thickness 0.25 µm). The temperature was programmed from 50°C after 5 min initial hold to 200°C at 4°C/min. Gas chromatography conditions were as follows: N_2 as carrier gas (1.8 ml/min); split mode was used (Flow: 72.1 ml/min, ratio: 1/50); temperature of injector and detector was 250°C. The machine was led by a computer system type ″HP ChemStation″, managing the functioning of the machine and allowing to follow the evolution of chromatographic analyses. Diluted samples (1/20 in Hexane) of 1 µl were injected manually.

GC/MS: The chemical composition of essential oils was analyzed using a gas chromatograph (TRACE GC Ultra) fitted to a mass spectrometer (Polaris Q-Ion Trap MS). Operating in electron-impact E. I (70 eV) mode. VB-5 (Methylpolysiloxane 5% phenyl) and a column (30 m × 0.25 mm × 0.25 µm thickness) were used (CNRST, Rabat, Morocco). The chromatographic conditions were as follows: Injector and detector temperatures at 220 and 300° C respectively; carrier gas, helium at flow rate of 1.4 ml/min; temperature program ramp from 40 to 300°C with gradient of 4°C/min (holding the initial and final temperature for 4 min). The relative amount of individual components of the total oil was expressed as a percentage peak area relative to total peak area. Library search was carried out using the combination of NIST MS Search and literature.

Oils constituents were identified by their retention indices relatives to n-alkanes (C8-C24) and by comparison of their mass spectral fragmentation patterns with those reported in literature [18].

2.3.3.Antibacterial Activity

2.3.3.1. Disc diffusion method

To highlight the essential oils antibacterial and antimycobacterial activity, the disk diffusion method [19,20] was used because of its simplicity and efficiency to test bacteria sensitivity [11].

For this purpose, sterile Whatman paper discs of 6 mm diameter were placed in the center plate of 90 mm in diameter containing 30 ml of L. B. medium previously inoculated with 100µl liquid cultures of bacterial test strains. The discs are then impregnated with 10µl of essential oil. The control corresponds to a disc impregnated with 10µl of sterile distilled water. After incubation, the inhibition zones formed around the disks were measured.

Three replicates were performed for each strain.

2.3.3.2. Test Statistics:

The demonstration of significant differences between the means of inhibition diameter of the individuals tested is obtained by analyzing variance using the software ‘Stat-graphics’.

2.3.3.3. The MIC Determination

The essential oils minimum inhibitory concentrations (MIC) were determined according to the method reported by Zaouali et al., [9] with a slight modification at the dilution. In fact, DMSO was substituted by 0.2% agar.

A dilution range was prepared in the agar solution, to obtain the following concentrations: 1/ 2, 1/ 4, 1/ 5, 1/ 8 and 1/ 10 (v/v) and 10 ml of each dilution was placed on sterile paper discs Watthman (6mm diameter) placed on the surface of Petri plates of 90mm in diameter containing 30ml of L. B. medium previously inoculated with 100 µl liquid cultures of tested strains. The control corresponds to a disc impregnated with sterile agar solution at 0.2%. After incubation, the inhibition zones formed around the disks were measured. Three repetitions were performed for each strain.

RESULTS AND DISCUSSION

3.1. The Yield Essential Oil

Average yields in essential oil of four provenances of *R. Officinalis* were calculated based on the dry plant material of the aerial part of the plant. The results obtained are summarized in the table1.

Table 1: Yields essential oils obtained by hydro-distillation of four provenances of *R. officinalis* Var. *prostratus*

Samples	Yields (%)
RosmarinusOfficinalis (Garden Jacky)	1.45±0.02
RosmarinusOfficinalis (AyouneCharkiya)	1.8±0.03
RosmarinusOfficinalis (WadiLaou)	1.9±0.02
RosmarinusOfficinalis (Sefrou)	2.4±0.1

The obtained average yields vary based on four sources (Table 1). Indeed, samples of rosemary from Sefrou gave a better yield in essential oil (2.4%) compared to those of AyounCharquia, of WadiLaou and Agadir's Jacky Garden with respectively 1.8% concentrations of 1.9 % and 1.45%.

These results are similar to those found by Khiaet al. [21] and found that the yields of essential oil of Rosemary of three Moroccan origins (RachidiaBerkine and Aknoul) are 2.21 %, respectively, 1.87% and 1.29%. Fechtal et al. [22] indicated that the essential oil yields of two sources of the eastern (El Ayat and Debdou) range from 0.5% to 2.9%. However, other studies have yields that do not exceed 1%. Indeed Derwich et al.[23], found that the performance of Rosemary Taferdoust the region is 0.5% and Tahiri et al. [24], found that Rosemary El Had and BeniBoudass have yields of 0.65 % and 0.82 respectively.

Table 2 : Chemical composition of essential oïlfrom four provenances of *R. Officinalis* Var. *prostratus*

Compounds	RI	Agadir	OuedLaou	AyouneCharquia	Sefrou
Monoterpenes					
Tricyclene	926	-	-	-	0.29
α-Thujene	931	0.21	0.49	0.53	0.12
α-Pinene	939	36.15	15.79	16.61	12.19
Camphene	953	6.10	13.06	13.67	6.81
Sabinene	976	3.40	7.11	6.04	0.4
β–Pinene	980	-	-	-	4.05
β–Myrcene	986	-	-	-	5.1
α-Phellandrene	1005	0.12	-	-	1.3
δ-3-Carene	1011	-	4.54	1.43	-
limonene	1031	-	-	-	4
Isobornyl formate	1233	0.13	-	0.2	-
Total		**46.11%**	**40.99%**	**38.48%**	**34.26%**
Oxygenated Monoterpenes					
1.8-Cineole	1033	33.93	1.24	35.91	18.35
Trans - Sabinene -hydrate	1068	0.44	0.15	-	1.6
Chrysanthenone	1123	0.71	0.42	-	-
Camphor	1143	5.08	3.31	6.40	22.1
Trans-verbenol	1144	0.46	0.58	-	-
Borneol	1165	4.18	3.59	4.84	-
Terpinen-4-ol	1177	-	0.41	0.56	0.8
Myrtenol	1194	-	1.14	1.39	-
Verbenone	1204	4.30	0.25	-	-
Trans-2-Caren-4-ol	1227	-	-	0.2	-
BornylAcetate	1285	-	31.21	-	0.8
α-TerpinenylAcetate	1354	1.51	2.88	-	-
Total		**50.61%**	**45.18%**	**49.3%**	**43.65%**
Hydrocarbon Sesquiterpene					
α–copaene	1376	-	0.10	0.15	-
β-Caryophyllene	1418	0.83	4.09	2.60	-
γ–Gurjenene	1473	0. 44	0.2		-
Germacrene-D	1480		0.22	1.4	-
α-Cadinene	1512	-	-	0.42	-
γ-Cadinene	1513	0.13	0.8	0.46	0.78
Total		**0.96%**	**5.41%**	**5.03%**	**0.78%**
Oxygenated Sesquiterpenes					
Caryophyllene Oxide	1581	-	4.71	3.80	6.3
γ-eudesmol	1630	-	0.21	0.14	-
α-eudesmol	1652	-	0.1	0.13	-
τ-Cadinol	1653	-	-	0.72	-
Total			**4.92%**	**4.79%**	**6.3%**
	TOTAL	**97.7**	**96.5**	**97.6**	**85.07**

RI: Retention index

Nevertheless, yields on four sources of Morocco are slightly higher than those of three localities of Tunisia. Indeed, Ayadi et al. [25] received variable yields according to yield region, or 1.35% in the region of SidiBouzid, Bizerte 1.25% and 1.27% for the region Zaghouan.

In Algeria, studies have recorded very lower yields compared to those obtained in this study with a return of 0.8 % for the Rosemary Honaine station and 0.6 % for the Rosemary Tlemcen station [26], However, our yields are slightly lower than those obtained by Jamshidi[27], which are 2.1 and 2.6% respectively in the regions of Kerman and Lalehzar Iran. In Turkey Ozcan and Chalchat, [28] found a yield of 1.9%.

The variability of returns of Rosemary from different regions may come from the origin of the plant [27], from the climate [29], environmental and agronomic conditions [30], the time of harvest [31], the plant development stage [32], or the extraction method[33].

Chemical composition of essential oil

The results for the chemical composition of essential oils of *R. Officinalis* four provenances (Agadir's Jacky Garden, Wadi Lou, AyounCharquia and Sefrou) are summarized in thetable 2.

The results for the chemical composition of essential oils of *R. Officinalis* four sources have identified seventeen compounds representing a total of 97.7 % for the Rosemary Garden Jacky, twenty four compounds representing a total of 96.5 % for Rosemary WadiLaou, twenty-one compounds representing 97.6 % of the essential total oil of rosemary from theAyounCharquiaand sixteen compounds with a total of 85.07 % of Rosemary from Sefrou.
It is apparent from the analysis of these results that the majority compounds are:

-The α-Pinene (36.15 %), the 1.8-Cineole (33.93 %), the Camphene (6.1 %), the Camphor (5.08%) and the Sabinene (3.4 %) for the Rosemary ofAgadir's Garden Jacky.

-the Bornyl Acetate (31.21 %), the α-Pinene (15.79 %), the Camphene (13.06 %), the Sabinene (7.11%), the δ, 3-Carene (4.54%), the Caryophyllene Oxide (4.71%), the□-Caryophyllene (4.09 %), and Borneol (3.59 %) for the Rosemary from WadiLaou.

-The 1.8 Cineole (35.91 %), the α-pinene (16.61 %), the Camphene (13.67 %), the Camphor (6.4 %), the sabinene (6.04 %), the Borneol (4.81 %) and the CaryophylleneOxide (3.8 %) for the Rosemary AyounCharquia.

- the Camphor (22.1 %), 1,8- Cineole (18.35 %), the α-Pinene (12.19 %), the Camphene (6.81 %), the CaryophylleneOxide (6.3 %) for Rosemary Sefrou.

In Morocco, Fechtal[34] and Khia[21] confirm the wealth of essential oils of oriental 1.8-Cineole, α and β-Pinene, Camphor andCamphene. Derwichet al., (2011) showed that Rosemary from the Tafersoust consists mainly of α-Pinene (18.25%) followed by Camphor (6.02 %), 1.8-Cineole (5.25%), Camphene (5.02%) and β-pinene (4.58%). Elamrani[35] showed that Moroccan Rosemary have a high content of one of three compounds: α-Pinene (37.0 to 40.0%, Rabat), Cineole (58.7 to 63.7% El Ateuf), Camphor (41.7 to 53.8 %, Taforhalt).

The detected variation in the chemical composition of the essential oil from *R. Officinalis* outcome of different regions is linked to several parameters such as: the environmental factor [36, 37], the climatic and geographical conditions that vary from country to country and time of harvest [38]. The extraction method significantly affects the essential oil composition [39] and processes, which use water, can induce ester hydrolysis ... etc.

3.2. Principal Component Analysis (PCA)

The study of province effect about chemical quality of essential oils of *Rosmarinus Officinalis* from four Moroccan regions shows differences in total concentrations of essential oils. To highlight a potential variability of the chemical composition of these essential oils and study statistically the existing similarities between the different oils and correlations among variables, we conducted a Principal Component Analysis (PCA).

We considered each compound as a quantitative dependent variable (Table 3).

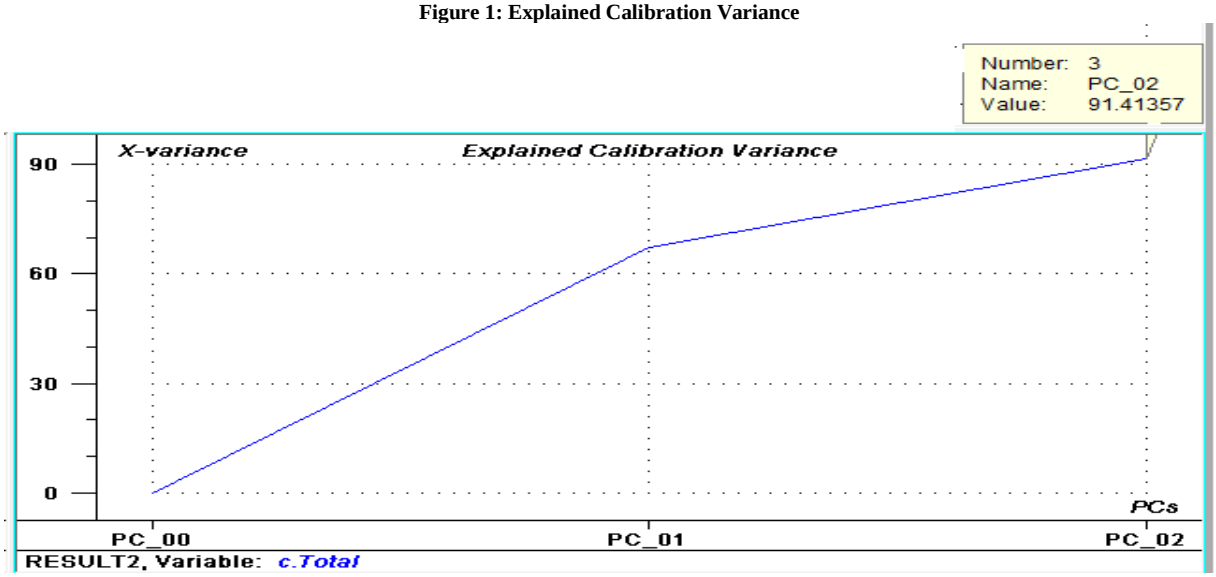
3.2.2.Variability explained

In the graph of the variance explained according to the number of selected principal components (figure 1); we note that the first component explains 69.77 % of the variability of the data; while the second component explained only 21.81%. So we can be satisfied to retain these two components explaining 91.59 % of the total variability.

Table 3: Compounds of the essential oil used in the statistical analysis

Var 1	Tricyclene	Var 17	Borneol
Var 2	α-Thujene	Var 18	Terpinene-4-ol
Var 3	α-Pinene	Var 19	Myrtenol
Var 4	Camphene	Var 20	Verbenone
Var 5	Sabinene	Var 21	Trans-2-Caren-4-ol
Var 6	β–Pinene	Var 22	Bornyle Acétate
Var 7	β–Myrcene	Var 23	α-TerpinenylAcetate
Var 8	α-Phellandrene	Var 24	α–Copaene
Var 9	δ-3-Carene	Var 25	β-Caryophyllene
Var 10	Limonene	Var 26	γ–Gurjenene
Var 11	Isobornyl Formate	Var 27	Germacrene-D
Var 12	1,8-Cineole	Var 28	α-Cadinene
Var 13	Trans-Sabinene-hydrate	Var 29	γ–Cadinene
Var 14	Chrysanthenone	Var 30	Caryophyllene Oxide
Var 15	Camphor	Var 31	γ-Eudesmol
Var 16	Trans-Verbenol	Var 32	α-Eudesmol
		Var 33	τ-Cadinol

Figure 1: Explained Calibration Variance

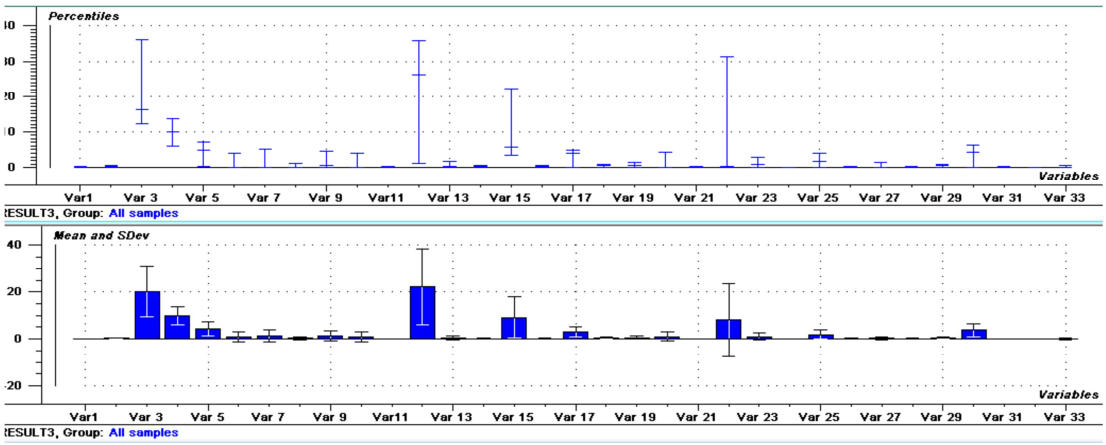


3.2.3.Study of variables

3.2.3.1. Meanand Standard deviation

The essential oil composition of Rosemary of the four provinces presents a significant difference. The identified components show great variability (Figure 2). 1.8-Cineole (Var 12) is the component that has the most variation within samples, followed by α-Pinene (Var 3), Camphene (Var 4), Camphor (Var 15), BornylAcetate (Var 22) and CaryophylleneOxide. The other components have little variability

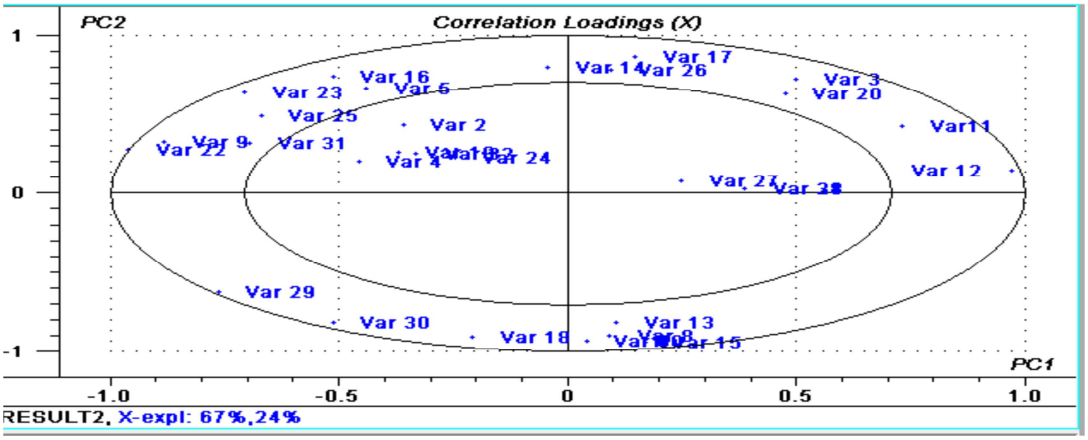
Figure 2: Mean and Standard deviation for different variable



3.2.3.2. Circle of Correlation

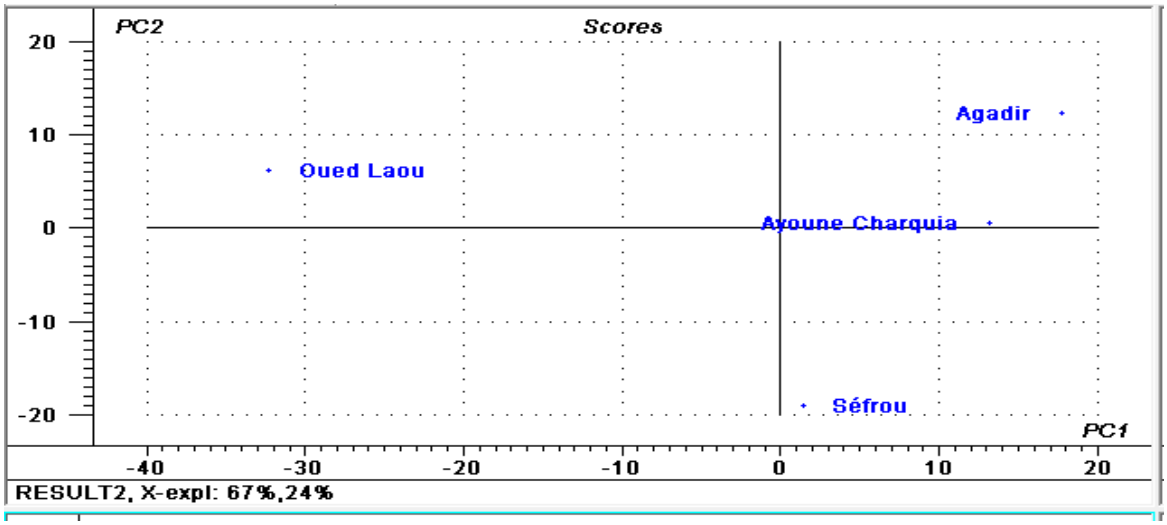
The representation of the correlation circle of essential oils variables (Figure 3), shows that the axes $PC_1$and PC_2 respectively carry 67 % and 24% of the total information and that all components of this oil contributes to form the firstaxis and reveals the existence of correlation between some variables studied, such as the correlation between Borneol (Var 17) Chrysanthenone (Var 14) γ–Gurjenène (Var 26).

Figure 3:Correlation Loading



3.2.4.Study of samples

Figure 4: Scores plot



Rosemary wadiLaou and AyounCharquia are explained by the component 1 or Rosemary Agadir and Sefrou are explained by the component n° 2(Figure 4).

The principal component analysis showed a good classification of the essential oils of rosemary from the different regions according to the chemical composition. The principal component analysis showed a good classification of Rosemary essential oils of the different regions according to the chemical composition.

Therefore, these oils can be classified according to their chemotype:
- In AyounChaquia region, we find *R. Officinalis* to 1.8-Cineole (Cineoliferum).
- In Sefrou region, we find *R. Officinalis* to camphor (Camphoriferum).
- In the region of Agadir, we find*R. Officinalis*to α-Pinene.
- In the region of WadiLaou, we find *R. Officinalis* var. *Prostratus* to Bornyl Acetate.

Figure 5: Loading plot

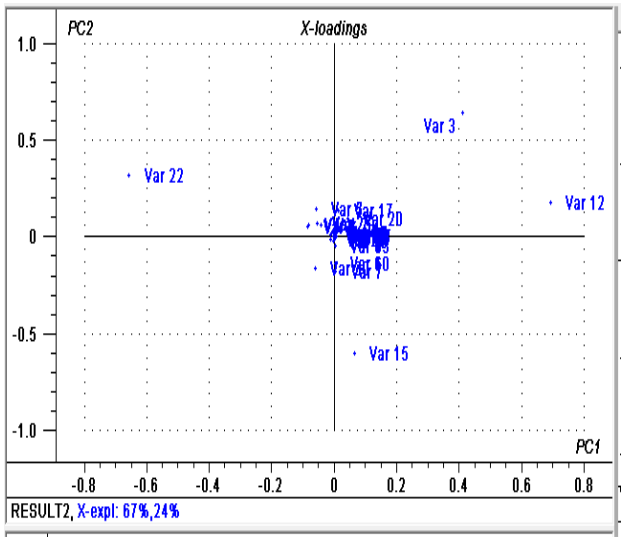
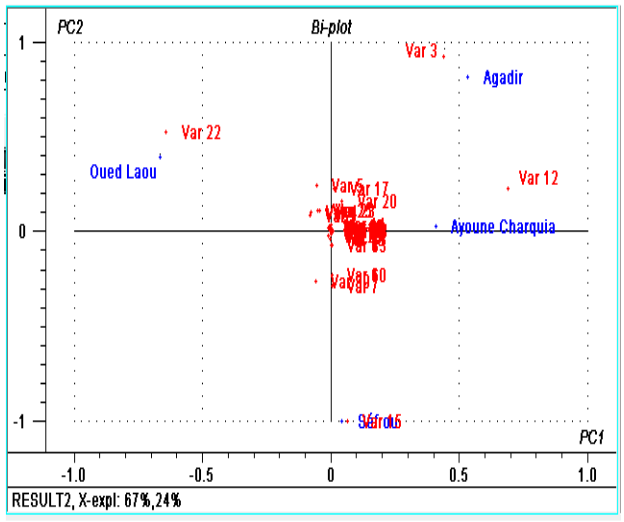


Figure 6: Scores and Loading plot



Elamrani et al., [40] studied the chemotaxonomy of *Rosemary*essential oil from different Moroccanregions. They defined three chemotype: α- pinene(37-40 %), Camphor (41-53 %), and 1.8-Cineole (58-63 %).

Pintore et al.,[41], compared the *Rosmarinus*Sardinia and Corsica and were able to identify respectively chemotypes α-Pinene, Verbenone, and BornylAcetate. This demonstrates the chemotype resemblance with that of provenance of WadiLaou.

Tucker and Maciarello[42],examined essential oils of *R. Officinalis*, from 23 cultivars.These authors showed that these cultivars were derived from five botanical varieties showing large variations in their main component: α-pinene (0.06 - 57.45 %), 1.8-Cineole (3.55 - 42.69 %), Camphor (0.20 - 56.45 %),Bornyl acetate (0.66- 21.03 %) and Borneol (0.40 to 14.69 %).

The essential oils were grouped so into six chemical groups (chemotypes):
1-α-Pinene> 1.8-Cineole ,
2-1,8-Cineole > α–pinene
3-α-Pinene>Camphre + Camphene > 1,8-Cineole,
4-Camphre + Camphene > α-Pinene> 1,8-Cineole,
5-Camphre + Camphene > 1,8-Cineole > α-Pinene,
6-Borneol + bornyl acetate > Camphor

This procedure is useful when using essential oils in aromatherapy. The chemotype differentiates the therapeutic properties of several varieties or cultivars as in the case of rosemary and which are characterized by the same form of leaves, stems, flowers, color and odor.

3.3. Study of antibacterial activity by the disk method

In this study, the antibacterial activity of essential oils of R. officinalis from different Moroccan regions are assessed vis-à-vis the three bacterial strains (*E. Coli, B. Suptilis and M. smegmatis*).

The study of this activity was, first, evaluated qualitatively by the disk diffusion method in the objective to select among the essential oils tested those that are most active. The results obtained are expressed in terms of inhibition zone diameters measured. They are shown in Table 4.

Table4:Antibacterial effect of four essential oils of R. Officinallis by the disk method

Samples of essential oils	Diameter of inhibition (mm)*		
	E. Coli	*B. Subtilis*	*M. smegmatis*
R. Sefrou	15.66 ± 0.57	30.33 ± 2.08	37 ±1
R. Ayoune	18 ± 1	22 ± 2	26.3 ±1.52
R. OuedLaou	16.33 ± 1 .15	27.0 ± 1.1	18.66 ±2.08
R. Agadir	12 ±1	13.33 ± 1.15	16 .66 ±1.52

**The average results available after three repetitions*

In order to check if there are significant differences between the averages of inhibition diameter for four different regions of *Rosmarinus Officinalis*tested on the three strains (*E. Coli, B. Subtilis et M. Smegmatis*),we used analysis of variance (ANOVA) with a factor. The results of this test are shown in Table 5.

Tableau 5:Variance Analysis of ANOVA with a Factorial combination of treatment

Strains	**Variance analysis**					
	Source	**Sum of Squares**	Ddl	**Average Square**	F	**Probabilities**
M. smegmatis	Inter-groups	764.667	3	254.889	101.96	0.0000
	Intra-groups	20.0	8	2.5		
B. Subtilis	Inter-groups	503	3	167	60.97	0.0000
	Intra-groups	22	8	2.75		
E. Coli	Inter-groups	367	3	122	146	0.0000
	Intra-groups	6.66	8	0.83		

The ANOVA table decomposes the variance to two components: the inter-groups component and the intra-groups component. Since the probability value for the F test was less than 0.05, there is a statistically significant difference between the means of inhibition diameters from one region to another at confidence level of 95.0% for the three tested strains.

The following figures describe the average of the diameter for the inhibition zone (mm) as a provenance function and this for each strain.

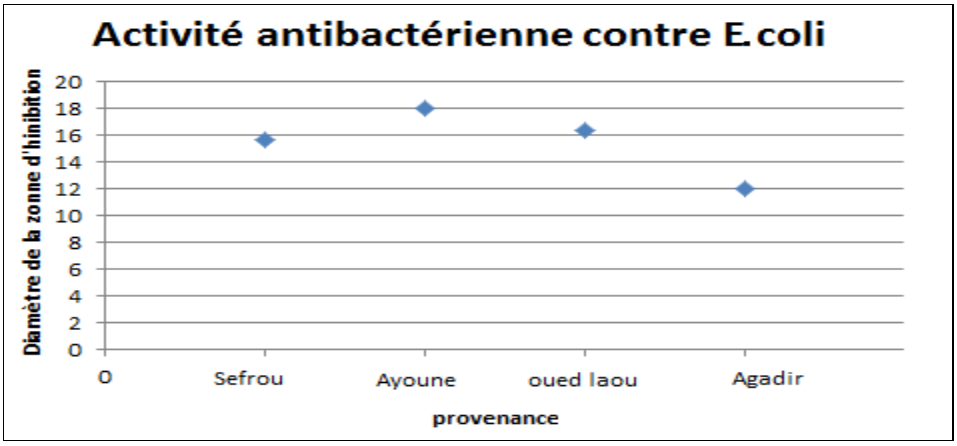


Figure 7: Antibacterial effect of R. *Officinalis'*essential oils from differentMoroccan regions against *E. Coli* by the disk method

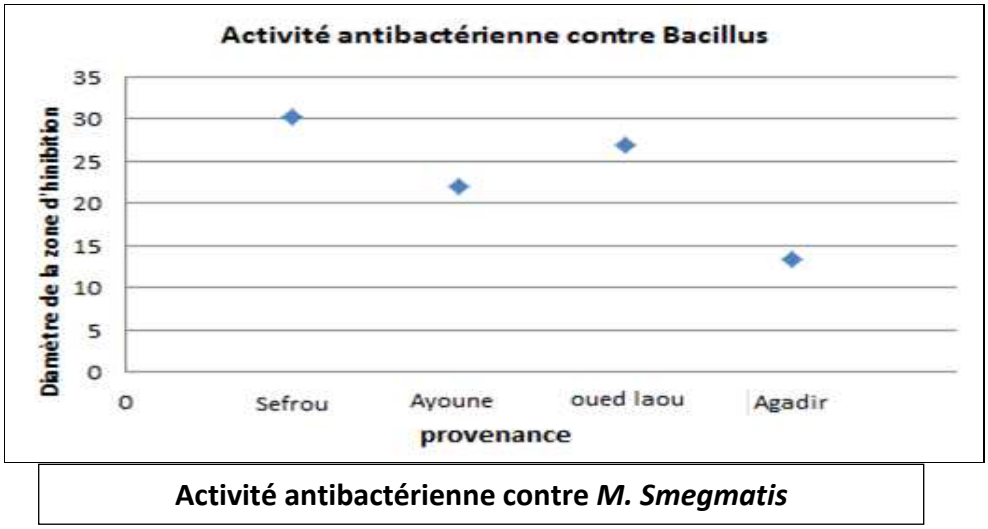


Figure 8: Antibacterial effect of *R. Officinalis's* essential oils from different Moroccan regions against *M. Smegmatis* by the disk method

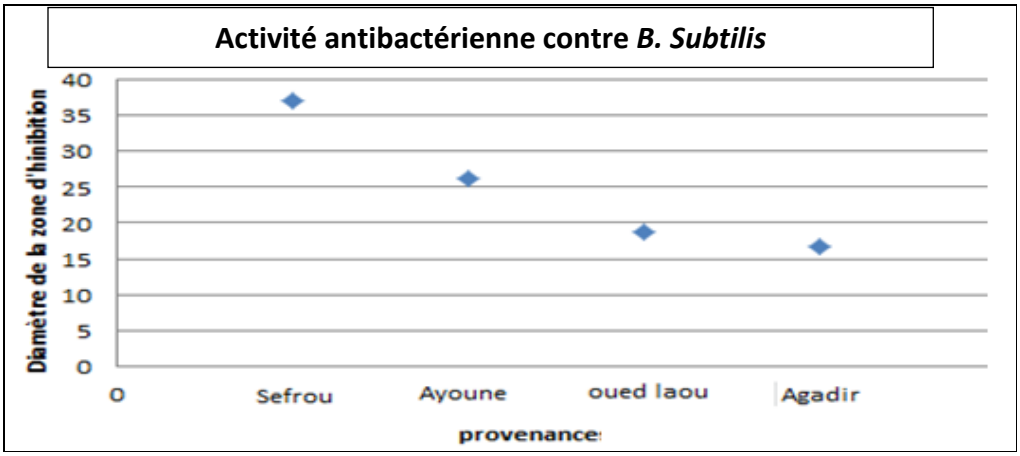


Figure 9: Antibacterial effect of R.*Officinalis's* essential oils from different Moroccan regions against *B. Subtilis* by the disk method

Antibacterial activity results of essential oil of R. Officinalis from four regions showed an inhibitory power over all studied strains. This activity differs from one region to another statistically. Indeed, the highest antibacterial activity was observed for the Sefrou's Rosemary and the lowest activity was observed for the Agadir's Rosemary (Table 4 and Figures 7, 8 and 9) and this for the three strains used .

The Rosemary essential *B. Subtilis*oil of the region Sefrou shows the most important activity with halos 15.66 ± 0.57 and 30.33 ± 2.08 for *E. Coli*and*B.Subtilis* respectively and37 ± 1 mm for *M.Smegmatis*.

For essential oil of Rosemary AyounCharkiya , the greatest activity was observed against *M. Smegmatis* with diameters of inhibition of 26.3 ± 1.52 while the lowest activity was observed against *E. Coli* with diameters of inhibition of 18 ± 1 mm.

The same results were reported for the essential oil of Agadir' Rosemary. In fact, the diameters of zones of inhibition obtained for *M. Smegmatis* are higher than those obtained for*B. Subtilis*and *E. Coli*. These diameters are respectively 12 mm ± 1.13, 33 mm ± 1.15 and 16.66mm ± 1.52 for *E. Coli*, and*M. Smegmatis.*

It is clear from the results presented in Figures (7, 8 and 9) that the essential oil of *R. Officinalis*wadiLaou has had a significant inhibitory activity against all tested strains.Furthermore, the Gram + bacterium has proved most susceptible to the action of this oil in comparison with the other bacteria, with significant inhibition of diameter of the order of 27.66 mm ± 1.1. While *E. Coli* (gram -) is the most resistant with an inhibition diameter of 16.33 ± 1.15mm.

These essential oils were tested against Gram + bacteria (*B. Subtilis*) and gram – bacteria (*E. Coli*). The latter showed some resistance against the inhibitory power of this essential oil. The same observation was reported by

Delamare et al., [43]. Similar results have been reported against the same strain respectively in Sardinia and in Iran [44, 45].

Most studies of the action of essential oils against the agencies responsible for food spoilage and pathogens agree that, generally, the essential oils are more active against Gram + bacteria than against Gram – [46- 49]. The low sensitivity of gram-negative microorganisms vis-à-vis the antibacterial agents could be explained by the presence of the double membrane in Gram-negative bacteria in contrast to Gram- positive bacteria which contain only one [50] .This makes easier the action of the essential oil.

Indeed among the reasons given for the action of essential oils on bacterial strains, reaching the membrane permeability through disruption of membrane structures and the loss of chemiosmotic control is the most important [51]. Moreover, by their lipophilic nature, essential oils can easily pass through cell walls and cell membranes, leading to disorders of polysaccharide structure, fatty acids and phospholipids as well as their permeability [52].

3.4. MIC determination

The MIC is the lowest concentration of compound at which there is no visible growth with the naked eye after the incubation time. Its determination was made by observing the disturbance induced by the growth of bacteria in each tube. The results are shown in Table 6.

Essential oils have shown an inhibitory effect against the studied microorganisms. Indeed, all microbial strains were inhibited at a concentration of 1/250 v / v. The most sensitive organism with all essential oils is *M. Smegmatis* whose growth was stopped at the low concentration of 1/500 v / v. The most resistant strain is *E. Coli* with an MIC value corresponding to 1/250.

This shows that these essential oils have valuable antibacterial properties on the tested microorganisms. These results are in agreement with those of the disk method.

Several authors have shown the relationship between the antibacterial activity of essential oils and their chemical composition. Indeed the changing nature of the constituents and their contents induce a variation of the microbiological activity. The presence of a number of components in the chemical composition promotes this activity.

Table 6: MIC of Rosemary essential oils from different regions

		MIC v/v						
	Strains	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{125}$	$\frac{1}{250}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$
R. Officinalis Agadir	*M. Smegmatis*	-	-	-	-	-	+	+
	E. coli	-	-	-	+	+	+	+
	B. Subtilis	-	-	-	-	+	+	+
R. Officinalis Oued Laou	*M. Smegmatis*	-	-	-	-	-	+	+
	E. coli	-	-	-	+	+	+	+
	B. Subtilis	-	-	-	-	-	+	+
R. Officinalis Ayoune	*M. Smegmatis*	-	-	-	-	+	+	+
	E. Coli	-	-	-	-	+	+	+
	B. Subtilis	-	-	-	-	-	+	+
R. Officinalis Sefrou	*M. Smegmatis*	-	-	-	-	-	+	+
	E. Coli	-	-	-	-	-	+	+
	B. Subtilis	-	-	-	-	-	+	+

+ Growth; - No Growth

It has been shown that the antimicrobial activity of the essential oils is often due to its terpene compounds [53]. In general the activity of the essential oils is primarily due to the presence of phenolic compounds, aldehyde and alcohol [54, 55]. Furthermore the terpenol are known to be very good antimicrobial agents [56, 57].

Numerous studies have shown the antibacterial properties of some compounds such as: α-Pinene [58, 59], 1.8-cineole [60], the camphor and its derivatives [61, 62],Caryophyllene Oxide [63], Borneol [61]. In addition, Magiatis et al.[64] found that CaryophylleneOxide is the most effective , followed by camphor and 1,8- Cineole. This can therefore explain the activity of essential oils extracted recorded in all regions.

CONCLUSION

During our work, we first determined and compared yields, chemical compositions and antibacterial properties of essential oils of Rosemary from four different regions in Morocco (Agadir's Jacky Garden, The AyounCharquia, WadiLaou and Sefrou). These essential oils were obtained by steam distillation and analyzed by gas chromatography coupled to mass spectrometry.

We found that the average yields obtained vary according to four sources. In fact, samples of rosemary from Sefrou gave a better yield in essential oil (2.4 %) compared to those of the AyounCharquia, wadiLaou and Agadir'sGardenJacky, with values respectively 1.8% , 1.9% and 1.45 %.

Chromatographic analysis of these essential oils showed that the chemical compositions are different from one region to another. The majority compounds are:

- The α-Pinene (36.15%), the 1.8-Cineole (33.93%), the Camphene (6.1%),the Camphor (5.08%) and the Sabinene (3.4 %) for the Rosemary of Agadir's Garden Jacky.
- The Bornyl Acetate (31.21 %), the□□-Pinene (15.79 %), the Camphene (13.06 %), the Sabinene (7.11%), the □, 3-Carene (4.54%), the Caryophyllene Oxide (4.71%), the □-Caryophyllene (4.09 %), and Borneol (3.59 %) for the Rosemary from WadiLaou.
- The 1.8 Cineole (35.91%), the α-Pinene (16.61%), the Camphene (13.67 %), the Camphor (6.4%), the Sabinene (6.04%), the Borneol (4.81%) and the Caryophyllene Oxide (3.8 %) for the Rosemary AyounCharquia.
- The Camphor (22.1 %), 1.8-Cineole (18.35 %), the α-Pinene (12.19 %), the Camphene (6.81%), the Caryophyllene oxide (6.3%) for Rosemary Sefrou.
-

The descriptive and comparative statistical study of the chemical composition of Rosemary of the different regions through the main component analysis method showed a good classification of rosemary essential oils from different regions depending on the chemical composition. Thus, these oils may be classified according to their chemotype:

- In AyounChaquia region, we find *R. Officinalis* to 1.8-Cineole.
- In Sefrou region, we find *R. Officinalis* to camphor.
- In the region of Agadir, we find *R. Officinalis* to α-pinene.
- In the region of WadiLaou, we find *R. Officinalis* var. *Prostratus* to Bornyl Acetate.

The study of antibacterial activity of essential oils of Rosemary from four regions showed an inhibitory power over all the studied strains.

This activity differs from one region to another statistically.

Indeed, the highest antibacterial activity was observed for the Sefrou's Rosemary and the lowest activity was observed for Agadir's Rosemary and this for three strains used.

REFERENCES

[1] T. Michel, Nouvelles méthodologies d'extraction, de fractionnement et d'identification: Application aux molécules bioactives de l'argousier (Hippophaërhamnoides). Thèse de doctorat, Université D'Orléans École Doctorale Sciences Et Technologies, Institut de Chimie Organique et Analytique, **2011**.
[2] M. Fennane, M. Ibn Tattou, A. Ouyahya, J. El Oualidi, Flore pratique du Maroc, manuel de détermination des plantes vasculaires, Volume 2, Série Botanique N°38, ISBN 9954834745, Rabat, **2007**, 636.
[3] H. Lahsissene, A. Kahouadji, M. Tijane, S. Hseini, Catalogue des plantes médicinales utilisées dans la région de Zaër (maroc occidental). Lejeunia, revue de botanique, Nouvelle série,**2009**, N° 186, BE ISSN, 0457-4184.
[4]A. M. Scherrer, R. Motti,C.S. weckerle, *J. Ethnopharmacol*., **2005**, 97, 129-143.
[5] A. Legssyer, A. Ziyyat, H. Mekh, M.Bnouham, C. Herrenknecht, V. Roumy, C. Fourneau, A. Laurens, J. Hoerter, R.Fischmeister, *Phytother Res*, **2004**, 18(11), 889-94.
[6] M. Bnouham, FZ. Merhfour, A. Legssyer, H. Mekhfi, S. Maâllem, A. Ziyyat, *Pharmazie*, **2007**, 62(8), 630-632.
[7] H. Mekhfi, M. El Haouari, M. Bnouham, M. Aziz, A. Ziyyat, A. Legssyer, *Phytother. Res*, **2006**. 20(2),135-9.
[8]M. El Haouari, J. J.López, H. Mekhfi,J.A. Rosado, G. M. Salido, *Ethnopharmacol*, **2007**, 113(2), 325-31.
[9]Y. Zaouali, T. Bouzaine, M. Boussaid, *Food Chem. Toxicol*.,**2010**, 48(11), 3144-52.
[10]J. Delcampo, M. J. Amiot, C. Nguyen, *Food Protect*, **2000**, 10,1359–1368.
[11]T. Rožman, B. Jeršek,.*Acta agriculturae Slovenica*, **2009**, 93 (1),51-58.
[12]M. Ghanmi, B. Satrani, A. Aafi, Phytotérapie, **2010**, 8, 295–301.

[13]A. Gianmario, S. Silvio, P.A. Rita, M. Teresa, D. Roberto, T. Aurelia, *Journal of Agricultural and Food Chemistry*, **2007**, 55(5), 1718.
[14]M. R.Al-Sereiti,K. M. Abu-Amer,P.Sen, *Exp Biol*, **1999**. 37(2), 124-130.
[15]W. Wang, N. Wu , Y. G. Zu, Y. J. Fu, **2008**, 108 (3), 1019-1022.
[16]S. B. Eva, M. H.Tulok,A. Hegedus, *Acta Biologica Szegediensis*, **2003**, 47, 111–113.
[17]M. Ghanmi, B. Satrani, M. Aberchane, Plantes Aromatiques et Médicinales du Maroc: Les milles et une vertus. Collection Maroc Nature, édition du Centre de Recherche Forestière, **2011**, ISBN 978-9981-824-28-7, 128.
[18] L. A. Mitscher , W. R. Baker, *Pure Appl. Chem*,**1998**, 70(2), 365-371.
[19]Adams, R. P, Identification of essential oil components by Gas Chromatography/ Mass Spectroscopy,**1995**, Allured Stream, IL.
[19] K.Bayoub, T.Baibai, D.Mountassif, A.Retmane, A. Soukri, *Afr. J. Biotechnol*,**2010, 9** (27), 4251-4258.
[20] A. Bauer, W. Kirby, W. M. M., Sherris, J. C. M. Turck, *Am. J. Clin. Pathol*.**1966,** 45(4), 493-496.
[21]A. Khia, M. Ghanmi, B. Satrani, A. Aafi, M. Aberchane, B.Quaboul, A. Chaouch, N. Amusant, Z. Charrouf ,*Phytothérapie*,**2014,** 12 (6),341-347.
[22]M.Fechtal, A. Zine el Abidine, M. Hachmi, A.Sesbou, R. Karkouzi, Variabilité infra-spécifique du rendement et de la composition chimique des huiles essentielles du Romarin (Rosmarinus officinalis L.). Annales de la recherche forestière au Maroc, **2005**,36, 98-106.
[23]E. Derwich, Z. Benziane, R. Chabir, *IJABPT*, **2011**, 2(1), 145-153.
[24]T, Tahiri, Estimation de la biomasse et la production en huile essentielle de Rosmarinus officinalis et de lanvanduladentata var typica dans le Parc National de Talassemtane, Mémoire de 3^{éme} cycle, ENFI, sale, **1994**.
[25]S. Ayadi ,C.Jerribi ,M.Abderrebba, *Soc Alger Chim* **2011**. 21(1), 25–33.
[26]F. Tomi, P. Bradesi, A. Bighelli, J. Casanova, *J. Magn. Reson*, **1995**, Anal.,1, 25-34.
[27]R. Jamshidi, Z. Afzali, D. Afzali, *Journal of Agriculture and Environment Science* 5,**2009**, (1),78–81.
[28] M. M. Özcanet J. C. Chalchat, *Inter. J. Food sci. nutria*. **2008**,59, 691 – 698.
[29] M. J. Jordan, V. Lax, M.C. Rota, S. Loran, J.A. Sotomayor, *Food Control*, 30463-30468,**2013**.
[30] M. Moghtader, D. Afzali, *Journal of Agricultural and Environmental Science*,**2009**, 5 No. 3, 393-397.
[31] O. Y. Celiktasa,E.E. Kocabasa, Hames, E. Bedirb, F. Sukanb, Vardar, T. Ozekc, K.H. C.Baserc, *Food Chem*, **2007**, 100(2), 553-559.
[32] G. Ruberto, M.T. Baratta, *Food Chem*., **2000**, 69, 167-174.
[33]O. Okoh, A. P. Sadimenko, A. J. Afolayan,, *Food Chem*, **2010**, 120, 308-312.
[34]M. Fechtal, R.Ismaili, A. Zine el Abidine,Effet de la transplantation sur la qualité et le rendement en huilesessentielles du romarin (Rosmarinusofficinalis L.),**2001**,Annales de la rechercheforestière au Maro 34,94-102.
[35]A. Elamrani, S. Zsira, B.Benjilali, M.A.Berrada,*Journal of Essential Oil Research,***2000**, 12, 487–495.
[36]B. Bernath,E.Danos, Hethelyi, Herba Hung.Herba Hung,**1991**,30, 35-46.
[37]G. E. Grella, V.Picci,*Phytoterapia*,**1988**, 59, 97-102.
[38]S. Fellah , M.Romdhane,M.J.Abderrabba, *Soc. Alger. Chim*,**2006**,16(2), 193-202.
[39] J. Bruneton ,Pharmacognosie et phytochimie. Plantes médicinales. Paris, France, Lavoisier, **1993**, 278- 279.
[40] A. Elamrani, S.Zsira, B. Benjilali, M.A. Berrada, *Journal of Essential Oil Research*,**2000**, 12, 487–495.
[41]G. Pintore, M. Usai, P. Bradesi, C. Juliano, G. Boatto, F. Tomi, M. Chessa, R.Cerri R, *J..Flav. Fragr. J.*,**2002**, 17, 15-19.
[42]A.O. Tucker A.O., M. J. Maciarello M. J., The essential oils of rosemary cultivars.*Flavour Fragrance J*, **1986**, 1, 137–142.
[43]A. P. L. Delamare, I. T.Moschen-Pistorello, L. Artico, L. Atti-Serafini, S. Echeverrigaray, *Food Chem*, **2007**, 100, 603–608.
[44]E. A. Hayouni, M.Abedrabba, M. Bouix, M. Hamdi, *Food Chem*, **2007**, 105, 267–273.
[45]L. E. Nikitina, *Pharm. Chem. J.*, **2009**, 43(5), 20.
[46] A. B.Hsouna, M. Trigui, R. B. Mansour,R.M. Jarraya, M. Damak, S. Jaoua, *Int.. J. Food Microbiol*, **2011**, 148, 66–72.
[47]V. Cardile, A. Russo.C., Formisano, D. Rigano, F. Senatore,N.A. Arnold, F. Piozzi, *Ethnopharmacol*, **2009**, 126(2), 265-72.
[48]J. M. Wilkinson, M. Hipwell, R. Trachy, H. M. A. Cavanagh, *J. Agric. Food Chem*., **2003**, 51, 76-81.
[49] G. Pintore, M. Usai, P. Bradesi, C. Juliano, G. Boatto, F. Tomi, M.Chessa, R. Cerri, J. Casanova, *Flav. Fragr. J.*, **2002**, 17, 15-19.
[50]C. Bagamboula, M. Uyttendaele, J.Debevere J.Inhibitory, *Food Microbiol*,**2004**, 21, 33–42.
[51]S. D. Cox, C. M. Mann, J. L. Markham, H. C. Bell, J. E. Gustafson, J. R. Warmington, S. G. Wyllie,*Appl. Microbiol*, **2000**, 88, 170–175.
[52]F. Bakkali, S.Averbeck,D.Averbeck ,M.Idaomar, *Food chem. Toxicol*.,**2008**, 46, 446-475.
[53]M. Cowan, Plant Products as Antimicrobial Agents, *Clin.Microbiol Re*,**1999**, 12 (4), 564- 582.
[54]G. Sacchetti, S. Maietti, M.Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, R. Bruni, *Food Chem*.,**2005**, 9, 621– 632.

[55]R. Bruni, A. Medici, E. Andreotti, C. Fantin, M. Muzzoli, M. Dehesa, *Food Chem*, **2003**,85, 415–421.
[56]M. Kelen, B. Tepe, *Bioresour. Technol*, **2008**, 99 (10), 4096–4104.
[57] B. Satrani, A. Farah, M. Talbi, *ActaBotanicaGallica*, **2006**, 153, 235–242.
[58] N.Aligiannis, E.Kalpoutzakis, I. B.Chinou, S.Mitakou, E.Gikas, A.Tsarbopoulos, *J. Agric. Food Chem*, **2000**,49, 811-81.
[59]R. Martin, L.R. Salgueiro, M.J. Goncalves, R. Vila, F. Tomi , J. Adzet, Casanova,*Planta Medica*, **2000**. 66(7), 647-650.
[60] D. Prudent, F. Perineau,J.M. Bessiere, G. Michel, R. Bravo,*J. Ess.Oil. Res*,**1993**, 5, 255-264.
[61] S. Felice, N. Francesco, A. A. Nelly., B. Maurezio , H. Werner,*Flav. And Fragr. J*, **2004**, 20 (3), 291-294.
[62] A. Tantaoui-Elaraki, H. Ferhout, A. Errifi, *J. Essent. Oil. Res*,**1993**, 5, 45-53.
[63] A. Ulubelen, G. Topcu, C. Eris, U. Sonmez, M. Kartal, S. Kurucu, C. Bozok-Johansson, .*Phytochemistry*, **1994**, 36, 971-974.
[64] P. Magiatisa, L. Alexios, C. Ioanna, A.H. Serkos,*Z. Naturforsch*, **2002**, 57 c, 287-290.