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Extraction and study of the essential oil *Rosmarinus Officinalis* Cuellie in the Region of Taza, Morocco

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ABSTRACT

Yield, chemical composition and antibacterial properties of the essential oil extracted from Rosmarinus officinalis in the region of Taza (Morocco) were studied. The average content of essential oil from the leaves of this species is 0.86% relative to the dry matter. Thirty-seven compounds were identified by GC / MS. The Dimenthol (38.83%), the Campholène aldehyde (16.02%), the α-pinene (11.05%), and borneol (10%) are the major chemical compounds of Rosmarinus oil. Strong inhibitory activity against eight pathogenic microorganisms tested was recorded.

Keywords: Rosmarinus officinalis essential oil, chemical composition, antimicrobial properties, Morocco.

INTRODUCTION

Rosemary belongs to the mint family, and it occurs as a shrub, sub-shrub or herbaceous. The leaves are narrowly lanceolate-linear, brittle and tough. Flowers are pale blue; purple spotted internally and arranged in short dense clusters. They bloom almost throughout the year [1].

Rosemary essential oil has been the subject of numerous studies, and various compositions have been described in terms of the major constituents, namely:

- More than 40% cineole (Morocco, Tunisia, Turkey, Greece, Yugoslavia, Italy, France)
- α-pinene, 1,8-cineole and camphor in similar proportions (Spain, France, Italy, Greece, Bulgaria)
- Myrcene is high in (Spain, Portugal, Argentina)
- Camphor, cineole, borneol (Cuba)
- Cineole, borneol, p-cymene (Turkey)
- α-pinene, verbenone, bornyl acetate (Corsica, Sardinia) [2].

Rosemary (Rosmarinus officinalis L.) grows worldwide, it has been cultivated for a long time to benefit from its strong antioxidant and antimicrobial activities. Antiviral, anti-inflammatory and anti-carcinogenic activities of this plant species have also been reported [3-5].

It has also been used to relieve symptoms caused by respiratory disorders and to stimulate hair growth. Rosemary extracts are used in aromatherapy to treat conditions related to anxiety and increase alertness [6].

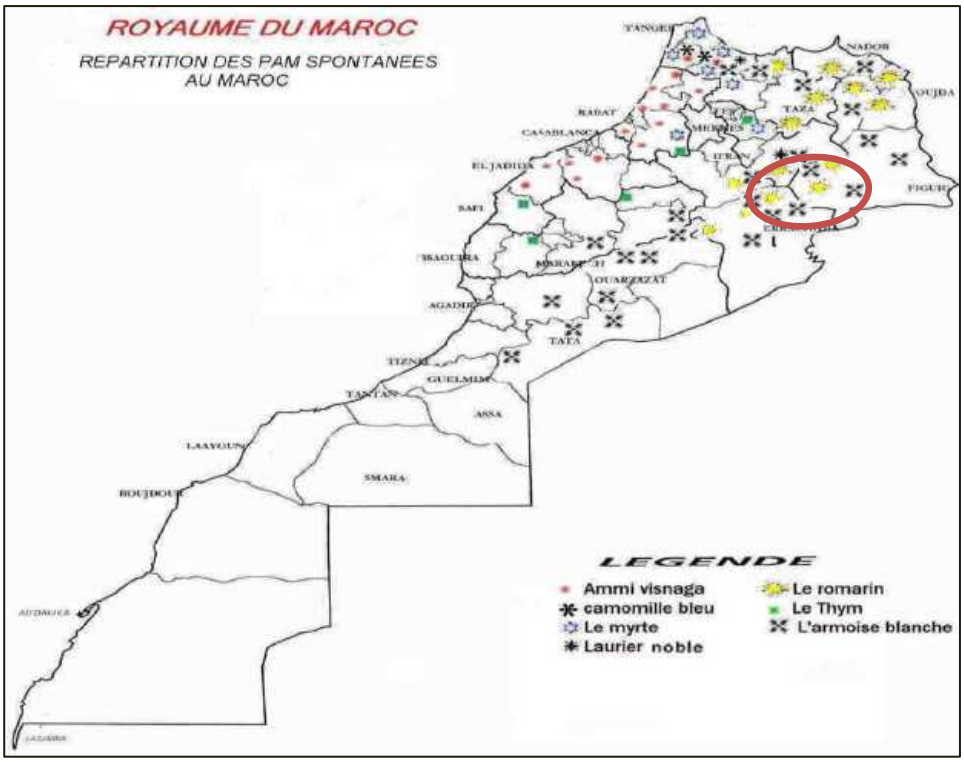
Morocco has a very great potential in the field of medicinal and aromatic plants (MAP). Currently, significant productions in MAP and their derivatives are realized, and this country is considered as one of the leading suppliers worldwide of rosemary, verbena, and rose [7]. 90% of professionals use uncultivated rosemary,which is located on the banks of the Moulouya the Rif Atlas, the Middle Atlas, the great Atlas, and the Eastern region, and more than 60 tonnes are exported annually [7].

The present study has two subjects, the first one is to characterize the Rosmarinus officinalis plant through the analysis of its essential oil using Gas Chromatography coupled with mass spectroscopy, and the second one is to determine the anti-bacterial effect of its essential oil. The chosen species for this study grows in the region of Taza, Morocco.

MATERIALS AND METHODS

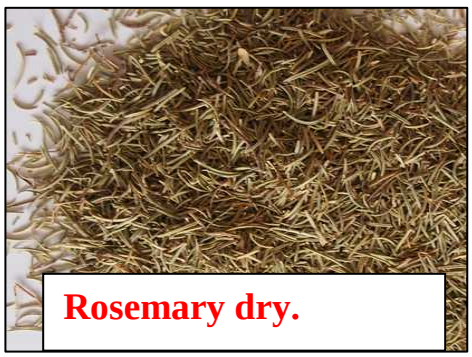
Plant material

Rosemary leaves used in this study were harvested in the region of Taza (Morocco) in the month of February 2012, this area is known by a significant production of the plant.



Map 1. Distribution of aromatic and medicinal plants in Morocco.

The studied samples were dried in the shade for ten days.



Extraction of essential oils
Essential oils extraction was carried out by hydro distillation in a Clevenger-type apparatus [8]. Three distillations were realized by boiling a sample of 200 g of rosemary in one liter of distilled water for 3 hours. The distillation system is comprised of a balloon of 2 liter surmounted by a column of 60 cm length which is connected to a refrigerant. The yield of essential oil was determined relative to the dry matter. The essential oil was stored at 4 °C in the dark in the presence of anhydrous sodium sulfate.

Chromatographic analysis
The essential oil was analyzed using gas chromatography (GC Ultra Trace) coupled to mass spectrometry GC / MS (Polaris Q ion trap MS), fitted with a capillary column VB-5 (5% methylpolysiloxane phenyl). The carrier gas was helium; the oven temperature was programmed at 200 °C for 6 min and increased to 300 °C with a rate of 20 °C / min for 10 min [UATRS].

Studied microorganisms
The microorganisms used in this study were: Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Enterobacter, Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae. The selected strains cause several nosocomials infections (urinary, intestinal, Respiratory, etc.) [9,10].

These germs usually have a resistance to antibiotics; they were maintained by subculture on nutrient agar favorable to their growth.

Microbiological procedure
- Dissemination method used on sterile discs.
We seeded flood Muller-Hinton agar with a pathogenic bacterial strain in a titer known nutrient broth. The disks were then organized on sterile agar. The discs are formed by sterilized filter paper of 6 mm diameter.

These discs were soaked with essential oil solutions. After incubation at 37 °C for 24 hours, zones of inhibition appear around these sterile discs. The tests are repeated three times.

- *The method of serial dilution*
- *The method of serial dilution*

Oil emulsification has previously been achieved through a solution of agar agar 0.2 % [11,12]. Since this oil is not miscible with water, and therefore the culture media, it provides a homogeneous distribution of compounds in the dispersed state in the medium and maximizes the contact germ / compound. The first dilution of the oil in agar agar solution was 1/10. Amounts of this dilution were added to test tubes containing nutrient agar for bacteria and mold to the PDA. They were then sterilized, cooled to 45 °C and poured into Petri dishes. The final concentrations of essential oils were 1/100, 1/250, 1/500, 1/1000 and 1/5000 (v/v). Controls containing the culture medium and the agar agar solution 0.2 % alone were also prepared. The assembly was done by streaking with a platinum loop calibrated to withdraw the same volume of inoculum. The latter is in the form of culture broth of the bacteria for 24 hours. The incubation temperature was 37 °C for 24. Each test was repeated three times to minimize the experimental error.

RESULTS AND DISCUSSION

The yield
The average yield of essential oil obtained from the leaves of Rosmarinus officinalis was 0.86 %. This is lower than that quoted by Bekkara et al [13], but it is higher than that quoted by Ayadi et al [14].

The above authors have shown in a study using the leaves of Rosmarinus officinalis in the region of Sidi Bouzide in Tunisia, that the essential oil content after extraction during 4h is 1.35 %. It was only 0.6 % for Rosmarinus officinalis in the area Tlemcen in Algeria [13, 14].

This variation in performance can be attributed not only to the origin of the plant and the extraction technique but also to the period of collection of the vegetable plant.

Chemical composition of the essential oil
The results of the chemical analysis of the essential oil from rosemary leaves by gas chromatography coupled to a mass spectrum are summarized in Table 1.

The Analysis by GC/MS, revealed 37 compounds in the essential oil of Rosmarinus officinalis, and the major compounds obtained: Dimenthol (38.83%), Campholène aldehyde (16.02%), pinene (11.05%) and Borneol (10%) were studied.

The chemical composition of the essential oil of Rosmarinus officinalis in the region of Taza shows qualitative and quantitative differences compared to the work of Bekkara et al [13], Ayadi et al [14]. Pintore G. et al [15] and O. Yesil Celiktas [16].

The authors cited above have shown in a study on the leaves of Rosmarinus officinalis in the region of Sidi Bouzide in Tunisia that essential compounds are 1,8-cineole (58.1%) , α-pinene (11.5 %) and camphor (7.8%) [13]. In Tlemcen (Algeria) they consists of camphor (13.8 %), α-pinene of (12.6 %), cineole (11.8 %) and borneol (10.8 %) [14].

The analysis of the Italian species showed that it consists essentially of α - pinene (13.7 %) ; 1,8- cineole (3.4%) ; camphor (2.9 %) and verbanone (20.3 %) . [15]. The Turkish is mainly composed of α-pinene (7.8%); 1,8- cineole (60.9 %) and camphor (7.1%) . [16].

Variations encountered in the qualitative and quantitative composition of our plant in comparison with other reported in the literature may be due to several factors such as Environment [17,18], climatic and geographical conditions, collection period [19] and extraction method [20] .

Table 1. Chemical composition of the essential oil of Rosmarinus officinalis of Taza region (Morocco)

Constituents	Retention time	%
Δ ^{3}-carene	7.66	0.21
α -pinene	**8.10**	**11.05**
camphene	**8.55**	**5.31**
sabinene	9.50	1.72
1, 3,6-octatriene, 3,7-dimethyl	10.15	0.48
&-phellandrene	10.51	0.16
Γ-terpinene	10.98	0.36
di menthol	**11.59**	**38.83**
1,4-cyclohexadiene, 1-methyl-4 (1-methylethyl)	12.51	0.11
Santolina triene	14.06	1.48
2,4-Hexadiene, 2,3-dimethyl	14.47	0.76
Bicyclo (2, 2,1) heptane-3-methylene-2 ,2-dimethyl-5-ol acetate	14.83	0.11
Campholène aldehyde	**15.50**	**16.02**
isobornyl formate	15.92	0.02
Bicyclo (3.1.1) hept-3-en-2-one,4,6,6-trimethyl, 1S	16.08	0.17
borneol	**16.34**	**10.00**
Trans-hydrate Sabinene	16.67	1.06
& Terpinenyl acetate	**17.20**	**4.42**
2-Isopropylidene-3-methyl-hexa-3,5-dienal	17.74	0.17
1, 3,6-Ontatrienne, Z 3,7-dimethyl	19.44	0.37
Bicyclo (221) heptane-2-ol, 1,7,7 - trimethyl-acetate	20.37	0.97
Germacrene-D	23.31	0.09
Aromadendrene	24.66	1.20
azulene	25.70	0.17
naphthalene	27.57	0.16
cadinene	27.85	0.18
Tetracyclo (6, 3, 2,0) tri-decan-9-ol 4 ,4-dimethyl	28.64	0.06
caryophyllene oxide	29.53	0.87
	29.81	0.23
caryophyllene oxide	30.28	0.09
Cubenol	30.48	0.07
Calarene epoxide	30.96	0.23
caryophyllene oxide	31.07	0.24
cadinene	31.20	0.91
6-isopropenyl-4, 8 - dimethyl-1, 2,3,5,6,7 octahydro naphthalene-2-ol	31.68	0.81
	32.06	0.26
Androst-5-en-17-one, 3-hydroxy (3a)	39.57	0.06

Antibacterial activity

It consists of inhibition diameter determination by the diffusion method on sterile discs. The antimicrobial effect of the essential oil of Rosemary was tested on seven bacterial strains.

In general, essential oils of Rosemary officinalis have shown a broad spectrum of activity against the tested microorganisms [21].

The antibacterial properties of Rosemary essential oil are attributed to the presence of &-pinene, 1,8-cineole, camphor, borneol and verbinone [22].

Concerning our study, the experimental results presented in Table 2 show that the extracted rosemary essential oil is active on all strains except Pseudomonas aeruginosa. Other strains behave differently with diameters between 12 and 22mm.

The largest zone of inhibition is observed in the case of Enterobacter, followed by two strains of Staphylococcus aureus and Proteus vulgaris. These species are the most sensitive to this oil.

The antimicrobial activity of this essential oil is mainly due to its richness in the following components: The α-pinene, camphor, borneol and esters. Indeed, all these compounds are known for their antimicrobial properties.

Table 2. Antibacterial activity of essential oils of Rosmarinus officinalis. (Zones of inhibition in mm

Gram negative	**Zones of inhibition in mm**
- *Escherichia coli*	12
- *Enterobacter*	22
- *Pseudomonas aeruginosa*	_
- *Acinetobacter baumannii*	20
- *klebsiella pneumonia*	12
- *Proteus Vulgaris*	16
Gram positive	**Zones of inhibition in mm**
- *Staphylococcus* aureus	18

The obtained results using the method of serial dilutions are summarized in Table 3. This method shows that the essential oil of Rosmarinus officinalis has a significant antibacterial activity against the tested strains. However, the studied microorganisms did not show the same sensitivity with respect to the essential oil used. Staphylococcus aureus, exhibits a high sensitivity in comparaison with Proteus and Enterococcus. It should also be noted that Escherichia coli and Klebsiella have presented the same susceptibility to this oil. Proteus is inhibited from the concentration of 1/10.

Table 3. Antibacterial Activities of Rosemary essential oil in the region of Taza Morocco:

	1/10	**1/25**	**1/50**	**1/100**	**1/200**	**1/300**	**1/500**	**T**
Bacteria								
E. coli	_	_	+	+				+++
Proteus Vulgaris	_	+	+	+	+	+	+	+++
Entérocoques	_	_	+	+	+	+	+	+++
Staphylocoques	_	_	_	_	+	+	+	+++
Klebsiella	_	_	_	+	+	+	+	+++
Acinetobacter B	_	_	+	+	+	+	+	+++

T: witness (-) inhibition; (+) Growth

CONCLUSION

The present study aimed to determine the yield, chemical composition and antibacterial properties of the essential oil of rosemary grown in the region of Taza in Morocco.

The analysis of essential oils by GC / MS showed that the main compounds in its organic phase are Campholène aldehyde (16.02 %), α-pinene (11.05 %), borneol (10%), camphene (5.31 %) and Terpenyl acetate (4.92 %). The study of the antibacterial activity of the essential oil on gram-positive and gram negative bacteria showed the existence of an antibacterial activity especially against Enterobacter, Acinetobacter baumannii and Staphylococcus aureus. . This observed antimicrobial activity is mainly due to the main oil components namely: α-pinene, camphor, borneol and esters. These compounds are known for their antimicrobial properties.

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