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COMPARATIVE STUDY OF THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *ORIGANUM COMPACTUM* FROM THE SEVEN REGIONS OF MOROCCO AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The aim of this work is to determine the chemical composition of the essential oil of *Origanum compactum*, from the region of Meknes (Morocco), and their antimicrobial effect, and on the other hand, to carry out a comparison between the chemical composition of this essential oil with the six regions of Morocco: Larache, Chefchaouen, Al Hoceïma, Tetouan, Taounate and Rabat.
The essential oils of *Origanum compactum* thus obtained were analyzed and identified by gas chromatography coupled with mass spectrometry (GC/MS).
To determine the similarities and dissimilarities between the chemical compositions of the essential oil of *Origanum compactum* from the Meknes region with the other six regions of Morocco, we performed the principal component analysis (ACP).
Regarding antimicrobial activity, the essential oils of *Origanum compactum* have shown significant inhibitory activity against the bacteria and molds studied.

Keywords: *Origanum compactum*, Chemical composition, GC/MS, ACP, Antimicrobial

INTRODUCTION

Origanum compactum is an endemic plant from Morocco and southern Spain (Jahandiez and Maire, 1932). It is common in the North and the Center of the Country. The vernacular names are «zaatar tadlawi» and «zaatar», this dialect is carried by other origins of Morocco and various thyms (Bellakhdar, 1997). It is considered by Moroccans as the real zaatar.
It is a perennial plant with a pubescent stem covered with long hairs, stem leaves oval-ovoid, hairy, inflorescences in dense and short spikes, very purple, floral bracts oval-lanceolate, rigid, leathery, large flowers, calyx with five triangular teeth and sub-equal, with ciliolated margins. This plant is found in forests, scrub and matorrals, on well-drained soils, under semi-arid and subhumid bioclimates with hot and cool variants and at the level of thermomediterranean and mesomediterranean vegetation stages (Benabid, 2000). It is distributed in the Middle Atlas, Middle Atlantic Morocco, North Atlantic Morocco and the Rif (Fennane *et al*., 2007).
All over Morocco, this oregano is considered a panacea. It is widely used in folk medicine due to its multiple therapeutic effects. It is mainly used as an infusion or decoction in the treatment of dysentery, colitis, gastrointestinal complaints, gastric acidity and bronchopulmonary diseases, against colds, flu, otolaryngeal diseases (O.R.L.) and bronchitis. It is also administered as a fumigation. It is used as a gargle against affections of the mouth (canker sores and gingivitis) (Bellakhdar, 1997). In the region of Zaër, the leafy stem, in infusion or decoction, is also used as a hypoglycé miant (Lahsissene *et al*., 2009).
In the literature, it has been reported that *Origanum compactum* has antibacterial (Bouhdid *et al*., 2008; Sfeir *et al*., 2013), antifungal (Salghi *et al*., 2013), antioxidant, anti malarial (El Babili *et al*., 2011), antiproliferative activities against breast cancer cells (Chaouki *et al*., 2010) and molluscicides (Hmamouchi *et al*., 2000).

MATERIAL AND METHOD

Plant material

The plant material consists of all the aerial parts of *Origanum compactum* growing spontaneously in the Meknes region. It was then dried in the laboratory in the dark in a well-ventilated place at room temperature. The identification of this botanical species was carried out at the biology department of the Faculty of Sciences of Meknes, Morocco.

Extraction of essential oils

The essential oil was extracted from the aerial parts of *Origanum compactum* by hydrodistillation using a Clevenger type device. In fact, three distillations were carried out by boiling 100 g of each plant material impregnated with a sufficient amount of water for three hours. The essential oil yield (volume in mL) was determined relative to 100 g of the dry matter. Then the oil obtained was dried over anhydrous sodium sulfate (Na_2SO_4) and stored at a temperature of -4 °C in a dark bottle until needed.

Liquid-solid chromatography on silica gel

The essential oils obtained by hydrodistillation were fractionated by column chromatography. The operating conditions are presented below:
Column diameter: 4cm
Silica: 230-400 mesh (Janssen, Chimica)
Silica height: 30cm
Eluents: Hexane (100%); Ether/Hexane (3/97); Ether/Hexane (5/95); Ether/Hexane (8/92); Ether/Hexane (13/87); Ether/Hexane (20/80); Ether (100%).

Chromatographic analysis

The chromatographic analyzes were carried out on a gas chromatograph with electronic pressure regulation of the Hewlett-Packard type (HP 5890 series), equipped with a capillary column of fused silica of the VB-5 type (Methylpolysiloxane at 5% phenyl) of 30 m long, 0.25 mm in diameter and 0.25 µm film thickness, a flame ionization detector (FID) set at 260 °C and supplied with a mixture of H_2/air^{-1} gas and a split-splitless injector set at 250 °C. The injection mode is split (leakage ratio: 1/50, flow rate: 66 mL/min^{-1}). The carrier gas used is nitrogen with a flow rate of 1 mL/min^{-1}. The column temperature is programmed from 50 to 250 °C at a rate of 5 °C/min^{-1}. The device is controlled by a computer system of the «HP ChemStation» type managing the operation of the device and making it possible to follow the progress of the chromatographic analyzes. The fragmentation is carried out by electronic impact under a field of 70 ev. The carrier gas is helium, the flow rate of which is set at 1.4 mL/min^{-1}. These fragment ions are then separated as a function of their masse/charge ratio by the application of an electronic field (quadrupole), then collected by a

detector. All of these fragment ions constitute the mass spectrum. The device is connected to a computer system managing a library of NIST mass spectra.

Principal component analysis (PCA)

Principal component analysis (PCA) is one of the multivariate descriptive analyzes. The purpose of using this analysis is to summarize as much information as possible while losing as little as possible to facilitate the interpretation of a large amount of initial data or to give more meaning to the reduced data.
This analysis consists in transforming the «p» initial quantitative correlated correlates into «p» quantitative variables correlated or not called «principal components».
It aims to highlight, in graphical form, the maximum of the information contained in a Data Table of a large number of descriptors, to know the amount of variance explained by the few independent main axes and to identify the relationships between variables and records. In fact, it makes it possible to obtain a representation of the point cloud in a space of reduced dimension so that the inertia carried by this space is as large as possible. It is used when it comes to describing a Table of continuous numeric variables of type «quantitative variables x individuals».
Statistical studies were carried out with XLSTAT Version 2014. The PCA were performed with Pearson-type matrices. The CAH and dendrograms were performed with dissimilarity matrices calculated in Euclidean distance and the method of aggregation chosen systematically is the average link.

Biological test

The minimum inhibitory concentrations (MIC) of essential oils were determined according to the method reported by Remmal *et al.* (1993) and Satrani *et al.* (2001). Due to the immiscibility of essential oils in water and therefore in the culture medium, the emulsification was carried out using a 0.2% agar solution in order to promote the germ/compound contact. Dilutions are prepared 1/10, 1/25, 1/50, 1/100, 1/200, 1/300 and 1/500 in the agar solution.
In test tubes each containing 13.5 mL of TSA (Tryptic Soy Agar) agar medium, autoclaved for 20 minutes at 121 °C and cooled to 45 °C, 1.5 mL of each of the dilutions are added so as to obtain the final concentrations of 1/100 to 1/5000 (v/v). The tubes are then shaken well before being poured into petri dishes. Controls, containing only the culture medium supplemented with the 0.2% agar solution alone were prepared. The bacteria are streaked with a calibrated platinum loop to collect the same volume of inoculum. The latter is taken from a young culture in culture broth (6h at 37 °C). For fungi the agar medium (MEA) is seeded with a 6mm diameter fragment taken from the periphery of a fungal culture in the MEA medium seven days old. Each test was repeated three times to minimize the experimental error.

RESULTS AND DISCUSSION

Essential oil yield

The aerial part of *Origanum compactum* provided a limpid yellow essential oil with a very strong odor with an average yield of around 3.62%. The comparison of this essential oil yield with those reported in the literature, shows that *Origanum compactum* from the Meknes region is richer in essential oil. Indeed, this rate is higher than that found by Bakhy *et al.* (2014) on thirty-six samples from north-west Morocco (Rif) (0.31% to 2.44%). This increase in yield is probably due to the altitude, the soil, the climate and the harvest period.

GPC/MS analysis of the essential oil of *Origanum compactum* from the Meknes region

Chromatographic analyzes of the essential oil of *Origanum compactum* from the Meknes region have shown the appearance of seventeen peaks relating to seventeen constituents representing approximately 88.72% of the chemical composition of the essential oil (Figure 1). This composition is characterized by the predominance of Thymol (56.41%) and (+)-3-Carene (13.56%). Among the different classes of essential oil are ten monoterpene hydrocarbons, a sesquiterpene hydrocarbon, four oxygenated monoterpenes and two oxygenated sesquiterpenes representing respectively 27.18%, 3.67%, 57.21%, and 0.66% of the chemical composition (Table 1).

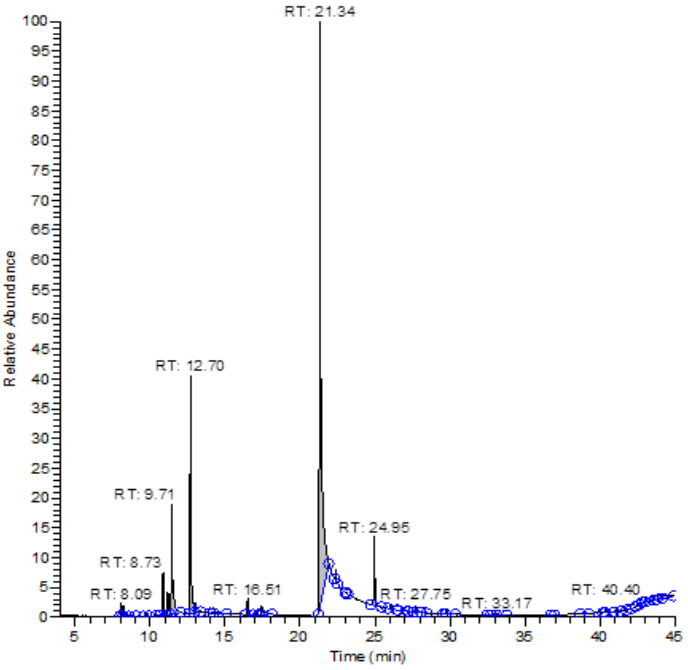


Figure 1 Chromatogram of the essential oil of *Origanum compactum* from the Meknes region

Table 1 Chemical composition of the essential oil of *Origanum compactum* from the Meknes region

IR	Compound	Formula	Percentage (%)
925	α-Thujene	$C_{10}H_{16}$	0.76
933	α-Pinene	$C_{10}H_{16}$	0.61
946	Camphene	$C_{10}H_{16}$	0.11
967	Sabinene	$C_{10}H_{16}$	0.18
999	α-Phellandrene	$C_{10}H_{16}$	8.64
1011	(+)-3-Carene	$C_{10}H_{16}$	13.56
1051	γ-Terpinene	$C_{10}H_{16}$	1.29
1089	α-Terpinolene	$C_{10}H_{16}$	1.51
1129	E,Z-Alloocimene	$C_{10}H_{16}$	0.12
1142	E,E-Alloocimene	$C_{10}H16$	0.40
1163	Terpinen-4-ol	$C_{10}H_{18}O$	0.15
1268	Thymol	$C_{10}H_{14}O$	56.41
1279	Carvacrol	$C_{10}H_{14}O$	0.57
1287	p-Cymen-7-ol	$C_{10}H_{14}O$	0.08
1434	β-Caryophyllene	$C_{15}H_{24}$	3.67
1581	Cedrol	$C_{15}H_{26}O$	0.44
1604	Cedrenol	$C_{15}H_{24}O$	0.22
Total			**88.72**
Monoterpene hydrocarbons			**27.18**
Sesquiterpene hydrocarbons			**3.67**
Oxygenated monoterpenes			**57.21**
Oxygenated sesquiterpenes			**0.66**

TR: Retention time, (-): Absence

In order to find new compounds of the essential oil of *Origanum compactum*, we chromatographed 2 g of the essential oil of *Origanum compactum* on a column in liquid phase at atmospheric pressure (CPL). Three fractions were recovered: F_1 (0.85 g), F_2 (0.66 g) and F_3 (0.39 g). Analysis by GPC/MS of the fractions F_1, F_2, and F_3 of the essential oil of *Origanum compactum*, made it possible to identify respectively 46.43%, 92.46%, and 56.41% of their chemical composition.
The analysis of the different fractions allowed us to identify sixteen new compounds compared to seventeen already identified in the crude essential oil. Indeed, the fraction F_1 mainly consists of β-Caryophyllene (19.42%) and of γ-Terpinene (11.25%) (Figure 2), On the other hand, the fraction F_2 consists mainly of Thymol (91.25%) (Figure 3). For fraction F_3, the presence of α-Terpineol (33.66%), Cubenol (10.35%) and Borneol (5.19%) is noted (Figure 4).

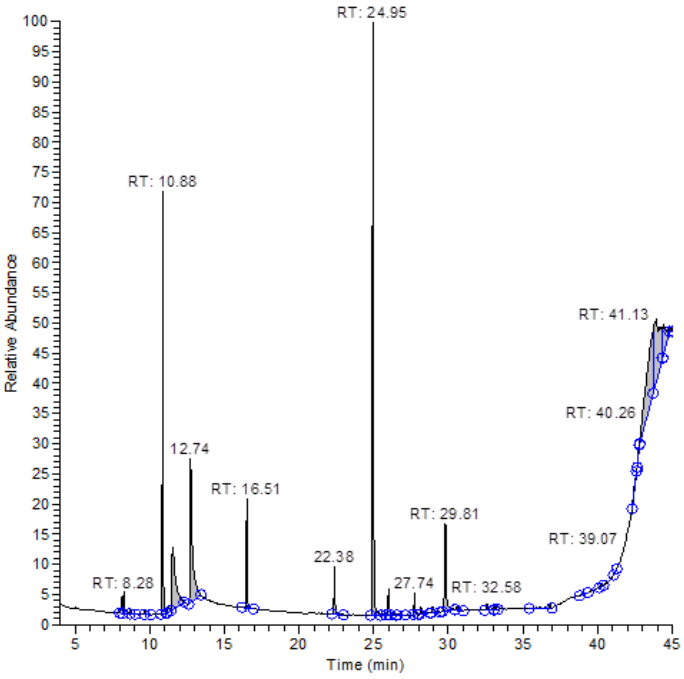


Figure 2 Chromatogram of the F_1 fraction obtained by CPL of the essential oil of *Origanum compactum* from the Meknes region

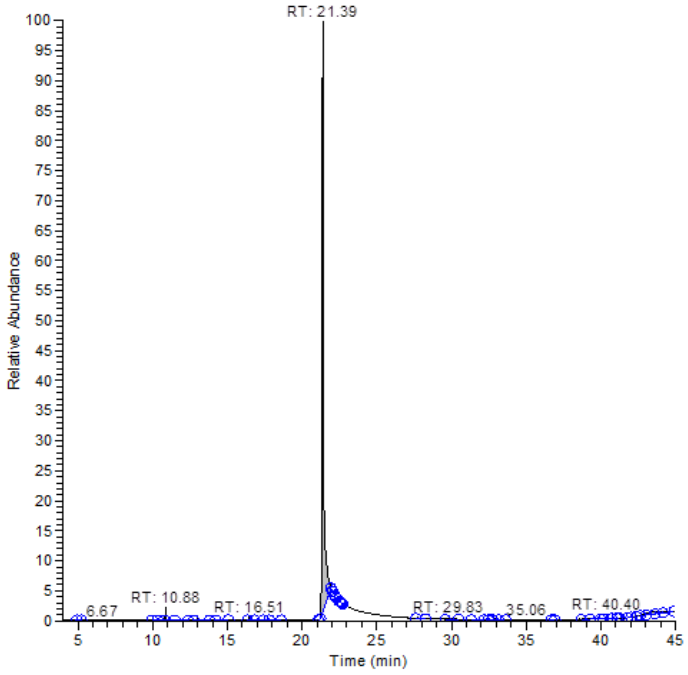


Figure 3 Chromatogram of the F_2 fraction obtained by CPL of the essential oil of *Origanum compactum* from the Meknes region

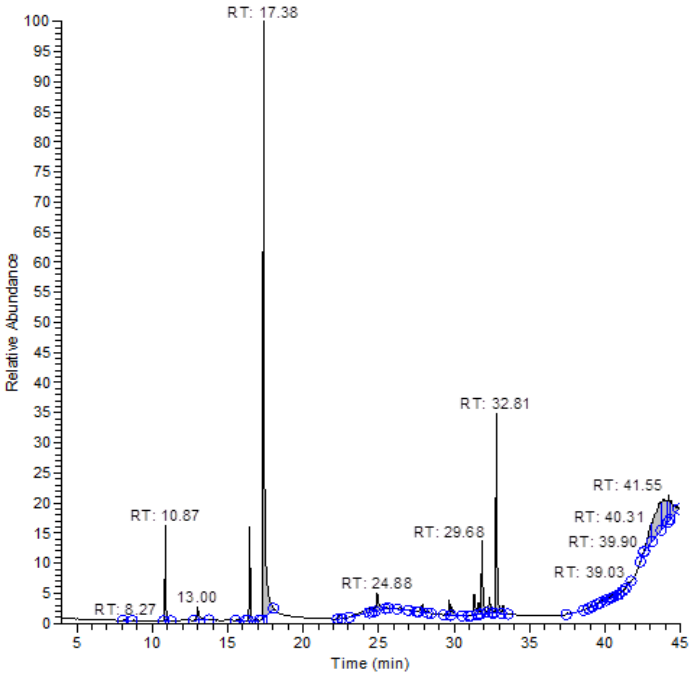


Figure 4 Chromatogram of the F_3 fraction obtained by CPL of the essential oil of *Origanum compactum* from the Meknes region

We summarize the results obtained by gas chromatography coupled to mass spectrometry (GC/SM) of the three fractions in table 2 below:

Table 2 Chemical composition of the fractions obtained by CPL on silica gel of the essential oil of *Origanum compactum* from the region of Meknes

IR	Compound	Formula	Percentage (%)		
			F_1	F_2	F_3
925	α-Thujene	$C_{10}H_{16}$	0.70	-	-
933	α-Pinene	$C_{10}H_{16}$	0.97	-	0.14
1014	p-Cymène	$C_{10}H_{14}$	7.92	-	-
1051	γ-Terpinene	$C_{10}H_{16}$	11.25	-	-
1054	Trans Sabinene hydrate	$C_{10}H_{18}O$	-	-	1.44
1163	Terpinen-4-ol	$C_{10}H_{18}O$	-	0.20	-
1165	Borneol	$C_{10}H_{18}O$	-	-	5.19
1173	α-Terpineol	$C_{10}H_{18}O$	-	-	33.66
1242	Carvone	$C_{10}H_{14}O$	-	0.03	-
1268	Thymol	$C_{10}H_{14}O$	-	91.25	0.43
1279	Carvacrol	$C_{10}H_{14}O$	-	0.02	1.56
1287	p-Cymen-7-ol	$C_{10}H_{14}O$	-	-	0.46
1288	p-Menth-2-en-1-ol	$C_{10}H_{18}O$	-	0.28	-
1420	Longifolene	$C_{15}H_{24}$	-	-	0.90
1434	β-Caryophyllene	$C_{15}H_{24}$	19.42	-	-
1436	trans-α-Bergamotene	$C_{15}H_{24}$	-	-	0.17
1439	α-Guaiene	$C_{15}H_{24}$	1.00	-	-
1524	õ-Cadinène	$C_{15}H_{24}$	0.52	-	-
1569	Caryophyllene oxide	$C_{15}H_{24}O$	4.65	0.43	-
1576	Spathulenol	$C_{15}H_{24}O$	-	-	1.00
1581	Cedrol	$C_{15}H_{26}O$	-	0.07	0.30
1604	Cedrenol	$C_{15}H_{24}O$	-	0.18	-
1642	Cubenol	$C_{15}H_{26}O$	-	-	10.35
1885	8,14-Cedrandiol	$C_{15}H_{26}O_2$	-	-	0.81
Total			**46.43**	**92.46**	**56.41**
Monoterpene hydrocarbons			**20.84**	**0.00**	**0.14**
Sesquiterpene hydrocarbons			**20.94**	**0.00**	**1.07**
Oxygenated monoterpenes			**0.00**	**91.78**	**42.74**
Oxygenated sesquiterpenes			**4.65**	**0.68**	**12.46**

TR: Retention time, (-): Absence, Fi: Fraction number i

By comparing the chemical composition of the essential oil of the Meknes region with that of the six other regions of Morocco (Larache, Chefchaouen, Al Hoceïma, Tetouan, Taounate and Rabat), Bakhy *et al*. (2014) have shown the presence of twenty-five constituents in the essential oil of *Origanum compactum* from the Larache region, representing approximately 95.60%. The main compounds are: γ-Terpinene (30.10%), Carvacrol (29.70%) and p-Cymene (11.50%).

The identification of the various constituents revealed twelve monoterpene hydrocarbons, three sesquiterpene hydrocarbons, nine oxygenated monoterpenes and one oxygenated sesquiterpene representing respectively 48.10%, 1.90%, 45.40% and 0.20% of the chemical composition.

Similarly Bakhy *et al*. (2014) have shown the appearance of twenty-five constituents representing 98.10% of the chemical composition of the essential oil of *Origanum compactum* from the Chefchaouen region. The main constituents are: Thymol (28.30%), γ-Terpinene (26.70%) and p-Cymene (11.60%). Among these constituents are thirteen monoterpene hydrocarbons, one sesquiterpene hydrocarbon, ten oxygenated monoterpenes and one oxygenated sesquiterpene representing respectively 45.70%, 1.10%, 51.20% and 0.10% of the chemical composition of the essential oil.

Lahlou (2002) revealed that the essential oil of *Origanum compactum* from the Al Hoceïma region contains fourteen constituents representing approximately 97.70% of the chemical composition of the essential oil. This composition is characterized by the predominance of Carvacrol (59.10%) and p-Cymene (11.70%). Among the different classes of essential oil there are six monoterpene hydrocarbons, one sesquiterpene hydrocarbon, six oxygenated monoterpenes and one oxygenated sesquiterpene representing respectively 22.30%, 1.60%, 73.20% and 0.60% of the chemical composition.

Amakran *et al*. (2014) have shown the appearance of twenty-one constituents representing approximately 99.74% of the essential oil of *Origanum compactum* from the Tetouan region. The main compounds are: Carvacrol (68.99%) and Thymol (18.67%). The identification of the various constituents revealed nine monoterpene hydrocarbons, four sesquiterpene hydrocarbons, seven oxygenated monoterpenes and one oxygenated sesquiterpene representing respectively 7.87%, 1.28%, 90.19% and 0.40% of the chemical composition.

Ouedrhiri *et al*. (2016) identified twenty-two constituents representing approximately 99.45% of the essential oil of *Origanum compactum* from the Taounate region. The main compounds are: Carvacrol (47.85%), γ-Terpinene (17.25%), Thymol (15.75%) and p-Cymene (8.44%). The identification of the various constituents revealed fourteen monoterpene hydrocarbons, one sesquiterpene hydrocarbon and seven oxygenated monoterpenes representing respectively 32.22%, 1.44% and 65.79% of the chemical composition.

El Babili *et al*. (2011) recorded the appearance of forty-six constituents representing 98.08% of the chemical composition of the essential oil of *Origanum compactum* from the region of Rabat. The main constituents are: Carvacrol (36.46%), Thymol (29.74%) and p-Cymene (24.31%). Among these constituents, there are eleven monoterpene hydrocarbons, fourteen sesquiterpene hydrocarbons fourteen oxygenated monoterpenes and seven oxygenated sesquiterpenes representing respectively 27.88%, 1.20%, 67.94% and 1.06% of the chemical composition. All the results are summarized in table 3.

Table 3 Chemical composition of the essential oils of *Origanum compactum* from seven regions of Morocco

IR	Compound	Formula	La	Ch	Al	Te	Ta	Ra	Me
925	α-Thujene	$C_{10}H_{16}$	1.10	1.20	0.80	0.09	-	0.03	0.76
933	α-Pinene	$C_{10}H_{16}$	0.50	0.60	0.40	0.13	0.61	0.62	0.61
946	Camphene	$C_{10}H_{16}$	-	0.10	-	-	0.09	0.11	0.11
962	1-Octen-3-ol	$C_8H_{16}O$	-	0.80	1.10	0.08	-	0.23	-
965	3-Octanone	$C_8H_{16}O$	0.70	1.20	-	-	0.09	0.12	-
967	Sabinene	$C_{10}H_{16}$	0.10	0.30	-	-	1.06	-	0.18
971	β-Thujene	$C_{10}H_{16}$	-	-	-	-	0.21	-	-
973	β-Pinene	$C_{10}H_{16}$	0.10	0.10	-	-	0.21	-	-
980	3-Octanol	$C_8H_{18}O$	-	0.20	-	-	-	-	-
983	Myrcene	$C_{10}H_{16}$	1.70	1.90	1.20	0.44	1.42	0.33	-
999	α-Phellandrene	$C_{10}H_{16}$	0.20	0.20	-	0.05	0.24	0.04	8.64
1001	2-Carene	$C_{10}H_{16}$	-	-	-	-	0.12	-	-
1010	α-Terpinene	$C_{10}H_{16}$	2.20	2.30	1.10	0.48	2.19	0.36	-
1011	(+)-3-Carene	$C_{10}H_{16}$	-	-	-	-	0.07	0.04	13.56
1014	p-Cymene	$C_{10}H_{14}$	11.50	11.60	11.70	2.53	8.44	24.31	-
1023	Limonene	$C_{10}H_{16}$	0.30	0.40	-	0.11	0.25	-	-
1023	β-Phellandrene	$C_{10}H_{16}$	0.20	0.20	-	0.06	-	-	-
1038	(E)-β-Ocimene	$C_{10}H_{16}$	0.10	0.10	-	-	0.06	-	-
1051	γ-Terpinene	$C_{10}H_{16}$	30.10	26.70	7.10	3.98	17.25	1.10	1.29
1054	trans Sabinene hydrate	$C_{10}H_{18}O$	0.30	0.30	-	-	-	-	-
1063	3-Nonanone	$C_9H_{18}O$	0.10	-	-	-	-	-	-
1084	Linalool	$C_{10}H_{18}O$	1.00	1.70	2.00	1.10	1.36	0.57	-
1086	trans- Linalool oxide	$C_{10}H_{18}O_2$	-	-	-	-	-	0.01	-
1088	Dimethyl styrene	$C_{10}H_{12}$	-	-	-	-	-	0.13	-
1089	α-Terpinolene	$C_{10}H_{16}$	-	-	-	-	-	-	1.51
1108	1,3,8-p-Menthatriene	$C_{10}H_{14}$	-	-	-	-	-	0.81	-
1129	E,Z-Alloocimene	$C_{10}H_{16}$	-	-	-	-	-	-	0.12
1142	E,E-Alloocimene	$C_{10}H16$	-	-	-	-	-	-	0.40
1143	Camphre	$C_{10}H_{16}O$	-	-	-	-	-	-	-
1163	Terpinen-4-ol	$C_{10}H_{18}O$	0.20	0.30	0.90	0.58	-	-	0.15
1165	Borneol	$C_{10}H_{18}O$	-	-	-	0.19	0.15	0.03	-
1173	α-Terpineol	$C_{10}H_{18}O$	4.10	6.80	1.00	0.58	-		-
1183	p-Cymen-8-ol	$C_{10}H_{14}O$	-	-	-	-	0.43	0.14	-
1194	Myrtenol	$C_{10}H_{16}O$	-	-	-	-	0.16	-	-
1226	Carvacryl methyl oxide	$C_{11}H_{16}O$	7.10	4.90	-	-	-	-	-
1242	Carvone	$C_{10}H_{14}O$	-	-	-	-	-	-	-
1244	Methyl carvacrol	$C_{11}H_{16}O$	-	-	-	-	-	0.06	-
1252	Piperitone	$C_{10}H_{16}O$	-	-	-	-	-	0.03	-
1263	Geranial	$C_{10}H_{16}O$	-	-	-	-	-	0.04	-
1268	Thymol	$C_{10}H_{14}O$	2.20	28.30	9.10	18.67	15.75	29.74	56.41
1279	Carvacrol	$C_{10}H_{14}O$	29.70	6.70	59.10	68.99	47.85	36.46	0.57
1287	p-Cymen-7-ol	$C_{10}H_{14}O$	-	-	-	-	-		0.08
1288	p-menth-2-en-1-ol	$C_{10}H_{18}O$							
1364	Piperitenone	$C_{10}H_{14}O$	-	-	-	-	-	0.44	-
1367	Eugenol	$C_{10}H_{12}O_2$	-	-	-	-	-	0.06	-
1387	α-Copaene	$C_{15}H_{24}$	-	-	-	-	-	0.02	-
1420	Longifolene	$C_{15}H_{24}$							
1418	Caryophyllene	$C_{15}H_{24}$	1.70	1.10	1.60	1.09	1.44	-	-
1421	α-Gurjunene	$C_{15}H_{24}$	-	-	-	-	-	0.02	-
1434	β-Caryophyllene	$C_{15}H_{24}$	-	-	-	-	-	0.78	3.67
1436	trans-α-Bergamotene	$C_{15}H_{24}$	-	-	-	0.06	-	-	-
1439	α-Guaiene	$C_{15}H_{24}$							
1451	α-Humulene	$C_{15}H_{24}$	0.10	-	-	0.06	-	0.05	-
1470	allo-Aromodendrene	$C_{15}H_{24}$	-	-	-	-	-	0.02	-

1486	γ -Muurolene	$C_{15}H_{24}$	-	-	-	-	-	0.02	-
1494	β-Ionone	$C_{13}H_{20}O$	-	-	-	-	-	0.01	-
1500	β-Bisabolene	$C_{15}H_{24}$	0.10	-	-	-	-	0.02	-
1506	α-Muurolene	$C_{15}H_{24}$	-	-	-	-	-	0.02	-
1509	Ledene	$C_{15}H_{24}$	-	-	-	-	-	0.02	-
1520	γ-Cadinene	$C_{15}H_{24}$	-	-	-	-	-	0.07	-
1524	õ-Cadinene	$C_{15}H_{24}$	-	-	-	0.07	-	0.10	-
1541	α-Cadinene	$C_{15}H_{24}$	-	-	-	-	-	0.01	-
1551	α-Calacorene	$C_{15}H_{20}$	-	-	-	-	-	0.01	-
1569	Caryophyllene oxide	$C_{15}H_{24}O$	0.20	0.10	-	0.40	-	0.86	-
1576	Spathulenol	$C_{15}H_{24}O$	-	-	0.60	-	-	0.03	-
1581	Cedrol	$C_{15}H_{26}O$	-	-	-	-	-		0.44
1604	Cedrenol	$C_{15}H_{24}O$	-	-	-	-	-		0.22
1611	Humulene epoxide	$C_{15}H_{24}O$	-	-	-	-	-	0.05	-
1641	Caryophylla 4(14), 8(15) dien-5-ol	$C_{15}H_{26}O$	-	-	-	-	-	0.03	-
1642	Cubenol	$C_{15}H_{26}O$	-	-	-	-	-	-	-
1647	T-Cadinol	$C_{15}H_{26}O$	-	-	-	-	-	0.02	-
1653	α-Cadinol	$C_{15}H_{26}O$	-	-	-	-	-	0.01	-
1668	14-hydroxy-9-epi-(E)-caryophyllene	$C_{15}H_{24}O$	-	-	-	-	-	0.06	-
1680	Cadalene	$C_{15}H_{18}$	-	-	-	-	-	0.04	-
1683	Bisabolol	$C_{15}H_{26}O$	-	-	-	-	-	-	-
1885	8,14-cedrandiol	$C_{15}H_{26}O_2$	-	-	-	-	-	-	-
Total			**95.60**	**98.10**	**97.70**	**99.74**	**99.45**	**98.08**	**88.72**
Monoterpene hydrocarbons			**48.10**	**45.70**	**22.30**	**7.87**	**32.22**	**27.88**	**27.18**
Sesquiterpene hydrocarbons			**1.90**	**1.10**	**1.60**	**1.28**	**1.44**	**1.20**	**3.67**
Oxygenated monoterpenes			**45.40**	**51.20**	**73.20**	**90.19**	**65.79**	**67.93**	**57.21**
Oxygenated sesquiterpenes			**0.20**	**0.10**	**0.60**	**0.40**	**-**	**1.06**	**0.66**
Ester			**-**	**-**	**-**	**-**	**-**	**0.01**	**-**

La: Larache, Ch: Chefchaouen, Al: Al Hoceïma, Te: Tetouan, Ta: Taounate, Ra: Rabat, Me: Meknes, TR: Retention time, (-): Absence

Principal component analysis (PCA) for the main compound of *Origanum compactum* essential oil from seven regions of Morocco

The analysis of the links between the chemical composition of the essential oils of *Origanum compactum* from the Meknes region and six other regions of Morocco: Larache, Chefchaouen, Al Hoceïma, Tétouan, Taounate and Rabat was carried out using this method (PCA), only the discriminating variables were taken into account.

To carry out this analysis, two first factorial axes were chosen. The dispersion of the *Origanum compactum* species in the plane formed by these two axes in relation to the chosen variables explains 72.73% of the variability, including 47.94% on the first axis and 24.79% on the second axis (Figure 5).

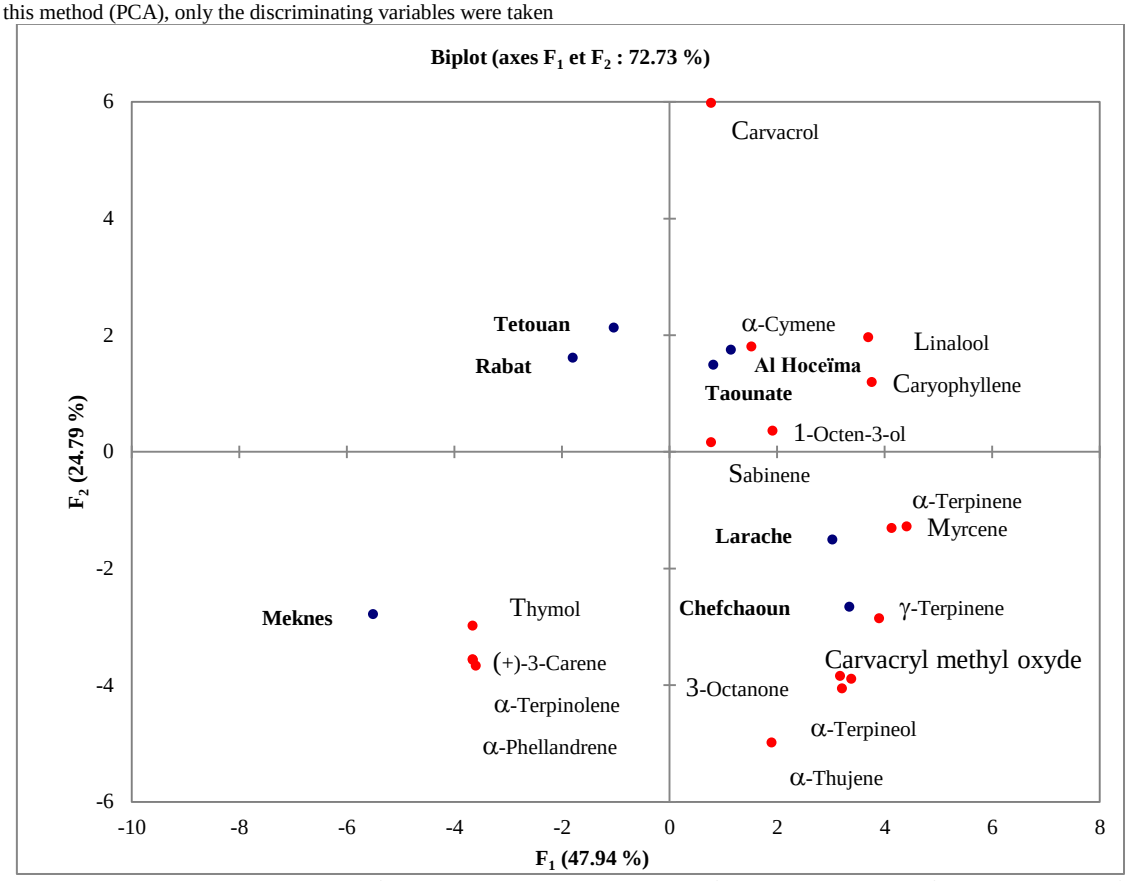


Figure 5 Principal component analysis for the main essential oil compound of *Origanum compactum* from seven regions of Morocco

This figure shows the separation of four groups in the two axis systems:

- The group formed by *Origanum compactum* from the Meknes region is characterized by the high rate of Thymol and (+)-3-Carene,
- *Origanum compactum* from Larache and Chefchaouen forms a group close to *Origanum compactum* from Meknes with a high Thymol content. The high level of γ-Terpinene made it possible to separate this group from the other groups,
- The group formed by *Origanum compactum* from Tetouan and Rabat is close to Meknes by a high rate in Thymol and close to the group formed by *Origanum*

compactum from the regions of Al Hoceïma and Taounate by a high rate in Carvacrol,

- The group formed by *Origanum compactum* from Al Hoceïma and Taounate is characterized by high levels of α-Cymene and Carvacrol. This group is close to the group formed by *Origanum compactum* from the regions of Tetouan and Rabat by a high content of Carvacrol.

The dendrograms have clearly visualized the links between *Origanum compactum* of the seven regions studied (Figure 6).

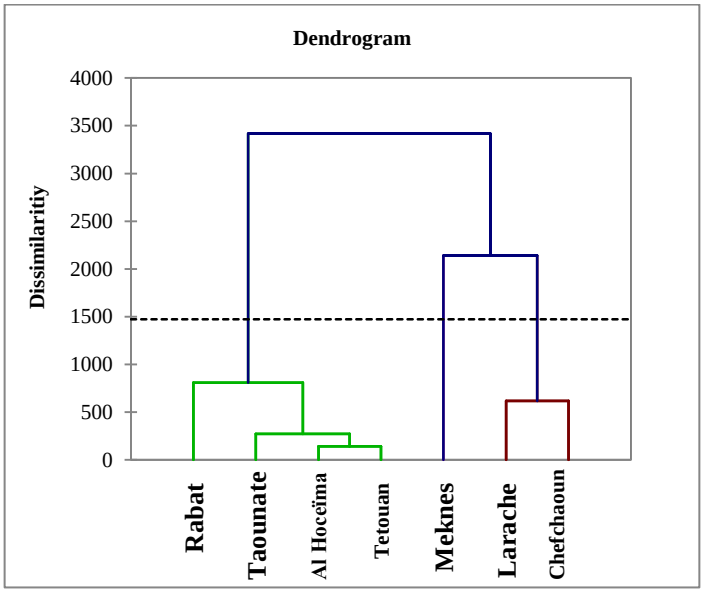
Antimicrobial activity of *Origanum compactum*

The essential oil of *Origanum compactum* has a significant inhibitory activity against the bacteria and molds studied. The result of this antimicrobial and antifungal activity is presented in table 4.

The results of the Table show that the essential oil of *Origanum compactum* inhibited the growth of *B. cereus* and *S. aureus* from a low concentration of the order of 0.30 mg/mL, while *E. coli*, *E. amylovora*, *P. savastoni* and *S. thyphi* are inhibited from a concentration of 0.45 mg/mL.

The seven molds showed different sensitivity behavior towards the essential oil of *Origanum compactum*. *P. expansum* is the most sensitive, with an MIC of 0.30 mg/mL, while *A. alternata*, *B. cinerea*, *P. digitatum*, *P. italicum*, *V. dahlea* and *A. niger* were inhibited from a concentration of 0.45 mg/mL.

The transfer of the mold discs to fresh MA medium has shown that the essential oil has a fungicidal effect on the mycelial growth of all molds from a concentration of 3.64 mg/mL, while an effect is obtained. Fungistatic from the essential oil concentration of 0.45 mg/mL.



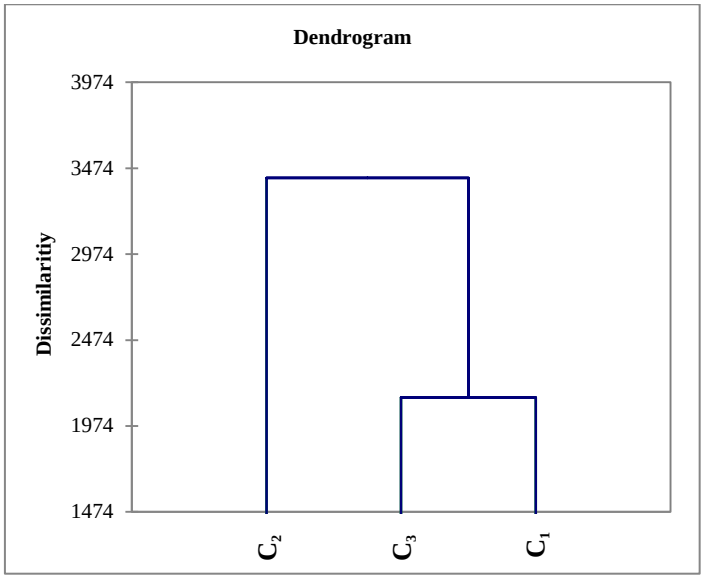


Figure 6 Dendrograms obtained from the analysis of the chemical composition of the essential oils of *Origanum compactum* from the seven regions of Morocco

The antifungal activity of essential oil from *Origanum compactum* has been demonstrated by several studies; against: the species of *Aspergillus* and *A. niger* (Charai *et al*., 1996), *P. italicum*, *A. alternata* (Sakkas and Papadopoulou, 2017), against Gram + bacteria and Gram- bacteria and also against resistant strains of *E. coli* (Ben Hammou *et al*., 2011; Bouhdid *et al*., 2008).

Laghmouchi *et al*., (2018) reported that the MIC for *S. aureus* decreases with increasing concentration of the essential of *Origanum compactum*. So this oil can be used as a substitute for chemical preservatives for the control of *S. aureus*: agent responsible for poisoning through the consumption of contaminated meat products. Antimicrobial activity has also been confirmed by other work (Bouyahya *et al*., 2016; Nayely *et al*., 2017; Lu *et al*., 2018; Teodora and Ralitsa, 2016).

Table 4 Minimum inhibitory concentrations of *Origanum compactum* essential oil on thirteen microorganisms

Concentration	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000	Witness
(mg/mL)	9.10	3.64	1.82	0.91	0.45	0.30	0.18	0.00
Bacteria								
B. cereus	-	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	+	+	+
E. amylovora	-	-	-	-	-	+	+	+
P.savastanoi	-	-	-	-	-	+	+	+
S.aureus	-	-	-	-	-	-	+	+
S. thyphi	-	-	-	-	-	+	+	+
Mushrooms								
A. alternata	-	-	-	-	-	+	+	+
A. niger	-	-	-	-	-	+	+	+
B. cinerea	-	-	-	-	-	+	+	+
P. digitatum	-	-	-	-	-	+	+	+
P. expansum	-	-	-	-	-	-	+	+
P. italicum	-	-	-	-	-	+	+	+
V.dahlea	-	-	-	-	-	+	+	+

(-): Inhibition, (+): Growth

CONCLUSION

The results showed that the essential oils of the Larache region are of the γ-Terpinene chemotype with a percentage of 30.10% and of the Chafchaoun and Meknes regions with the percentages 28.30% and 56.41%. Regions of Al Hoceïma, Tétouan, Taounate and Rabat have the Carvacrol chemotype with the percentages 59.10%, 68.99%, 47.85% and 36.46% respectively.

In terms of antimicrobial activity, essential oils extracted from *Origanum compactum* have shown significant inhibition of the six bacteria and seven molds studied from a concentration of 0.45 mg/mL.

At the end of this study, the essential oils of *Origanum compactum* can be recommended as antimicrobials against certain pathogenic microorganisms and

as alternatives to synthetic antibiotics. This approach can help reduce the amount of synthetic antibiotics applied, and subsequently decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution.

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