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EFFECT OF DOMESTICATION ON ESSENTIAL OIL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF ORIGANIUM COMPACTUM FROM TWO MOROCCAN LOCALITIES

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ABSTRACT. The aim of this work is to investigate the effect of domestication on the chemical composition and antibacterial activity of essential oils extracted from *Origanum compactum* collected in the Tidili and Chaouen regions of Morocco. The EOs chemical composition was determined by GC–MS. The antibacterial activity was tested against thirteen bacterial strains using the disc diffusion method. The minimum inhibitory concentration and the minimum bactericidal concentration were determined by the micro-dilution method. The results showed that the tested EOs exhibited a wide variation in their chemical composition, with carvacrol being the majority compound (46.1% - 59.0%) for all oils analyzed, followed by the two hydrocarbon monoterpenes, p-cymene (10.9% - 22.2%) and γ -terpinene (7.3% - 11.12%). Compared to wild forms, the essential oil of cultivated O. compactum contained a lower percentage of carvacrol and a higher content of its primary precursors, p-cymene and γ -terpinene. The EOs of O. compactum expressed important antibacterial activity against all thirteen strains studied. The diameters of the inhibitory zones varied from 8 to 45 mm, while the MIC values ranged from 518 to 1250µg/ml. Based on the results obtained, domestication can be suggested as a promising solution for the conservation of these endemic species of high socio-economic value.

Keywords: Essential oil, Origanum compactum, chemical composition, antibacterial activity, cultivated and wild plants.

INTRODUCTION

Nowadays, aromatic and medicinal plants (AMPs) are no longer just a remedy for poor communities in developing countries, but also a source of active biomolecules that are in high demand in the pharmaceutical, food, cosmetics and perfume industries [1]. These compounds having many promising pharmacological activities, such as antioxidants [2], antibacterial [3], antifungal [4], anti-inflammatory [5]. Their potential use in cosmetics formulation and as an alternative to chemical food additives has attracted the attention of a number of researchers.

Moreover, modern medicine makes use of the therapeutic virtues of EOs and their constituents. Indeed, many volatile compounds are now common ingredients in

pharmaceutical preparations. Among the main secondary metabolites used for their antiseptic, analgesic and antibacterial properties are thymol, eugenol, saponins, flavonoids, carvacrol, terpenes and their precursors [6] and are characterized by low toxicity for the host organism [7].

Morocco, due to its Mediterranean climate and geomorphological characteristics, benefits from favorable conditions for the development of a rich and varied flora including a large potential of aromatic and medicinal plants with a very marked endemism. Due to their very interesting biological activities, the PAMs differ greatly depending on species, genotypes and geographical origin [8]. Determining the chemical composition and biological activities of species from different provenances would certainly help in the discovery and development of new antimicrobial and/or preservative agents.

In fact, these AMPs have become subject to excessive over-exploitation associated with uprooting from their natural environments. This situation combined with soil degradation exposes these medicinal and endemic plants to the risk of extinction [9]. Moreover, it has been reported that they are mainly collected at the flowering stage, which jeopardizes the survival and regeneration of these plants [9, 10].

Domestication will be a promising solution for the conservation of AMPs while allowing their sustainable use. However, the cultivation of spontaneous medicinal plants requires a thorough knowledge of the different effects of domestication on the chemical composition and consequently on the biological properties of their essential oils. Accordingly, the low reproducibility of bioactive molecules is one of the main obstacles to the successful domestication of aromatic and medicinal plants [11].

Morocco is endowed with a floristic heritage as rich as it is varied, especially in the field of aromatic plants. The *Lamiaceae* family is represented in Morocco by 30 genera and 225 species, over 90 of which are endemic. These species are of great ecological and economic interest. In fact, it offers many medicinal and aromatic plants [12]. The genus *Origanum* is depicted by five species (three are endemic), including *O. Compactum*. Throughout Morocco, oregano, known locally as "Zaatar" and corresponding to the species *O. compactum*, is traditionally considered the most beneficial species for human health. It is widely used in Moroccan folk medicine for its multiple therapeutic effects. Indeed, it is recommended, among other things, in the treatment of diarrhea, respiratory, skin and urinary tract infections [13].

In this context, we proposed to conduct pioneering research on the effect of domestication on the chemical composition and antibacterial properties of essential oils extracted from *O. Compactum* from two Moroccan localities, Tidili and Chaoun.

MATERIALS AND METHODS

Crop experimental design

In July 2017, aerial parts of wildy Origanum compactum from two regions were harvested at the flowering stage. In October 2017, O. compactum seeds were sown in polyethylene bags (15 cm \times 10 cm) containing a mixture of sand and peat (2:1 w/w). The bags were placed in a shadehouse characterized by a temperature of 18-25°C, a photoperiod of 16/8 h, and 60-80% relative humidity. Crops were irrigated daily for the first week, then every other day. Two-month-old seedlings were transplanted into the field (6 m \times 6 m) in a randomized block design with 40 cm spacing between plants and between rows. The experimental area, located at the Dour Abyad (Marrakech, Morocco; 31.67N/-

7.95W), is characterized by an arid climate with an average temperature of 38.3 °C in the warmest month and of 4.5 °C in the coldest month, and a mean rainfall of 242 mm/year. Soil characteristics were as follows: sand, 37.3%; silt, 24.2%; clay, 38.5%; pH, 8.06; electrical conductivity, 0.28 ms/cm; organic matter, 2.9%; total nitrogen, 0.65%; P2O5, 18.2 mg/100 g; K2O, 48.9 mg/100 g; CaCO3, 35.1%. The crop was watered on alternate days for the first month to help plant establishment. Thereafter, irrigation was carried out as needed, depending on the rainfall.

Essential Oil extraction

Extraction of *O. compactum* essential oils was carried out by steam distillation in a Clevenger apparatus. Distillation was realized out using 200 g of the aerial part of the plant in 4 L of distilled water for 3 hours at 100°C [14].

Chemical analysis of Essential oil

Chemical analysis of the essential oils of the plant studied (wild and cultivated) was performed out by Agilent gas chromatography (19091S-433: 2169.66548) coupled with mass spectrometry (model HP 5973). The chromatograph is equipped with an Argilent DB5 MS 5% phenyl capillary column (30 m long \times 250 μm , 0.25 μm). The GC oven temperature program includes an isothermal step of 3°C/min from 50°C to 250°C. The carrier gas was argon with a constant linear velocity of 36.445 cm/s. Detector and injector temperatures were maintained at 230°C. The injection volume was 1 μl . The division ratio was 1:50. Mass spectrometry was carried out using a mass range of 41 to 450 m/z, with an ionizing voltage of 70 eV.

Antibacterial activity

Microbial strains

In this study, thirteen pathogenic bacteria of significant importance for food were used. The bacterial strains assayed included six Gram-positive, namely *Staphylococcus aureus* (209PCIP 53156) *Staphylococcus aureus* (ATCC 29213), *Micrococcus luteus* (ATCC 381), *Bacillus cereus* (ATCC 14579), *Enterococcus faecalis* (ATCC 29212), *listeria monocytogenes* 19115. Seven Gram-negative bacteria: *Escherichia coli* (ATCC 8739) *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (DSM 50090) *pseudomonas aeruginosa* (27853) and *Klebsiella pneumoniae* a clinically isolated *klebsiella pneumonia* (CIP 104727), *Salmonella enteritidis* DMB 560.

Antibacterial screening

The disk diffusion method on solid media was employed for the determination of the EOs antibacterial activity [15]. Briefly, a suspension of the tested microorganism in log phase (0.1mL) was spread on Mueller Hinton Agar (MHA) for bacteria. Filter paper discs (6 mm in diameter) were individually impregnated with $2\mu L$ of each EO and placed on the inoculated plates. The treated Petri dishes were placed at $4\,^{\circ}C$ for 2h and then incubated at $37\,^{\circ}C$ for 24 h for bacteria. The inhibition zones diameters were measured in millimeters. Cefixime ($2\mu g/disk$) was used as standard antibiotic. All the tests were performed in triplicate.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The broth micro dilution method was used to determine the minimum inhibitory concentration (MIC) according to the NCCLS guide lines M7-A4 [16]. The investigated oils were dissolved in 4% dimethyl-sulphoxide (DMSO). The tests were performed in Mueller Hinton Broth (MHB). A fresh overnight culture, in log phase, of the tested microorganisms was used to prepare the bacterial suspension adjusted to 10^6UFC/mL in MHB, and $100~\mu\text{L}$ were added to each well of 96-well plates. Subsequently, $100\mu\text{L}$ of each dilution of EOs were added. Negative controls were prepared with the (DMSO) solvent and positive controls containing the (DMSO) and suspension of bacteria. Plates were incubated at $37\,^{\circ}\text{C}$ for 24 h for bacteria.

To determine the minimum bactericidal concentration (MBC), $100\mu L$ of each broth in which no microbial growth was observed, was spread on Mueller Hinton agar (MHA) and incubated at $37^{\circ}C$ for 24 h. The MBC was the lowest concentration of the EO at which no colonies formed on the petri dish. Each test was performed in triplicate. Cefixime served as positive standard antibiotic.

RESULTS

Essential Oil yield and chemical composition

The table 1 present the yield results for wild and cultivated O. compactum essential oils in two regions (chaoun and tidili). These EO showed a slightly yellow-brown, mobile, limpid color with a strong, pungent odor. Average yields of *O. compactum* EO range from 0.99% to 3.5%. The highest value was observed in the populations collected from the Chaoun region, with 3.5% for wild oregano and 3.36% for cultivated oregano. In contrast, the lowest EO yield was obtained from the *O. comapctum* species harvested in the Tidili region, with 0.99% for cultivated oregano and 1.6% for wild oregano. Our results revealed that domestication of *O. compactum* from the Tidili region had an impact on its essential oil yields.

Table 1. Yield of O. compactum essential oils

	Ch	aoun	Tidili								
	Origanum compactum										
	wild	cultived	wild	cultived							
Yield (% v/w)	3.5±0.06	3.36±0.01	1.6±0.01	0.99 ± 0.02							

Analysis of the chemical composition of cultivated and wild O. compactum from two regions (Tidili and Chaoun) was carried out by gas chromatography coupled with mass spectrometry. This analysis identified 33 compounds representing more than 98 % of the chemical composition of the essential oils studied (table 2). All these EOs are characterized by a high level of oxygenated monoterpenes (50.6% -61.7%). Carvacrol (46.0% - 59.0%) was the major compound for all the EOs analyzed, followed by the two hydrocarbon monoterpenes, p-cymene (10.9% - 22.2%) and γ -terpinene (7.3% - 11.2%).

Table 2. Chemical composition of wild and cultivated O. compactum from Chaoun and Tidili regions

		Tidili re	egions.	Chaoun		Tidili
				um		
		į	wild			cultivated
Compounds	RI*	RT**	Pe	ercentage %	Pe	ercentage %
Thujene	928	5,299	-	0.9		-
α-pinene	936	5,440	1.4	1.0	1.5	1.1
Camphene	951	5,725	1.8	1.3	2.3	2.1
Octenol	975	6,206	-	0.6		
Octanone	983	6,362	-	0.6		
P-myrcene	991	6,459	-	0.6		
3-octanol	993	6,524	-	0.2		
3-carene	1009	6,885	-	0.4		
α-terpinene	1018	7,004	1.3	1.3	1.9	
P-cymene	1025	7,167	18.4	22.2	17	10.8
D-limonene	1030	7,321	0.9			
1,8-cineole	1031	7,248	-	0.4		
Ocimene	1047	7.377			0.9	
γ-terpinene	1060	7,852	8.4	10.1	7.3	11.2
Sabinene hydrate	1067	8,027	-	0.5	1.7	
Linalool	1099	8,488	-	0.5		
α-Thujone	1108	8,645	0.9	1.0		1.2
Camphor	1146	9,661	2.1	2.0	2.5	2.7
Terpineol	1179	10,303	-	0.5	1.1	
α- terpineol	1188	10,560	2.3	2.9	2.2	5.2
Carvacrol-methyl ether	1244	11,595	2.8	4.2	6.6	8.0
Thymol	1290	12,485	-	0.8		6.5
Carvacrol	1301	12,713	59.0	45.3	46.0	42.6
Myrtenylacetate	1325	13,218	-	0.3		
Caryophyllene	1445	15,070	0.8	1.1	1 .7	5.1
Germacrene-D	1480	16.166			1.0	
Bicyclogermacrene	1494	16.437			1.6	
Delta-cadinene	1528	16,858	-	0.4	1.1	1.3
Selina-3,7(11)-diene	1548	17,225	-	0.4		
Spathulenol	1576	17.832			1.1	
caryophyllene oxide	1580	17,952	-	0.4	2.1	1.9
β-bisabolene	1653	25.949			1.1	
Total identified			97.3	96.0	100	99.7
Yield			3.5	3.36	1.6	0.98

^{*}Retention Index, **Retention Time

Antibacterial activity

Table 3 summarizes the qualitative (diameters of inhibition zones) and quantitative results (minimal inhibitory and bactericidal concentrations) of the antibacterial effect of essentials oils from *O. compactum* and positive control (Ciprofloxacin) on the thirteen bacterial strains assayed. The results of this study showed that all four EOs were effective against the twelve bacterial strains tested, with an inhibition diameter ranging from 14 to 44 mm. Both cultivated and wild EO in chaoun region showed high sensitivity, with inhibition diameters of 45 mm for *Micrococcus luteus* (*ATCC 381*), 44 mm (cultivated) and 41 mm (wild), for *Staphylococcus aureus* (209PCIP 53156). In contrast, EO from

Tidili region also showed high sensitivity with an inhibition diameter of 35 mm for *Staphylococcus aureus* (209PCIP 53156).

The results of the table 3 reveal that the essential oils studied expressed antibacterial activity against all strains used. In the majority of cases, the MIC values were equivalent to the MBC values, which demonstrated the bactericidal capacity of the essential oils of the oregano species studied. In fact, *O.compactum*, essential oils obtained from cultivated and wild aerial parts recorded a high activity on all strains tested. The minimum inhibition concentration (MIC) of the oil ranged from 214 to 1250 µg/ml.

DISCUSSION

Our results revealed that domestication of *O. compactum* from the Tidili region had an impact on its essential oil yields. Our findings concur with those of El babili [17] and Amakran [18] who used the same extraction method for O. compactum and obtained a yield of 2.1% and 5.6%, respectively. In fact, as reported by Rajabi [19], the essential oil yield can highly be affected by the environmental conditions. Moreover, previous researches revealed that temperature, rainfall, soil condition and altitude can significantly influence essential oil yield and their chemical constituents [19, 20]. For example, the yield of essential oil extracted from *Daucus gracilis* showed significant variability associated with environmental factors. Hence, the results showed the positive correlation between essential oil yield, altitude and soil type. It reaches its highest content in high altitude sites and in calcareous and brown fersialitic soils [21]. Soil pH has been also reported to have an impact on secondary metabolites production due to its influence on the solubility of certain soil nutrients, and thus on their bioavailability [22].

The chemical composition of EOs extracted from O. compactum harvested in their natural environment is in agreement with that mentioned in the work of Laghmouchi [23], who reported that this same oregano species, native to Morocco, contains 28.5% carvacrol, and 19% thymol. Bellakhdar & Il Idrissi [24], analyzed the EO of O. compactum from the region of Rabat (obtained with a yield of 1.6%), and found in its composition 53% thymol, 5.3% carvacrol, 18% para-cymene, 12% γ-terpinene and 2.2% α-cardinol. Our results are also consistent with those of Den Broucke & Lemeli [25] who reported that thymol (from 0 to 43.4%), carvacrol (from 3.8 to 71%), and p-cymene (from 0 to 25.4%) were the main components of the essential oil obtained from Origanum compactum from various sources in Morocco. Other authors have obtained similar results [26]. Indeed, Chebli [27] found the following proportions in the essential oil of *Origanum* compactum: carvacrol (58.1%), thymol (9%), p-cymene (11.4%) and γ-terpinene (7.1%). In another study, the 32 components of O. compactum EO included the same main compounds, carvacrol (30.53%), thymol (27.50%) and their precursor gamma-terpinene (18.20%) [28]. Variations in these constituents are linked to extraction techniques and geographical and climatic conditions.

Table 3. Diameters of inhibition zones (IZ), minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of cultivated and wild O.compactum essential oils from Chaoun and Tidili regions and Ciprofloxacin

	Chaoun							Tidili							
	Origanum compactum										Ciprofloxacin				
	wild			cultivated wild			cultivated								
	IZ mm	MIC μg/ml	MBC μg/ml	IZ mm	MIC μg/ml	MBC μg/ml	IZ mm	MIC μg/ml	MBC μg/ml	IZ mm	MIC μg/ml	MBC μg/ml	IZ mm	MIC μg/ml	MBC μg/ml
Staphylococcus aureus (209PCIP 53156)	44	625	625	41	625	625	35	625	625	20	625	625	13.97	225	225
Staphylococcus aureus (ATCC 29213)	24	518	518	22	518	518	20	518	518	20	518	518	22.67	7.03	7.03
Micrococcus luteus (ATCC 381)	45	625	625	45	625	625	25	625	625	22	625	625	12.07	225	225
Bacillus cereus (ATCC 14579)	23	1037	1037	19	1037	1037	17	1037	1037	16	1037	1037	29	0.85	0.85
Escherichia coli (ATCC 8739)	20	1250	1250	15	1250	1250	18	1250	1250	17	1250	1250	6	0.43	0.43
Escherichia coli (ATCC 35218)	25	1037	1037	25	1037	1037	17	1037	1037	17	1037	1037	26.33	0.85	0.85
Pseudomonas aeruginosa (DSM 50090)	8	>1	>1	8	>1	>1	8	>1	>1	8	>1	>1	10.17	28.12	28.12
pseudomonas aeruginosa (27853)	21	518	518	20	518	518	17	518	518	15	518	518	11.67	3.51	3.51
Klebsiella pneumonia	22	625	625	14	625	625	15	625	625	15	625	625	10	225	225
klebsiella pneumonia(CIP 104727)	22	518	518	21	518	518	20	518	518	14	518	518	6	0.85	0.85
Enterococcus faecalis (ATCC 29212),	18	518	518	18	518	518	16	518	518	16	518	518	21	0.43	0.43
listeria monocytogenes 19115	24	518	518	21	518	518	18	518	518	18	518	518	6	14.06	14.06
salmonella enteritidis DMB 560,	23	1037	1037	24	1037	1037	19	1037	1037	16	1037	1037	24.67	14.06	14.06

By comparing the composition of oils extracted from oregano collected in their natural environment with that of cultivated forms, it can be seen that cultivation induced quantitative variations, particularly in the contents of the major components. Thus, essential oils extracted from the aerial parts of cultivated O. compactum were characterized by a slight decrease in carvacrol content compared to the essential oils obtained from spontaneous plants. This decrease in carvacrol was accompanied by an increase in the p-cymene content. The chemical composition of EO was influenced by several factors such as stage of development, climate, and extraction methods [29]. However, there was a little information about the relationship between environmental factors and the EO composition of O. compactum. The only such study addressing the influence of altitudinal and edaphic factors on chemotypic variation in EO composition of this species was reported by Bakhy [30], on samples collected from 3 regions of Morocco: Tetouan, Chefchaouen and Larache. A study conducted by De Falco [31], propose that some changes in the EO composition of the Italian O. vulgare were due to both genotype and habitat influences. In this study, a trend of the major monoterpenes seems to accompany the transition of sampling sites from the Northern regions to sites in Southern Morocco. Aissi [32] showed that altitude was related to many environmental factors such as temperature, precipitation, wind exposure, solar radiation, air humidity ...etc. This altitude is an important factor influencing carvacrol content, since high carvacrol contents have been obtained at high altitude [32]. In contrast, thymol-rich chemotypes, α terpineol and carvacryl methyl ether were more abundant in low altitude areas. The chemical variation could also be due to the type of soil differences between populations. Soil macroelements and/or microelements are considered the most edaphic factors influencing the essential oil biosynthesis [33]. The involvement of metal ions, such as Mg^{2+} , Mn^{2+} , Fe^{2+} and K^+ , in monoterpene and sesquiterpene synthase pathways has been proved in numerous aromatic plants [34].

However, in some cases, the same population with similar environmental factors may yield plants with different chemical profiles. Variability in EO composition also appears to be related to local selective forces acting on secondary metabolite biosynthesis, suggesting that these populations have specific microclimates that influence the EO composition of plants. Indeed, local abiotic and/or biotic selective factors could act on the loci of terpene biosynthesis pathways that contribute to the emergence of different chemical models [35].

According to the recorded diameters of inhibitory zone of the antibacterial activity, we observed that the EOs tested had no inhibitory effect on *Ps. aeruginosa*, which was obvious resistance. In fact, this bacterium has an intrinsic resistance to biocides that is related to the nature of its outer membrane. The latter is composed of lipopolysaccharides that form a barrier form an impermeable barrier to hydrophobic compounds. In the presence of agents that permeabilize the outer membrane, substances that are inactive against *Ps. aeruginosa* become active [36]. The EOs studied showed inhibitory effects against all other bacterial strains with varying degrees of antibacterial activity.

The sensitivity of a microorganism to EO depends on the properties of the EO and the microorganism itself. It is well known that Gram-positive bacteria are more sensitive to EO than Gram-negative bacteria. Several studies testing the inhibitory activity of EOs confirm this phenomenon [37]. Indeed, the majority of essential oils tested for their antibacterial properties showed a more pronounced effect against Gram+ bacteria. The resistance of Gram- bacteria to essential oils has been attributed to their hydrophilic outer membrane, which can block the penetration of hydrophobic compounds into the target cell membrane [38].

The important antibacterial action demonstrated by the essential oil of *Origanum* compactum—was related to its high content of carvacrol (46.0% - 59.0%). These phenolic compounds were known to have a great action antibacterial action [39]. Antibacterial activity is certainly linked to the major compounds (thymol, carvacrol, γ -terpinene and p-cymene) present in the *O.compactum* EO, to the synergistic effects between these components and to

the additive effects of the minor compounds, which can reinforce the antibacterial action [40, 41]. EOs are complex mixtures of a wide variety of components and their antimicrobial activity is therefore related to their composition and interactions. These interactions can be of three natures: additive, synergistic or antagonistic [42]. The greatest effectiveness of p-cymene lies in its tendency to be incorporated into the lipid bilayer of *S. aureus* by facilitating the transport of carvacrol through the cytoplasmic membrane [43]. Other antibacterial mechanisms of EOs have been investigated as related to the capacity EOs components to deregulates the quorum sensing signaling pathways leading thus to decrease in bacterial resistance [44]. In addition, it has been reported that strains of *E. coli* that are not sensitive to the 1.8-linalool-1.8-cineole mixture are likely to be affected by linalool alone, suggesting that possible antagonistic and synergistic effects may occur depending on the microorganism tested [45].

CONCLUSION

Medicinal plants are among the most important sources of bioactive compounds. This study addressed for the first time the effect of domestication on the chemical composition and biological activities of *O. compactum* essential oils of two regions (Chaoun and Tidili). The results obtained showed that cultivation did not greatly affect the yield and chemical composition of the essential oils. The latter were essentially dominated by the presence of carvacrol in large quantities, followed by p-cymene then g-terpinene. Cultivation reduced carvacrol content, but slightly increased p-cymene content. In addition, these EOs have shown very interesting antibacterial activities, especially those dominated by carvacol. The various oregano essential oils showed highly promising synergistic interactions against all the strains tested, particularly the Gram-negative bacterium *P. aeruginosa*. These results suggest that domestication is a promising solution for the conservation of *O. compactum* endemic species with high socio-economic value. Furthermore, due to their abundance of bioactive chemical components such as carvacrol and thymol, EOs extracted from these species have shown considerable antibacterial activity, demonstrating their potential use in a variety of industries.

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