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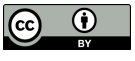
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Essential oil composition, antioxidant and antibacterial activities of wild and cultivated *Lavandula mairei* Humbert

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A B S T R A C T

Lavandula mairei is an endemic and rare plant species growing in the mountains from the southeast to the southwest of Morocco. It is an aromatic and medicinal plant widely used in traditional medicine for the treatment of various diseases. To our knowledge, no data are available on the chemical composition and the biological activities of *L. mairei* essential oils (EOs). The aim of this study was to investigate the effect of cultivation on the chemical composition, antioxidant and antibacterial activities of EOs isolated from *L. mairei*. The hydrodistilled oils obtained from wild and cultivated *L. mairei* were analyzed by GC/MS. Twenty-three compounds were identified representing more than 98% of both EOs (wild and cultivated). Oils were characterized by high amount of carvacrol (78.29 and 76.61% for wild and cultivated EO respectively). The antioxidant and antibacterial assays revealed that the two EOs tested showed significant activities. The results highlighted that cultivation affected neither the chemical composition nor the biological activities of *L. mairei*. Cultivation of *L. mairei* may constitute an alternative to the conservation of this species.

Keywords:
Lavandula mairei
Essential oils
Antioxidant
Antibacterial

1. Introduction

The genus *Lavandula* L. (Lamiaceae) is native to the Mediterranean region, Canary Islands and India, and it is now cultivated in different regions of the world (Palá-Paúl et al., 2004). The EOs of a number of *Lavandula* species are of economic importance in perfumery and fragrance industry, aromatherapy (Shellie et al., 2002) and known to have various biological properties such as antibacterial activity (Cherrat et al., 2014; Djenane et al., 2012; Hanamanthagouda et al., 2010; Varona et al., 2013), antifungal activity (Adam et al., 1998; Angioni et al., 2006; Zuzarte et al., 2012, 2013), antioxidant activity (Carrasco et al., 2015; Cherrat et al., 2014; Viuda-Martos et al., 2011), insecticidal activity (Badreddine et al., 2015; Cosimi et al., 2009; Khosravi and Sendi, 2013) and anti-inflammatory activity (Hajhashemi et al., 2003; Zuzarte et al., 2013).

The genus *Lavandula* is represented in Moroccan flora by 9 species and subspecies of which 5 are endemic (Fennane et al., 2007). Among these endemic lavenders, *Lavandula mairei* Humbert is considered as a rare species (Fennane and Ibn Tattou, 1998). The wild plant grows in saharian, arid and semi-arid bioclimates, from the southeast to the southwest mountains of Morocco. It is a perennial shrub growing, usually, up to 0.40–0.80 m high with spike violet flowers. It is widely used in traditional medicine for the treatment of various diseases such as gastrointestinal ailments, microbial infection, cough and asthma (Abouri et al., 2012).

Morocco is one of the countries where production of medicinal plants is based on the harvesting in the wild and several plant species were becoming rare and sparse due to overexploitation and, aridity and to the endangered medicinal plant species lack of

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conservation actions (Abouri et al., 2012; Bellakhdar et al., 1991). Therefore, cultivation of these medicinal plants might become a promising alternative for their conservation and sustainable use. Several studies have already shown the interest of culture practice on conservation of medicinal plants (Alaoui Jamali et al., 2014; El Abdouni Khiyari et al., 2014; El Bouzidi et al., 2013). Furthermore, the increasing concern on the development of resistance by bacteria to synthetic antibiotics has lead to an urgent need for alternative strategies for the control of bacterial infections. From this viewpoint, plants EOs have been suggested as alternative sources for bacterial pathogens control.

According to Fennell et al. (2004), transplanting a plant from a wild ecosystem to a cultivated field can affect the growth, the content and composition of secondary metabolites. Therefore, it is judicious to evaluate the effect of domestication and planting of medicinal plants, instead of the use of wild harvested plants, on plant growth, EOs composition and biological properties before any program of field cultivation.

To our knowledge, there is no report on the chemical composition and biological activities of *L. mairei* EO. Thus the aim of this study was to (i) determine the chemical composition and evaluate the antioxidant and the antibacterial properties of *L. mairei* EOs and (ii) investigate the effect of cultivation on EO composition and biological activities.

2. Materials and methods

2.1. Plant cultivation

The seeds of *L. mairei* were collected in southwest of Morocco, from Tafraoute village in June 2012. Seedlings were grown from seeds in 5 rows with 50 cm apart and 6 m length. For the first month, the crop was watered twice a week. For the next months the irrigation was carried with an interval of 15 or 21 days as needed. The experimental area is located in Faculty of Sciences, Agadir. The soil characteristics were pH (8.2), sand (29.0%), silt (38.5%), clay (32.5%), organic matter (1.8%), total nitrogen (0.9%), P_2O_5 (118.8 mg/kg), total $CaCO_3$ (13.8%). The experimental area is characterized by an aride bioclimate with a mean rainfall of 236.8 mm/year and an annual average temperature of 18.5 °C. Irrigation was carried out as required.

2.1.1. Plant materials

The aerial parts of cultivated and wild *L. mairei* were harvested at flowering stage at the end of May 2014 and voucher specimens were deposited in the laboratory of Biotechnology and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco, and referred as LM114 and LM214 for wild and cultivated plants respectively. Plant samples were air-dried in the shade and stored in the dark at 4 °C until use.

The seeds used for plant cultivation and the samples of wild plant material studied were collected from the same population of *L. mairei* (29°51′19.1″ N, 8°54′51.6″ W, elevation 1240 m), and the age of cultivated plants was 20 months.

2.1.2. Extraction of essential oil

The EOs of wild and cultivated *L. mairei* were obtained from dried aerial plant materials by hydrodistillation using a Clevenger type apparatus for 3 h. The EOs were dried over anhydrous sodium sulfate and stored in an amber bottle at 4 °C until used.

2.2. Gas chromatography/mass spectrometry (GC/MS) analyses

The analytical GC/MS system used was an Agilent GC/MSD system (Agilent Technologies 6890/5973) with helium (high purity) as the carrier gas at a constant linear velocity of 37 cm/s. The transfer line, ionization source and quadrupole temperatures were 280 °C, 230 °C and 150 °C respectively, operating at 70 eV ionization energy and scanning the m/z range 41–450. The column used was an Agilent DB5 MS capillary column (30.0 m × 0.25 mm ID x 0.25 μm film thickness) programmed from 60 °C to 246 °C at 3 °C/min. EO samples (60 μL) were diluted with acetone (2 mL). The injection volume was 1.0 μl, the split ratio was 1:50 and the injector temperature was 260 °C. Three replicates were performed for each sample. Identification of the individual components was based on: (i) comparison with the mass spectra of authentic reference compounds where possible and by reference to WILEY275, NBS75K, and Adams terpene library (Adams, 2007); (ii) comparison of their retention indices (RI) on a DB5 (apolar, 5% phenyl polysilphenylene-siloxane), calculated relative to the retention times of a series of C-9 to C-24 n-alkanes, with linear interpolation, with those of authentic compounds or literature data (Adams, 2007). For semi-quantitative purposes, the normalized peak area of each compound was used without any correction factors to establish abundances.

2.3. Antioxidant activity

2.3.1. DPPH assay

The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) scavenging ability was determined according to the method described by Şahin et al. (2004). Fifty microliter of the essential oil at various concentrations (0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 mg/mL) diluted in methanol were added to 2 mL of DPPH methanol solution (60 μM). After 20 min incubation in darkness, at ambient temperature, the absorbance was measured at 517 nm. Quercetin was used as a positive control while methanol as a negative one. All analyses were carried out in triplicate and results were expressed as mean ± SD. The scavenging percentage of DPPH radical was calculated using the following formula:

$$\text{Inhibition } \% = (A_0\text{-}A_S/A_0) \text{ x } 100$$

Where A_0 is the absorbance of the control and A_S is the absorbance of the tested oils.

The sample concentration providing 50% inhibition (IC_{50} μg/ml) was calculated by plotting the inhibition percentages against the concentrations of the sample.

2.3.2. Reducing power determination

In this assay, antioxidant activity of the EOs was determined by using the potassium ferricyanide-ferric chloride method as described by Oyaizu (1986). The sample concentrations (in methanol) used were: 0.003, 0.005, 0.007, 0.009, 0.011, 0.013, 0.015 and 0.017 mg/mL. Quercetin was used as a reference compound and methanol as a negative control. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance at 700 nm against the corresponding sample concentration. The test was carried out in triplicate and IC_{50} values were reported as means ± SD.

2.4. Antibacterial activity

2.4.1. Bacteria

The bacterial strains tested in this study included four Gram positive, namely *Listeria innocua* (CECT 4030), *Listeria monocytogenes* (CECT 4032), *Staphylococcus aureus* (CECT 976) and *Bacillus subtilis* (DSM 6633), and two Gram negative: *Proteus vulgaris* (CECT 484) and *Pseudomonas aeruginosa* (CECT 118). The standard bacterial species were cultivated in Tryptic Soy Agar (TSA) and incubated at 37 °C for 18 h under aerobic conditions. Original

cultures are maintained at −70 °C in glycerol.

2.4.2. Antibacterial screening

The agar disc diffusion assay was employed for the determination of the essential oils antibacterial activity according to the method of Gachkar et al. (2007). As a positive control Ampicillin (25 μg/disc), Penicillin (10 μg/disc), Tetracycline (30 μg/disc), Amoxicilline (25 μg/disc) and Chloromphenicol (30 μg/disc) were used. The antibacterial activity was determined by measuring the diameter of the inhibition zone in millimeters with a digital caliper, and the results were expressed as mean ± SD. Three plates were used for each treatment as replications and the experiment was repeated twice.

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

The broth macrodilution method was used to determine the minimum inhibitory concentration (MIC) according to the NCCLS guidelines M07-A8 (NCCLS, 2009). All tests were performed in Tryptic Soy Broth (TSB) supplemented with Tween 80, at a final concentration of 0.5%, to enhance oil solubility (Mourey and Canillac, 2002). Fresh overnight cultures, in log phase, of the tested microorganisms were used to prepare the cell suspension adjusted to 10^6 CFU/mL. The test tubes were incubated aerobically at 37 °C for 24 h. MIC was defined as the lowest concentration of the EOs which inhibits the growth of bacteria (Shin et al., 1998). Bacterial growth was indicated by the presence of turbidity. To determine the MBC, 100 μL of broth aliquots were taken from each test tube and incubated in TSA at 37 °C for 24 h. Bacterial viability controls included aliquots taken from growth control test tubes. MBC was defined as the lowest concentration of assayed samples which produced 99.9% reduction in CFU/mL as compared with the control (Bosio et al., 2000). Positive and negative growth controls were included in every test. The test was performed in triplicate and the experiment was repeated twice.

2.5. Data analysis

Data were subjected to variance analyses (ANOVA) using STATISTICA software, ver. 6 (Stat-Soft, 2001, Créteil, France).

3. Results and discussion

3.1. Chemical composition

The yields of EOs obtained from aerial parts, were 1.00 ± 0.26% for wild plant and 1.22 ± 0.20% for cultivated plant on a dry weight basis (v/w). Cultivation did not greatly affect essential oil production. Twenty-three compounds were identified representing more than 98% in both cases (wild and cultivated) using GC/MS and retention indices (Table 1). Oils were characterized by a large amount of monoterpenes (90.65 and 90.96%) including oxygenated monoterpenes as major compounds (82.98 and 82.67%). Sesquiterpenes represented only 5.27 and 4.13% for wild and cultivated EO respectively. *L. mairei* EOs are characterized by high amount of carvacrol (78.29 and 76.61%) (Figs. 1 and 2) followed by terpinolene (2.96–3.20%), octen-3-ol (1.84–2.31%), *cis*-β-ocimene (1.72–1.27%), *p*-cymen-8-ol (1.81–1.16%), β-caryophyllene (1.45–1.76%) and

Table 1
Chemical composition of essential oils obtained from wild and cultivated *Lavandula mairei*.

Compounds*	M+ (Parent Ion)**	RI***	% of Compound	
			Wild L. mairei	Cultivated *L. mairei*
α-Pinene	136	931	0.35 ± 0.02[a]	Tr[b]
Octen-3-ol	128	972	1.84 ± 0.04[a]	2.31 ± 0.02[b]
Octan-3-one	128	984	0.45 ± 0.02[a]	0.72 ± 0.05[b]
β-Myrcene	136	990	1.00 ± 0.07[a]	1.15 ± 0.09[a]
α-Phellandrene	136	1008	0.19 ± 0.02[a]	0.24 ± 0.03[a]
δ-3-Carene	136	1012	0.54 ± 0.02[a]	0.82 ± 0.02[b]
α-Terpinene	136	1016	0.16 ± 0.02[a]	0.30 ± 0.03[b]
p-Cymene	132	1021	0.77 ± 0.02[a]	0.78 ± 0.03[a]
Limonene	136	1025	0.31 ± 0.01[a]	0.28 ± 0.01[a]
cis-β-Ocimene	136	1042	1.72 ± 0.01[a]	1.27 ± 0.02[b]
trans-β-Ocimene	136	1051	Tr[a]	0.24 ± 0.01[b]
Terpinolene	136	1088	2.96 ± 0.02[a]	3.20 ± 0.01[b]
Linalool	154	1096	0.33 ± 0.02[a]	0.33 ± 0.02[a]
Mentha-1.8-dien-4-ol	154	1177	0.36 ± 0.02[a]	tr [b]
Terpinen-4-ol	154	1178	0.45 ± 0.02[a]	0.40 ± 0.02[a]
p-Cymen-8-ol	150	1184	1.81 ± 0.06[a]	1.16 ± 0.01[b]
α-Terpineol	154	1191	0.38 ± 0.01[a]	0.29 ± 0.02[a]
Carvacrol methyl ether	164	1244	1.36 ± 0.01[a]	3.86 ± 0.01[b]
Carvacrol	150	1302	78.29 ± 0.08[a]	76.61 ± 0.08[b]
β-Caryophyllene	202	1424	1.45 ± 0.02[a]	1.76 ± 0.03[a]
Spathulenol	224	1581	1.51 ± 0.02[a]	1.34 ± 0.03[b]
Caryophyllene oxide	216	1587	2.31 ± 0.04[a]	1.03 ± 0.01[b]
Manoyloxide	290	2036	0.44 ± 0.02[a]	Tr[b]
Monoterpene hydrocarbons			7.67 ± 0.36[a]	8.29 ± 0.08[a]
Oxygenated monoterpenes			82.98 ± 0.11[a]	82.67 ± 0.11[a]
Sesquiterpene hydrocarbons			1.45 ± 0.02[a]	1.76 ± 0.03[b]
Oxygenated sesquiterpenes			3.82 ± 0.03[a]	2.37 ± 0.03[b]
Oxygenated diterpenes			0.44 ± 0.02[a]	Tr[b]
Others			2.29 ± 0.05[a]	3.03 ± 0.10[b]
Total			98.64 ± 0.15	98.12 ± 0.03

*: Compounds listed in order of elution, **: Ionization mode: electron impact at 70 eV, ***: Retention Indices measured relative to *n*-alkanes (C-9 to C-24) on a non polar DB5-MS column. Tr = Traces.
The reported percentages of each compound were the mean value of three replicates for each population (wild and cultivated).
Different letters after values mean statistically significant differences with $p < 0.05$.

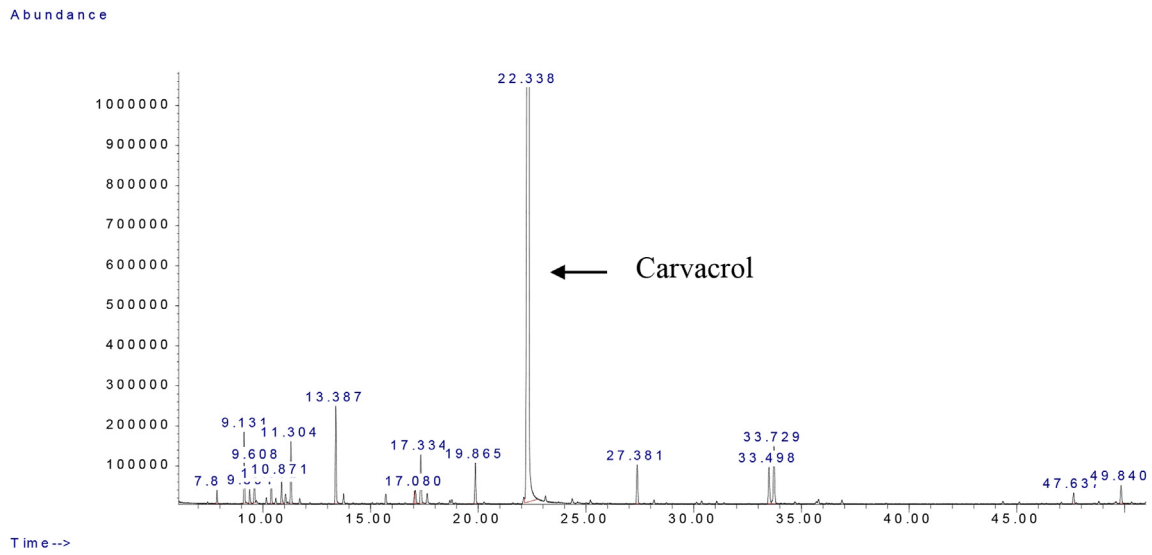


Fig. 1. GC/MS profile of wild *Lavandula mairei* essential oil.

carvacrol methyl ether (1.36–3.86%). Previous studies have shown that carvacrol does not usually appear as dominant compound in oils extracted from other *Lavandula* species. Thus, camphor and 1.8-cineole were the major components of essential oils from *Lavandula stoechas* from Tunisia (Badreddine et al., 2015) and *Lavandula latifolia* from Spain (Herraiz-Peñalver et al., 2013; Muñoz-Bertomeu et al., 2007). Cherrat et al. (2014) have reported that 10s,11s-Himachala-3(12),4-diene, cubenol, methyl eugenol, δ-cadinene and myrtenyl acetate were the main constituents of *Lavandula stoechas* from north of Morocco, while Camphene, α-Pinene, limonene, eucalyptol, fenchone and camphor were the principal compounds of *Lavandula stoechas* essential oil from Spain (Carrasco et al., 2015). The major constituents of essential oils from *Lavandula angustifolia* from Cyprus were 1.8-cineole, borneol, camphor, terpineol and myrtenal (Chrysargyris et al., 2016). The essential oils of *Lavandula pedunculata* from Portugal are characterized by three chemotypes: 1,8-cineole; 1,8-cineole/camphor and fenchone (Zuzarte et al., 2010). Trans-α-necrodyl acetate, β-selinene, trans-α-necrodol, fenchone, cineole, viridiflorol and camphor were determined as the most abundant compounds of the essential oils extracted from *Lavandula luisieri* from Portuguese populations (González-Coloma et al., 2011). Linalool and linalyl acetate, were determined as the two major compounds of essential oils of *Lavandula angustifolia* from Greece (Hassiotis et al., 2014). Chemical composition of essential oils from *Lavandula officinalis* from Iran shown that α-pinene, menthol and camphor were the main components (Miri, 2015). Ouedrhiri et al. (2017) have reported that β-pinene, 1,8-cineole and fenchone were the principal compounds of *Lavandula dentata* from north of Morocco. However, as previously reported, carvacrol was the major constituent of EOs obtained from Moroccan and Tunisian *L. multifida* samples (Belhadj Mostefa et al., 2014; Bellakhdar et al., 1985; Douhri et al., 2014; Sellam et al., 2013) and from *L. canariensis* (Palá-Paúl et al., 2004).

The EOs composition reveals very similar profiles for wild and cultivated *L. mairei*, the percentage of various constituents showed very low variations and carvacrol remained the main constituent with more than 76% in both cases. The presence of carvacrol in very substantial proportions presents a special interest, indeed carvacrol

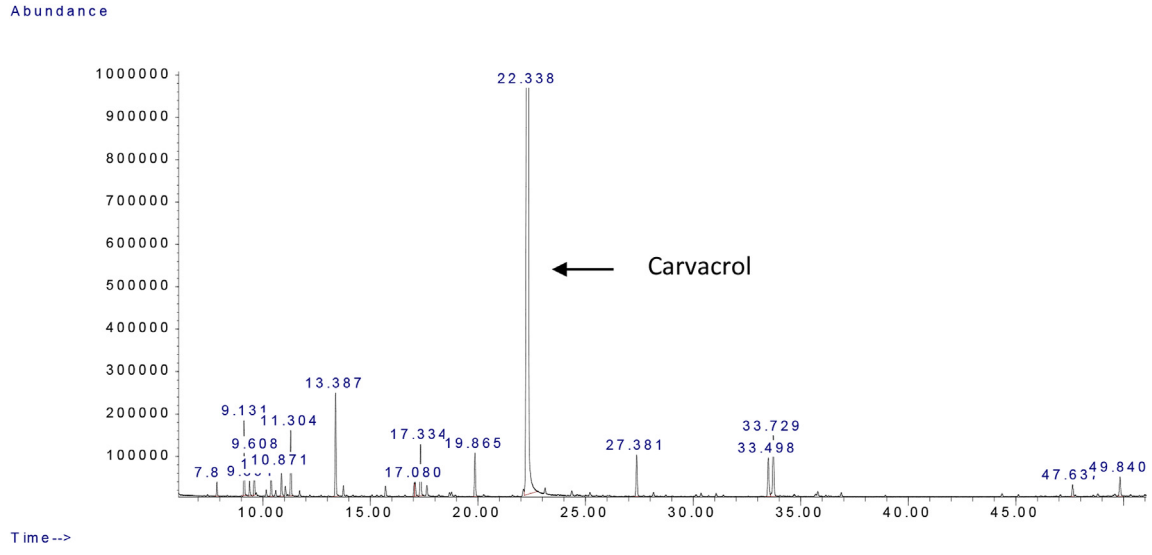


Fig. 2. GC/MS profile of cultivated *Lavandula mairei* essential oil.

Table 2
Antioxidant activity of essential oils obtained from wild and cultivated *Lavandula mairei*.

Antioxidant tests	Essential Oil		Standard antioxidant
(IC_{50})	Wild	Cultivated	Quercetin
DPPH (μg/mL)	107.54 ± 10.60^{c}	112.33 ± 2.30^{c}	1.66 ± 0.20^{a}
Reducing power (μg/mL)	5.22 ± 2.3^{b}	4.5 ± 0.80^{b}	3.16 ± 0.05^{b}

Values are given as mean ± SD (n = 3). Means in each column followed by different letters are significantly different (P < 0.05).

possesses very high cytotoxic, antibacterial, antifungal and antiviral activities (El Bouzidi et al., 2013; Jaafari et al., 2007; Regnier et al., 2014; Sánchez et al., 2015).

3.2. Antioxidant activity

L. mairei EOs were screened for their antioxidant activities *in vitro* using two different and complementary assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging and the reducing power determination. The obtained results (Table 2) showed that both EOs exhibited antioxidant activity across the two testing methods. The concentrations that led to 50% inhibition or effectiveness (IC_{50}) are given in Table 2. The lower IC_{50} values reflected better protective action. For the DPPH assay the IC_{50} value obtained was 107.54 ± 10.60 μg/mL for wild plant and 112.33 ± 2.30 μg/mL for cultivated plant but they were less effective than quercetin used as positive control, (IC_{50} = 1.66 ± 0.20 μg/mL). For ferric reducing ability both oils showed very high activity; IC_{50} = 5.22 ± 2.31 μg/mL for wild plant and IC_{50} = 4.5 ± 0.80 μg/mL for the cultivated plant, values relatively comparable to those obtained for quercetin, used as antioxidant standard, IC_{50} = 3.16 ± 0.05 μg ml^{-1}. The *L. mairei* EOs IC_{50} value of reducing power ability was only twice a value of quercetin, exhibiting a high reducing power. The degree of antioxidant activity was variable in *Lavandula* genus; indeed the antioxidant capacity of *L. mairei* oils accords to what has been reported for other species of the *Lavandula* genus (Cherrat et al., 2014; Miri, 2015; Mohammedi and Atik, 2011). However, Miliauskasa et al. (2004) detected weak antioxidant activity of EO obtained from *L. angustiflolia*. Also of interest, our results highlighted that cultivation did not greatly affect the antioxidant property of *L. mairei,* since the obtained values did not differ significantly between wild and cultivated plants.

As reported previously, the high antioxidant/reducing power activities of the oils could be explained by their high content of the phenol, carvacrol (Alaoui Jamali et al., 2012; Kulisic et al., 2004; Polatoğlu et al., 2013; Ruberto and Baratta, 2000; Sokmen et al., 2004; Tepe et al., 2005; Wang et al., 2008).

3.3. Antibacterial activity

The EOs from wild and cultivated *L.mairei* were screened against four Gram (+) and two Gram (-) bacteria. Both EOs showed antibacterial property on all tested bacteria with no significant difference between wild and cultivated plant (Table 3). Indeed, the EOs of wild and cultivated *L. mairei* inhibited the growth of tested bacterial strains with an inhibition zone diameter varying from 23.5 to 35.6 mm. The maximum activity was against *L. innocua* (35.3–34.6 mm) and *L. monocytogenes* (35.6–34.6 mm). *P. vulgaris* (23.5–24.0 mm) and *S. aureus* (24.5–23.5 mm) were the less sensitive strains. Overall, *L. mairei* EOs showed a better antibacterial potential compared to the positive controls. For example, *P. vulgaris* which is totally resistant (0 mm) to Ampicillin (Am 25), Penicillin (P10), Amoxicilline (Ax25) and slightly sensitive (9 mm) to Tetracycline (Te30) is sensitive to EOs obtained from the aerial parts of wild and cultivated *L. mairei* (Table 3). Our results were in accordance with results previously reported on *L. angustifolia* (Djenane et al., 2012), *L. stoechas* (Cherrat et al., 2014) *L. hybrida super* (Varona et al., 2013) and *L. bipinnata* (Hanamanthagouda et al., 2010).

3.4. Determination of MIC and MBC

The antibacterial activity of EOs of wild and cultivated *L. mairei* was studied by determining their MIC and MBC values by using broth macrodilution method according to the NCCLS guidelines M07-A8 (NCCLS, 2009). As shown in Table 4, the investigated *L. mairei* EOs exhibited remarkable antimicrobial activity against all tested bacteria, with MIC values ranging from 0.60 to 1.20 mg/mL. Both EOs (wild and cultivated) showed a significant antibacterial activity against Gram positive as well as Gram negative bacteria (Table 4). The MBC values of the two oils were similar or even higher than the corresponding MIC values, with MIC/MBC ratio very close to 1, confirming their bactericidal activity.

The best antibacterial activity (MIC = 0.60 mg/mL) was shown against *B. subtilis* and *P. aeruginosa.* The weakest activity was observed against *S. aureus* (MIC = 1.20 mg/mL). *L. mairei* EOs can

Table 3
Antimicrobial screening of studied essential oils.

	Inhibition zone diameter (mm)					
	Gram +				*Gram -*	
	Listeria innocua	*Listeria monocytogenes*	*Staphylococus aureus*	*Bacillus subtilis*	*Proteus Vulgaris*	*Pseudomonas aeruginosa*
Wild	35.3 ± 4.1^{a}	35.6 ± 2 ^{a}	24.5 ± 0.7 ^{a}	25.5 ± 0.7 ^{a}	23.5 ± 0.7 ^{a}	27.0 ± 0 ^{a}
Cultivated	34.6 ± 4.7^{a}	34.6 ± 2 ^{a}	23.5 ± 0.7 ^{a}	25.5 ± 0.7 ^{a}	24.0 ± 0.0 ^{a}	27.0 ± 0 ^{a}
Am25*	24 ± 1.0 ^{b}	23 ± 0.5 ^{b}	12 ± 0.5 ^{e}	27 ± 1.0 ^{a}	0 ± 0.0 ^{d}	25 ± 1.0 ^{a}
P10	22 ± 1.0 ^{b}	24 ± 1.0 ^{b}	14 ± 1.0 ^{d}	27 ± 1.5 ^{a}	0 ± 0.0 ^{d}	20 ± 1.0 ^{b}
Te30	25 ± 2.0 ^{b}	23 ± 0.0 ^{b}	21 ± 1.0 ^{b}	15 ± 1.0 ^{b}	9 ± 1.0 ^{c}	28 ± 2.5 ^{a}
Ax25	23 ± 0.0 ^{b}	20 ± 1.0 ^{c}	18 ± 1.0 ^{c}	27 ± 2.0 ^{a}	0 ± 0.0 ^{d}	27 ± 1.0 ^{a}
C30	24 ± 1.0 ^{b}	22 ± 1.0 ^{b}	25 ± 0.5 ^{a}	27 ± 0.5 ^{a}	20 ± 0.0 ^{b}	33 ± 00 ^{a}

Am25: Ampicillin at 25 μg/disc, P10: Penicillin at 10 μg/disc, Te30: Tetracycline at 30 μg/disc, Ax25: Amoxicilline at 25 μg/disc, C30: Chloramphenicol at 30 μg/disc.
Amounts of EOs used: 5 μl / sterile filter paper disc.
Different letters after values mean statistically significant differences with p < 0.05.

Table 4
Minimal inhibitory concentrations and minimal bactericidal concentrations of *Lavandula mairei* EOs against six different bacteria.

	Bacteria	*L. mairei* EOs				Antibiotic	
		Wild		Cultivated		Chloramphenicol	
		MIC	MBC	MIC	MBC	MIC	MBC
		(mg/mL)		(mg/mL)		(mg/mL)	(mg/mL)
Gram+	*L. innocua*	0.9 ± 0.02^{ef}	1.00 ± 0.00^{f}	0.90 ± 0.01^{ef}	1.00 ± 0.02^{f}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{ab}
	L. monocytogenes	0.80 ± 0.01^{e}	1.00 ± 0.01^{f}	0.85 ± 0.02^{e}	1.00 ± 0.03^{f}	0.01 ± 0.00^{a}	>1
	S. aureus	1.20 ± 0.01^{g}	1.20 ± 0.01^{g}	1.20 ± 0.00^{g}	1.20 ± 0.00^{g}	0.06 ± 0.00^{b}	>1
	B. subtilis	0.60 ± 0.02^{de}	0.60 ± 0.01^{de}	0.60 ± 0.02^{de}	0.60 ± 0.01^{de}	0.01 ± 0.00^{a}	0.25 ± 0.01^{c}
Gram -	*P. aeruginosa*	0.60 ± 0.03^{de}	0.60 ± 0.02^{de}	0.60 ± 0.02^{de}	0.60 ± 0.02^{de}	0.03 ± 0.00^{ab}	0.50 ± 0.01^{d}
	P. vulgaris	1.00 ± 0.01^{f}	1.00 ± 0.00^{f}	1.00 ± 0.02^{f}	1.00 ± 0.01^{f}	<0.01	>1

MIC: Minimal Inhibitory Concentrations. MBC: Minimal Bactericidal Concentrations.
Values are given as mean ± SD (n = 3). Means in each column followed by different letters are significantly different ($P < 0.05$).

thus be used as effective antibacterial agent against several bacterial species.

The strong antibacterial activity of the EOs of wild and cultivated *L. mairei* can be attributed to the presence of high concentration of oxygenated monoterpenes (carvacrol: 78.29–76.61%). Indeed, plants EOs with high amounts of carvacrol are known to posses antibacterial activity (Alaoui Jamali et al., 2014; Calo et al., 2015; El Bouzidi et al., 2013). However, some studies have concluded that the whole EOs have a greater antibacterial activity than the major mixed components (Dorman and Deans, 2000; Jirovetz et al., 2006) and the amount of small compounds should not be neglected.

In comparing wild and cultivated *L. mairei* oils, it appears that they exhibit comparable strong antibacterial activity. The same results were reported for wild and cultivated *Achillea ageratum* and *Thymus* spp. (El Bouzidi et al., 2012, 2013).

4. Conclusions

To the best of our knowledge, the composition and the biological activities of *L. mairei* EOs are reported for the first time. This study revealed that the EOs of this endemic and rare species is rich in carvacrol (more than 76%) a component of particular biological importance, making *L. mairei* EO as a potential industrial source of carvacrol. Our results highlighted that EOs from the cultivated samples maintained their biological activity with respect to the wild population. Therefore, cultivation may constitute an alternative solution to conservation of this valuable and threatened medicinal plant.

Conflicts of interest

We declare that we have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bse.2017.11.004.

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