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Chemical composition and antibacterial activity of the essential oils of *Lavandula pedunculata* and *Lavandula dentata*

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Abstract

The chemical composition and antibacterial activity of the essential oils of *Lavandula pedunculata* and *Lavandula dentata* collected in south of Morocco, were investigated. The essential oils were isolated by hydrodistillation and analyzed by GC and GC-MS. The minimal-inhibitory concentration (MIC) of the essential oils was evaluating the antibacterial activity against different strains, gram negative bacteria *Escherichia coli* (ATCC 4157) and *Pseudomonas aeruginosa* (ATCC 27853) and Gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Streptococcus fasciens* (ATCC 29212). The essentialoils were characterized by a high percentage of oxygenated monoterpenes, the main compounds being camphor for our oils, 53.1% and 50.3% for *L pedunculata* and *dentata* respectively. The essential oils of both species exerted significant antibacterial activity against the four bacteria strains. The inhibitory activity of the essential oils in Gram-positive bacteria was significantly higher than in Gram-negative. Our findings demonstrate that the essential oils of *L pedunculata* and *L dentata* possess antimicrobial activity.

1. Introduction

The Mediterranean region shows closer interrelations than any other region in the world between its flora and major landscapes and the human activities that have been molding them for nearly 10 000 years. Mediterranean basin countries contain many types of ecosystems: coastal, island, mountainous, desert, oasis and wetlands.
Indeed, Morocco, by its geographical location, is a natural setting that is genuinely offering a full range of Mediterranean bio-climates promoting a rich and varied flora with a marked endemism. Among the 7000 species, Subspecies and existing varieties, 537 are endemic to the country [1].
Lavandula genus (Lamiaceae) comprises 28 species, it provides valuable essential oils mainly for the food (flavouring), perfumery and cosmetic industries, and is also very popular in aromatherapy [2]. However, many other applications can be foreseen, as suggested in several reports on the biological activity of this genus [3]. Lavandula oils have been reported to have sedative and antispasmodic properties [4] as well as acaricidal [5], antibacterial [6], antifungal [7] and antioxidant [8] activities. *L. pedunculata* is more commonly found throughout the Moroccan regions and can reach up to 70 cm tall. Its long-stalked spikes, full of lilac flowers, can reach 24 cm and are covered by different kinds of glandular trichomes [9, 10].
Lavandula dentata, grows on rocky ground and phrygana of the arid Mediterranean or Saharan regions. It is a pseudo-shrub which forms clumps with quadrangular stems, ligneous, leafed at the bottom and lengthily bared under the floral spikes. Leaves are very narrow, with rolled up, dentate and crenelated edges. Bluish flowers form short and dense spikes topped with same color bracts [11]. *Lavandula dentata* is used in folk medicine against common cold [12] and its essential oil induces a spasmolytic activity [13].

In the last years, research in aromatic and medicinal plants, and particularly their essential oils, has attracted many investigators. Essential oils have traditionally been used during centuries for their antibacterial properties [14-15]. Essential oils almost exist only in the higher plant; the genres that are able to develop Essential oils are gathered in a small number of families such as: Lamiaceae, Lauraceae, Asteraceae, Rutaceae, Myrtaceae, Poaceae, Cupressaceae, Piperaceae [16].
Therefore, the aim of this investigation was to analyze the chemical composition and major bioactive compounds of lavender essential oils made from *Lavandula pedunculata* and *Lavandula dentata* grown in Morocco. Additionally, the inhibitory effects of the essential oils towards Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus* and *Streptococcus fasciens*) bacteria were studied.

2. Experimental details

2.1.*Plant materials*

The *Lavandula pedunculata* and *Lavandula dentata* were collected during the flowering period, April 2013, *Lpedunculata* from Tafraout (South west of Morocco), *L dentata* from Marrakech. Voucher specimens for each plant were deposited in the Herbarium of the Laboratory of Plants in the Scientific Institute in Rabat, Morocco. The entire Plants are dried in the shade and stored in the laboratory at room temperature (25°C).

2.2. Essential oils extraction

The E oils were extracted by hydrodistillation (3h) from flowers and dry stems of *Lavandula pedunculata* and *Lavandula dentata* using a Clevenger type distillation apparatus (500 ml of water for 100 g plant material). Sodium sulfate (Na_2SO_4) was added as a drying agent to the decanted essential oil. After extraction, the essential oil was stored in a brown glass bottle tightly closed in order to protect it from light and air and maintained at a temperature of 4°C until used.

2.3*Essential oils analysis*

2.3.1 Gas chromatography analysis (GC-FID)

GC analysis was carried out using a Perkin-Elmer Autosystem XL GC apparatus (Waltham, MA, USA) equipped with a dual flame ionization detection (FID) system and the fused-silica capillary columns (60m *0.22mm I.D., film thickness 0.25µm) Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). The oven temperature was programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Samples were injected in the split mode (1/50) using helium as a carrier gas (1 mL/min) and a 0.2 µL injection volume of pure oil. Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) (Restek, Lisses, France) with linear interpolation using the Van den Dool and Kratz equation and software from Perkin-Elmer.

2.3.2 *Gas chromatography mass spectrometry (GC-MS)*

Samples were analyzed with a Perkin-Elmer turbo mass detector (quadrupole) coupled to a Perkin-Elmer Autosystem XL equipped with the fused-silica capillary columns Rtx-1 and Rtx-wax. Carrier gas: helium (1 mL/min), ion source temperature: 150 °C, oven temperature programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C (35 min), injector temperature: 280 °C, energy ionization: 70 eV, electron ionization mass spectra were acquired over the mass range 35– 350 Da, split: 1/80, injection volume: 0.2 µL of pure oil.

2.3.3. Components identification

The identification of the essential oil constituents was based on the comparison of their retention index (RI), calculated relative to the retention times of a series of C-5 to C-30 n-alkanes, with linear interpolation, with those of our own library of authentic compounds or literature data [17 – 18].

2.4. Tests for antibacterial activity

2.4.1. Preparation of bacterial strains

The tested microorganisms included the following bacteria: Gram negative bacteria: *Escherichia coli* (ATCC 4157), *Pseudomonas aeruginosa* (ATCC 27853) and Gram positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Streptococcus fasciens* (ATCC 29212). All microorganisms were derived from the culture collection of the Biology Department (Microthec unity) at the Faculty of Sciences (Rabat, Morocco). Prior the experiment

working, cultures were prepared by culturing 1mL of each culture stock in 9 ml of BHI (Brain Heart Infusion) in order to obtain inoculate containing cultures in an exponential growth phase.

2.4.2. Disc diffusion method
The agar disc diffusion (ADD) method was employed for the determination of antimicrobial activities of the tested EO as described previously [19]. Briefly, the test was performed in sterile Petri plates containing BHI agar. Sterile filter paper discs (6 mm in diameter) were impregnated with 6 µl of oil and were placed on the Petri plates previously inoculated with a sterile microbial suspension. The suspension of bacteria was obtained from 18h cultures (one microorganism per Petri Plates). All Petri plates were sealed with sterile laboratory films to avoid eventual evaporation of the test samples, and then incubated at 37°C for 24 h. The diameters of inhibition (D) zones were measured in millimeters.

2.4.3. *Determination of Minimum Inhibitory Concentration*
For determining the minimum inhibitory concentration (MIC) values, of the essential oil of *Lavandula pedunculata* and *Lavandula dentata* against the four bacteria strains. We tested seven serial concentrations of each EO (40 µg/mL, 20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25µg/mL and 0.625 µg/mL) diluted in BHI broth with 0.15% agar and strongly mixed for 2 min using a vortex. For MIC determination, we adopted the technique of sterile micro-titeplates [20], using the tetrazolium (MTT) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) as an indicator of sustainability. In each well, poured 100 µl of liquid culture medium BHI more 100 µl of the test product. Serial dilutions were then performed. Each well is then inoculated with 10 µl of the bacterial suspension. At the end of the incubation period (24 h) at37°C, 10 µL of MTT (0.4 mg/mL) is added to each well. The plates were reincubated for 30 minutes at 37 °C. After this incubation time, Wells, where microbial growth occurs, show a blue-violet color. The MIC was then determined and corresponds to the lowest concentration substance which produces no bacterial growth. We used *penicillin-streptomycin* (PS) and *Ampicillin* (Amp) as a positive control.

3. Results and Discussion

3.1. Essential oils yields and chemical composition:
The EO yield (% v/w) was 0.66 % for *Lavandula pedanculata* and 0.58 % for *Lavandula dentata*; the chemical components identified are reported in Table 1.
The analysis of essential oil from *L pedanculata* and *L dentata* has been carried out by GC. The chemical composition of essential oil was characterized by the presence of 32 and 40 compounds, which accounted for 87% and 89.4 of the total oil for *L. Pedanculata* and *L. dentata* respectively. The retention index of volatile compounds (RIa and RIp) and their percentage are summarized in Table 1.
The EO of *L. pedanculata* was dominated by oxygenated monoterpenes (63 %), followed by monoterpenic hydrocarbons (28 %). The sesquiterpene hydrocarbons and oxygenated sesquiterpenes accounted only for 3% and 6% of the total oil, respectively. The essential oil has been characterized by a high amount of camphor (53.1 %). The other major components are: camphene (6.1 %); 1.8-Cineole (6.5 %). The 29 other compounds are present in the essential oil of *L. pedanculata*in low quantities. For the EO of *L.dentata*, The oxygenated monoterpenes represent (70 %), followed by oxygenated sesquiterpenes (15%) and monoterpenic hydrocarbons (12.5 %). The sesquiterpene hydrocarbons accounted only for (2.5%) of the total oil. camphor was the major compound (50.3 %) followed by other components with relatively small amounts: trans Pinocarveol (6.2 %); β-Eudesmol (4.1 %) ; Borneol (3.0 %); Linalol (2.2 %) . The 35 other compounds are present in the essential oil of (*L. dentata*) in minor amounts.
Camphor was also found as a major compound in the essential oil of *L. pedunculata* from Portugal [8], the essential-oil composition of *L. pedunculata* from Morocco shows similarities with that reported for *L. stoechas* subsp. *stoechas* from other Mediterranean regions, namely Spain [21], Cyprus [22], Greece [23], Corse [24], and Turkey [25]. In fact, even though *L. pedunculata* and *L.stoechas* subsp. *stoechas* are morphologically well distinct, their essential oils consist of the same major components (camphor and 1,8-cineol,) and the same chemical polymorphism.
The *L. dentata* from Tunisia and Algeria were rich in 1.8-cineole (38.4 and 33.5%, resp.)[26]. However, these oils differ by their concentrations in camphor, since the Tunisian oil contained a high concentration (18.9%), while the Algerian oil had a low content (1.6%) of this compound which represents the major component of our

L. dentata. In contrast, when we compare the results obtained for *L. pedunculata* and *L. dentata* with those reported for *Lavandula officinalis* and *L. angustifolia*, profound differences are found, particularly in the oil composition of *L.officinalis* the major components were: linalyl acetate (47.56%), linalool (28.06%), lavandulyl acetate (4.34%) and α- terpineol (3.7%) [27]. The main component for *L. angustifolia* EO was linalool (20.1-65.9%), followed by borneol (6.3-32%) and camphor (2.4-13.5%). These changes in the essential oil composition might arise from several environmental (climatic, seasonal, geographical) and genetic differences [28].

Table 1: Chemical composition (%) of the essential oils from *L. pedunculata* and *L. dentata.*

N°	Compounds	Ir (apol)	Ir (pol)	*L dentata*	*L pedunculata*
1	Tricyclene	922	1013	-	0.5
2	α-Pinene	929	1021	0.1	**2.0**
3	Camphene	943	1065	0.2	**6.1**
4	Thuja-2.4 (10) diene	948	1127	-	0.3
5	1-octen-3-ol	959	1439	0.3	-
6	β-Pinene	970	1109	0.5	**1.4**
7	α-Terpinene	1011	1180	-	0.1
8	p-Cymene	1011	1265	0.1	0.4
9	1,8-Cineole	1020	1207	1.9	**6.5**
10	Limonene	1020	1197	0.5	0.8
11	O-Cresol	1035	1919?	0.2	-
12	γ-Terpinene	1050	1245	-	0.1
13	Linalool oxyde E	1056	1432	1.0	0.4
14	Camphenilone	1060	1453	-	0.4
15	Fenchone	1067	1389	0.7	**1.3**
16	Linalool oxyde Z	1071	1460	0.8	-
17	Linalol	1085	1536	**2.2**	-
18	Hotrienol	1087	1613	0.2	-
19	α-Campholenal	1103	1479	0.2	-
20	Nopinone	1105	1571	0.5	-
21	Pinocarveol	1108	1651	0.7	-
22	trans Pinocarveol	1125	1641	**6.2**	-
23	Camphor	1125	1508	**50.3**	**53.1**
24	cis-Verbenol	1129	1647	0.3	1.2
25	p-Mentha-1,5-dien-8-ol (isomere I)	1130	1651	0.2	0.3
26	Pinocarvone	1139	1555	1.1	0.6
27	p-Mentha-1,5-dien-8-ol (isomere II)	1146	1717	0.8	-
28	Borneol	1150	1686	**3.0**	**1.5**
29	p-Methylacetophenone	1154	1752	0.5	-
30	Cryptone	1156	1663	0.7	0.5
31	p-Cymen-8-ol	1160	1829	1.2	0.7
32	Myrtenal	1170	1613	1.6	0.8
33	α-Terpineol	1172	1686	0.4	0.3
34	Myrtenol	1179	1776	1.6	0.6
35	Verbenone	1180	1681	0.5	**1.2**
36	trans Carveol	1197	1816	0.7	-

37	Cuminaldehyde	1211	1761	0.5	0.4
38	Carvone	1215	1715	0.6	0.4
39	Bornyl acetate	1270	1576	-	0.8
40	Carvacrol	1280	2211	-	1.2
41	β-Selinene	1479	1708	1.5	0.5
42	Caryophyllene oxyde	1567	1962	1.5	-
43	1.10 -Di-epi-Cubenol	1603		**-**	**1.2**
44	β-Eudesmol	1634	2206	**4.1**	0.9
45	β-Bisabolol oxyde	1639	2105	0.8	-
46	Muurol-5-en-4one	1659	2254	0.3	-
47	α-Bisabolol	1664	2194	0.5	-
48	Cryptomerione	1699	2255	0.4	-
49	cis-p-Mentha-1(7)8-dien-2-ol	1200	1898	-	0,5
			TOTAL	**89.4**	**87.0**

RI *a* = retention indices on the apolar column (Rtx-1)
RI *p* = retention indices on the polar column (Rtx-Wax)

3.2. *Antibacterial activity of the essential oils and MIC*

Figure 1 presents the inhibition zone of *L. pedanculata* and *L. dentata* essential oil determined for 2 Gram positive and 2 Gram negative bacteria using the Disc diffusion method. The results showed that both essential oil had a considerable inhibitory effect on all assayed bacteria strains noted by large growth inhibition halos.

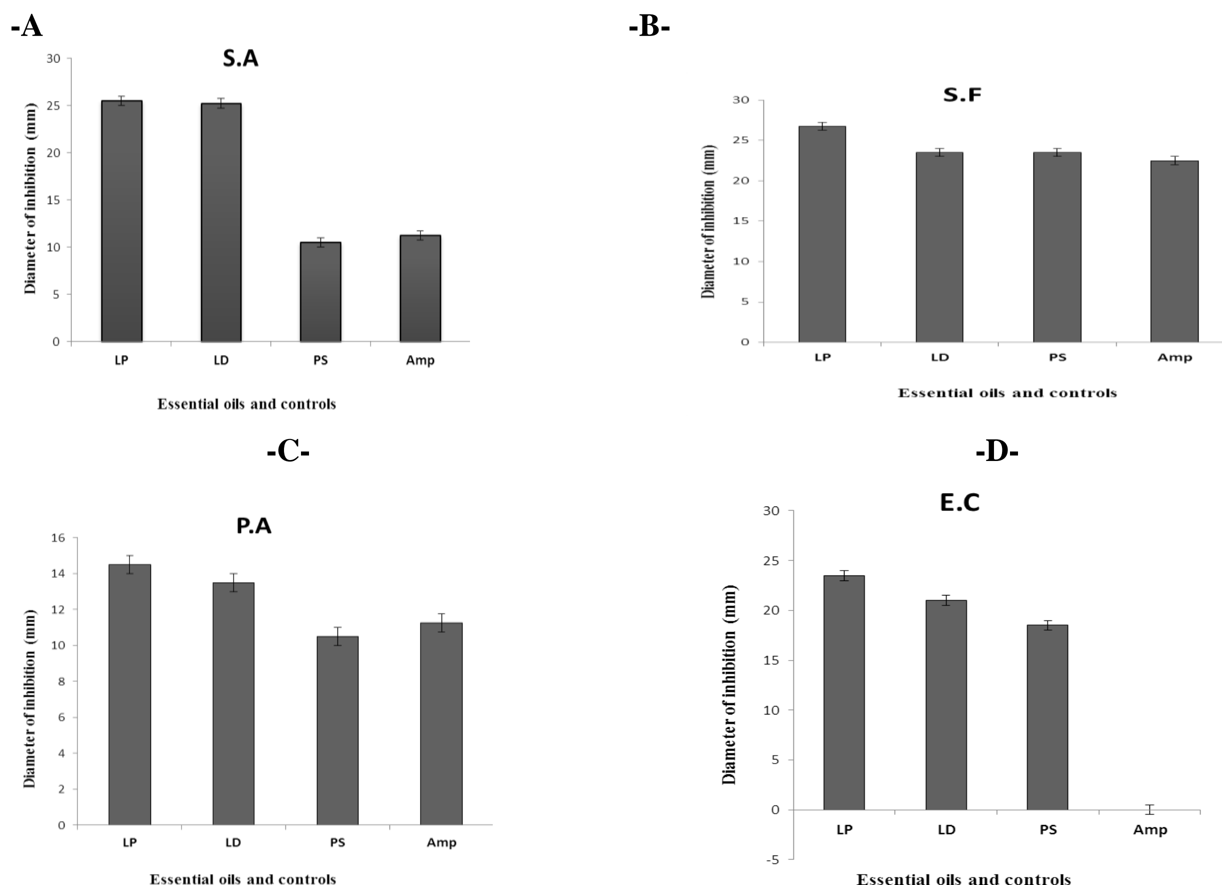

Figure 1: Antibacterial activity of EO from (LP) and (LD) against *Staphylococcus aureus* (A), *Streptococcus fasciens* (B), *E.coli* (C) and *Pseudomonas aeruginosa* (D). *: Mean zone of inhibition (Ø mm) and standard deviation. Control (PS: *Penicillin-streptomycin*, Amp: *Ampicillin*)

The data indicated that Gram-positive *Streptococcus fasciens* was the most sensitive strain tested to the E oil of *L. pedanculata* and *Staphylococcus aureus* was the most sensitive strain tested to the essential oil of *L. dentata* with the strongest inhibition zone (26.7 mm and 25.25 respectively). The *Staphylococcus aureus* was, in general, found to be less sensitive to the control (*Penicillin-streptomycin* and *Ampicillin*). Both oils also exhibited high antimicrobial activity against *Escherichia coli*. Modest activities were observed against *Pseudomonas aeruginosa*, with inhibition zones of 14.5 mm and 13.5 mm for EO of *L. pedanculata and L. dentata* respectively.
The antibacterial activity of *L. pedanculata* and *L. dentata* essential oil could be attributed to the presence of camphor and 1-8cineole for *L. pedanculata* and camphor and Trans Pinocarveol for *L. dentata*. It has been reported that camphor is one of the most efficient antimicrobial agents of various plants [29]. The mechanism of antibacterial activity of camphor is not completely understood in great detail. It has been demonstrated that the mechanism of action of camphor on the growth microorganisms includes the destabilization of the phospholipid bilayer structure, interaction with membrane enzymes and proteins, and its act as a proton exchanger reducing the pH gradient across the membrane [30]. In other studies, 1,8-cineole and camphor has found to possess some antimicrobial activities [31]. Thus, the antibacterial properties of the essential oils of *Lpedanculata* is probably connected with their high content of 1,8 cineol and camphor. Based on the Minimal inhibitory concentrations values (MIC), *L. pedanculata* was found to be more active against *Escherichia Coli* than *L.dentata*, (5 µg/mL for *L. pedanculata* against 10 µg/mL for *L.* d*entata*)
Finally, both oils were found less active against the *Pseudomonas aeruginosa*, (MIC values 20 µg/mL for both oils).

Table 2: Minimal inhibitory concentrations (MIC) (µg/mL) of selected essential oils.

	***Test organism* (MIC en µg/mL)**			
Plants species	***Staphylococcus aureus***	***Streptococcus fasciens***	***Escherichia coli***	***Pseudomonas aeruginosa***
L. pedanculata	5	5	5	20
L.dentata	5	5	10	20

Conclusions

Our results revealed that camphor is the major components of *Lavandula pedanculata* and *Lavandula dentata* essential oil grown in Morocco. Those oils possess rather a significant activity against different microorganisms. They may be suggested as a new potential source of a natural antimicrobial for the food industry after testing the toxic and irritating effects on humans.Therefore, further studies are necessary to estimate the potential for utilizing *L. pedanculata* and *L. dentata* essential oils as additives for extending the safety and shelf-life of food products.

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