



## **Antioxidant and Antibacterial Activities of Essential Oils from Three Moroccan Species (*Lavandula mairei* Humbert, *Lavandula dentata* L. and, *Lavandula stoechas* L.)**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### **ABSTRACT**

This study aimed to evaluate the antioxidant and the antibacterial activities of the essential oils from three Moroccan species of the genus *Lavandula* (*Lavandula dentata* L., *Lavandula mairei* Humbert and, *Lavandula stoechas* L.). The antibacterial activities were evaluated by the minimal inhibitory concentration (MIC) and the disc diffusion method, against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter koseri*) and Gram-positive bacteria (*Staphylococcus aureus*). The antioxidant potential was evaluated using free radical

Scavenging against 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing activity power (FRAP), and total antioxidant capacity (TAC). The antibacterial activity of the essential oils showed higher activity against tested nosocomial bacteria especially against *Klebsiella pneumoniae*, *Citrobacter koseri*, and *Staphylococcus aureus*. The essential oil of *Lavandula stoechas* L. revealed the higher antioxidant activity as measured by DPPH (IC<sub>50</sub> = 3,11 ± 0,13 mg/ml), FRAP (EC<sub>50</sub> = 6,88 ± 0,8 mg/ml) and TAC (443,2 ± 38 mg AAE/g EO) assays. The essential oil of Moroccan *Lavandula stoechas* L. could have potential as antimicrobial compounds for the treatment of many infectious bacterial diseases, including multi-resistant bacterial strains of nosocomial origin.

**Keywords:** Antioxidant activity; antibacterial activity; *lavandula mairei*; *lavandula stoechas*; *lavandula dentate*.

## ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picrylhydrazyl  
 FRAP: Ferric reducing activity power  
 TAC : Total antioxidant capacity  
 AMP : Ampicillin  
 CEC : Cefaclor  
 PR : Pristinamycin  
 IMP : Imipenem

## 1. INTRODUCTION

Nosocomial infections also called healthcare-associated infections (HAI), are infections acquired during the time of receiving healthcare that was not already present at the moment of hospital admission [1]. These infections are commonly caused by bacteria that are resistant to several antibiotics and can lead to delayed recovery or even death [2]. The WHO has launched in May 2015 the Global Antibiotic Research and Development Partnership, to fight antimicrobial resistance through new antibiotics and other therapeutic products [3].

Many studies have shown the potential of aromatic and medicinal plants as sources of compounds with antimicrobial activity [4,5]. One of these is the genus *Lavandula* that belongs to the Lamiaceae family and comprises more than 40 species [6]. *Lavandula* species have been used from Antiquity to flavor and preserve food and to cure illnesses, including microbial and viral infections [7]. The different medicinal and therapeutic effects of these plants are attributed to their richness in secondary metabolites [8]. Therefore, the evaluation of essential oil against a microbe is an evaluation of all the existing compounds in this oil at the same time against this microbe (Houël et al. 2015).

The present work aimed to evaluate the antioxidant potential (DPPH, FRAP, and total antioxidant capacity) and the antibacterial activity

of the three *Lavandula* species (*Lavandula mairei* Humbert, *Lavandula dentata* L. and *Lavandula stoechas* L.) against multi-resistant bacterial strains of nosocomial origin.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

Aerial parts of *Lavandula mairei* Humbert, *Lavandula dentata* L. and *Lavandula stoechas* L. were collected in Mai 2019 from the Al-Haouz region (Morocco). The determination was carried out by Professor Ahmed Ouhammou in the regional herbarium "MARK" of the FSSM-UCA (Morocco). Plant material was dried for three weeks in the shade at room temperature. The samples dried were stored in the dark at 5°C until use.

### 2.2 Extraction of Essential Oils

Dried shoot (200 g) was hydro-distilled for 3 h with 700 ml of water using a Clevenger-type apparatus, according to the method recommended by the European Pharmacopoeia (1975). The essential oils (Eos) obtained were dried with anhydrous sodium sulfate and stored in a refrigerator at 5°C until the analysis.

### 2.3 Antioxidant Activities

#### 2.3.1 DPPH radical scavenging activity

The ability of the essential oil to scavenge the DPPH radical was measured using the method described by Wu et al. (2003). A volume of 100 µl of various concentrations of the essential oil or standard was added to 1.4 ml of the ethanolic solution containing 0.1 mM of DPPH (2, 2-diphenyl-1 picrylhydrazyl). The mixture absorbance was measured at 517 nm by a spectrophotometer after 30 min of incubation in

the dark at room temperature. The percentage of inhibition was expressed as  $I\% = ((A_0 - A_s)/A_0) \times 100$ . Where  $A_0$  is the Blank absorbance and  $A_s$  is the sample absorbance. Butylated hydroxytoluene (BHT) was used as a positive control and the IC<sub>50</sub> values were calculated as the concentration causing 50% inhibition of the DPPH.

### 2.3.2 Ferric reducing antioxidant power (FRAP) assay

The reducing power activity of the tested oils was measured using the method of Oyaizu 1986. A set of 500  $\mu$ l of phosphate buffer (0.2 M, pH 6.6) and 500  $\mu$ l of potassium ferricyanide [ $K_3 Fe(CN)_6$ ] 1% were mixed with 200  $\mu$ l of the essential oils. The mixture was incubated in a bain-marie for 20 min at 50°C. A volume of 500  $\mu$ l of trichloroacetic acid (TCA) 10% was used to acidify the mixture. The obtained solution was then centrifuged for 10 min at 3000 rpm. 500  $\mu$ l of the solution's upper layer was mixed with 100  $\mu$ l of  $FeCl_3$  (0.1%) and 500  $\mu$ l of distilled water. The absorbance was measured at 700 nm and Quercetin was used as a standard. EC<sub>50</sub> (mg/ml) was calculated by plotting the absorbance against the corresponding concentration (EC<sub>50</sub> concentration corresponding to 0.5 of absorbance).

### 2.3.3 Total antioxidant capacity

The analysis was based on the reduction of Mo (VI) to Mo (V) and the resulting formation of a green phosphate/Mo(V) complex at acidic pH [9]. A volume of 25  $\mu$ l of essential oil was added to 1 ml of reagent solution (sulfuric acid 0.6 mol/L, sodium phosphate 28 mmol/L, and ammonium molybdate 4mmol/L). The mixtures were incubated in a bain-marie for 90 min at 95°C and then cooled to ambient temperature. The absorbance was measured at 695 nm, and the total antioxidant activity was given as ascorbic acid equivalence number (mg AAE/g EO).

## 2.4 Antibacterial Activity

### 2.4.1 Bacterial strains

The antibacterial activity of *Lavandula mairei* Humbert, *Lavandula dentate* L., and *Lavandula stoechas* L. was tested against; Gram-negative bacteria included *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter koseri* and *Acinetobacter baumannii*, and Gram-

positive *Staphylococcus aureus*. The inoculum suspension was prepared by using colonies from 24 hour cultures. The colonies were first suspended in a sterile aqueous solution (0.9%) of NaCl and then shaken for 20s. The density was adjusted at the Standard turbidity of a 0.5 McFarland ( $1.5 \times 10^8$  CFU/ml) [10].

### 2.4.2 Agar disc diffusion assay

The method described by Furtado and Medeiros (1980) was employed for the agar disc diffusion assay in triplicate. The microbial suspensions ( $1.5 \times 10^8$  CFU/ml) were inoculated by flooding onto the surface of Mueller Hinton (MH) agar. Sterile 6 mm diameter filter discs were impregnated with 10  $\mu$ l/disc of the EOs and were delivered onto the inoculated agar MH. The plates were incubated for 18h at 37°C. Antibacterial activity was evaluated by measuring the zones of inhibition against the tested strains. The standard antibiotics for comparison were the discs antibiogram of imipenem (IMP), ampicillin (AMP), cefaclor (CEC), pristinamycin (PR).

### 2.4.3 Minimum inhibitory concentration (MIC)

The MIC was obtained using a micro-dilution assay in plates of 96-well according to the National Committee for Clinical Laboratory Standards (NCCLS). The different concentrations of the essential oils were prepared in a suspension including 0.2% of agar in sterile distilled water to scatter the components without adding any solvent or detergent [11]. They were performed by successive dilutions 1/2 from 90 to 0.08 mg/ml. The obtained concentrations in the well were from 22.5 to 0.02 mg/ml. The bacterial suspensions were produced in the same way as previously described, diluted in MH broth and placed in 96-well plates at a density of  $1.5 \times 10^6$  CFU/ml. The plates were incubated for 18 h at 37°C, bacterial growth was visualized by adding 20 $\mu$ l of aqueous 2,3,5-triphenyltetrazolium chloride (TTC) solution (1%) to each well, with further incubation for 1 hour. MIC was defined as the lowest concentration that does not produce a red color [12].

### 2.4.4 Statistical analysis

The mean values and standard deviations were calculated by using SPSS 20. The results were analyzed by one-way ANOVA followed by Tukey-test, using the same software. Differences were considered to be significant at  $P < 0.05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1 Antioxidant Activities

##### 3.1.1 Essential oils composition

The phytochemicals contained in essential oils of the three *Lavandula* species are estimated by more than 23 compounds for *L. mairei* [4] and 52 for both *L. dentata* and *L. stoechas* [13]. Among these phytochemicals, carvacrol, 1,8-cineole and fenchone were reported as the major components of these species, respectively [4,18,19].

##### 3.1.2 The free radical-scavenging activity

Fig. 1 represents the percentage of inhibition of the essential oils of three studied species according to their concentration (*L. stoechas*, *L. dentata* and *L. mairei*). BHT, known for its antioxidant power [14], was used as a reference to compare the inhibition results of the essential oils. The IC<sub>50</sub> value represents the concentration capable of inhibiting 50% of DPPH. The highest antioxidant capacity against DPPH was observed in the essential oil of *L. stoechas* (3.11 mg/ml), *L. dentata* (4.75 mg/ml) and *L. mairei* (6.62 mg/ml), respectively. The difference between the antioxidant capacities of the three essential oils was significant at P < 0.05 (Table 1).

The antioxidant capacity of the investigated essential oils is due to their ability to act as donors of hydrogen atoms or electrons to transform DPPH<sup>\*</sup> (stable free radical) into its reduced form [15]. The study conducted by El Hamdaoui et al. (2018) on the scavenging of DPPH by the essential oil of *L. mairei* showed a lower IC<sub>50</sub> compared to our results, but remained high compared to the IC<sub>50</sub> of the essential oils of *L. stoechas*, and *L. dentata*.

##### 3.1.3 Ferric reducing/antioxidant power (FRAP)

Ferric Reducing Antioxidant Power (FRAP) Assay is based on the capacity of the antioxidant to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> [16]. This colorimetric method was used to compare the effective concentrations (EC<sub>50</sub>) to reach an absorbance of 0.5 of the different compounds (Fig. 2).

The essential oil (EO) of *L. stoechas* showed the highest antioxidant capacity (6.88 mg/ml), followed by EO of *L. dentata* (9.23 mg/ml) and EO

of *L. mairei* (13.4 mg/ml), while Quercetin (0.04 mg/ml) was used as reference (Fig. 3). The difference in the ferric reducing antioxidant capacities between the essential oils of *L. stoechas* and *L. dentata* was not significant, whereas the difference between these two EOs and the essential oil of *L. mairei* was significant at P < 0.05 (Table 1).

The antioxidant capacity of *L. stoechas* and *L. dentata* has been revealed by several previous studies [18–20]. This activity is related to the chemical composition of the essential oils which are rich in phenolic compounds [21]. In addition, the antioxidant capacity of EOs is of high interest since they can preserve foods from the risks of oxidants [22].

##### 3.1.4 The total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of the three essential oils was expressed as ascorbic acid equivalents (Table 1). The highest antioxidant activity was observed in the essential oil of *L. stoechas* (443.2 mg AAE/g EO) followed by *L. dentata* (262.1 mg AAE/g EO) and *L. mairei* (125.9 mg AAE/g EO), respectively. The difference in TAC between the three essential oils was significant (P < 0.05), whereas the difference between the EO of *L. stoechas* and the reference compounds (BHT and Quercetin) was non-significant (P < 0.05).

The essential oil of *L. stoechas* has a great antioxidant power which is in correlation with its chemical composition [23–25]. However, the phytochemicals that give essential oils their antioxidant activities can be influenced by the environment, agronomic conditions and the harvesting stage [26–28].

### 3.2 Antibacterial Activity of Essential Oils

#### 3.2.1 Disc diffusion technique

Table 2 shows the results of antibacterial activity by the agar disc diffusion technique. All essential oils showed significant antibacterial activity. The inhibition diameter of the essential oils ranged from 10 ± 0.1 mm to 18 ± 0.5 mm. The tested essential oils have in the majority of cases a higher antibacterial activity compared to the three antibiotics Ampicillin (AMP), Cefaclor (CEC), Pristinamycin (PR). The imipenem (IMP) antibiotic showed the highest antibacterial activity compared to EOs and the other antibiotics against all tested strains. The obtained results in

this paper are in agreement with other studies that revealed the high antibacterial potential of essential oils of the genus *Lavanlula* [4,23, 29].

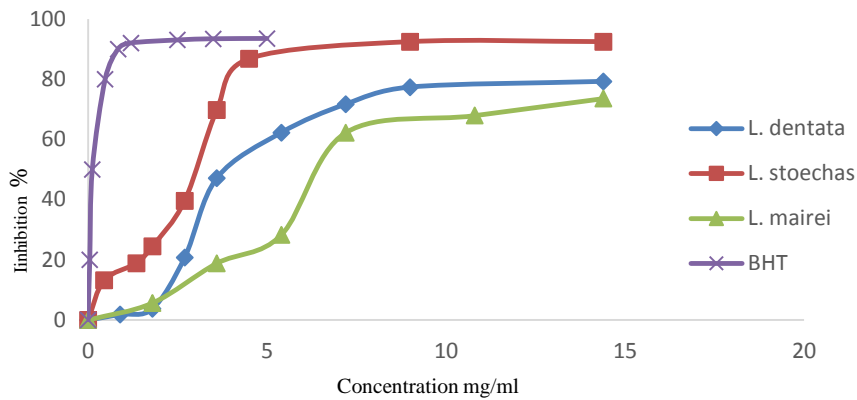


Fig. 1. DPPH free radical scavenging activity of *L. mairei*, *L. dentata* and *L. stoechas*. BHT was used as a reference

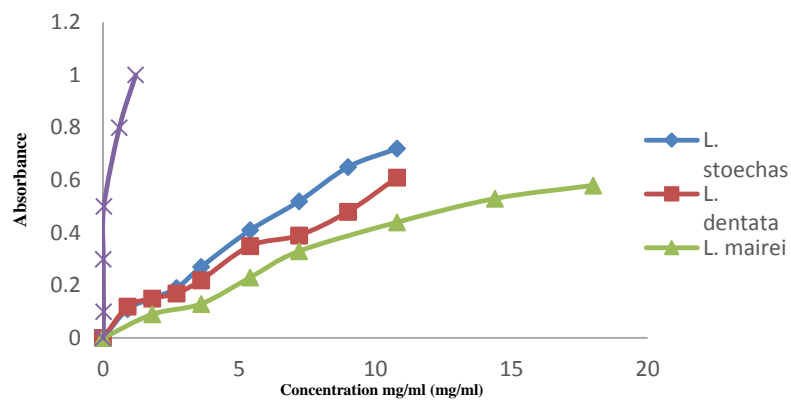


Fig. 2. Reducing power of *L. mairei*, *L. dentata* and *L. stoechas*. Quercetin was used as reference

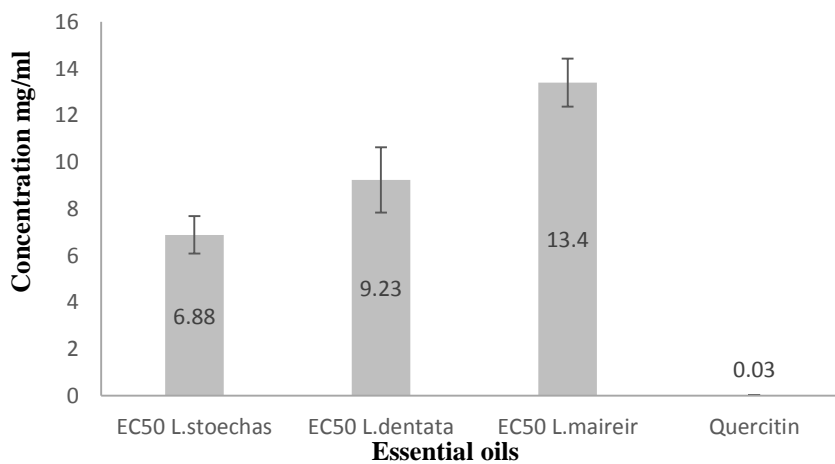


Fig. 3. EC50 of the Essential oils of *L. mairei*, *L. dentata* and *L. stoechas*. Quercetin was used as reference

**Table 1. Antioxidant activities of essential oils of three *Lavandula* species**

	DPPH (mg/ml)	FRAP (mg/ml)	Total antioxidant capacity (mg AAE/g EO)
<i>L. stoechas</i>	3,11 ± 0,13 <sup>a</sup>	6,88 ± 0,8 <sup>a</sup>	443,2 ± 38 <sup>a</sup>
<i>L. dentata</i>	4,75 ± 0,1 <sup>b</sup>	9,23 ± 1,4 <sup>a</sup>	262,1 ± 31 <sup>b</sup>
<i>L. mairei</i>	6,62 ± 0,21 <sup>c</sup>	13,4 ± 1,03 <sup>b</sup>	125,9 ± 18 <sup>c</sup>
BHT	0,12 ± 0,01 <sup>d</sup>	-	540,6 ± 40 <sup>a</sup>
Quercetin	-	0,03 ± 0,001 <sup>c</sup>	512,7 ± 15 <sup>a</sup>

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

**Table 2. Inhibition zone diameter (mm) of essential oils of three *Lavandula* species**

Bacterial strains	Inhibition zone (mm)						
	Essential oils			Antibiotics			
	<i>L. stoechas</i>	<i>L. dentata</i>	<i>L. mairei</i>	IMP	AMP	CEC	PT
<i>E. coli</i>	12±0.2	14±0.5	13±0.2	18	0	0	12
<i>K. pneumonia</i>	16.3±0.6	14±0.5	16 ±0.5	30	0	12	10
<i>S. aureus</i>	18±0.1	18±0.5	15±0.5	39	0	13	16
<i>P. aeruginosa</i>	14±0.3	12±0.4	10±0.1	28	8	0	10
<i>A. baumannii</i>	15.6±0.3	13.3±0.1	10±0.2	22	0	0	0
<i>C. koseri</i>	14±0.5	15.5±0.9	13±0.86	17	8	15	8

The inhibition zone includes the diameter of the disk (6 mm); IMP: Imipenem, AMP: Ampicillin, CEC: Cefaclor, PR: Pristinamycin.

**Table 3. Minimal inhibitory concentration (mg/ml) of essential oils of three *Lavandula* species**

Bacterial strains	<i>L. stoechas</i>	<i>L. dentata</i>	<i>L. mairei</i>
<i>Escherichia coli</i>	0,156	0,156	0,156
<i>Klebsiella pneumonia</i>	0,039	0,156	0,039
<i>Staphylococcus aureus</i>	0,039	0,039	0,078
<i>Pseudomonas aeruginosa</i>	0,156	0,156	0,624
<i>Acinetobacter baumannii</i>	0,156	0,312	0,624
<i>Citrobacter koseri</i>	0,039	0,039	0,078

### 3.2.2 Minimal inhibitory concentration (MIC)

Table 3 represents the minimum inhibitory concentration of the three essential oils tested. The EO of *L.stoechas* was the most effective against all strains compared to the other EOs. The minimum inhibitory concentration of *L. stoechas* was between 0.039 mg/ml (for *C. koseri*, *S. aureus* and *K. pneumonia*) and 0.156 mg/ml for the rest of the tested strains.

The antibacterial capacity of the essential oil of *L. mairei*, *L. dentata* and *L. stoechas* have revealed strong activity against several pathological Gram-negative and Gram-positive bacteria, such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* [4,30,31].

## 4. CONCLUSION

This study is the first to evaluate the essential oil of *L.mairei* against some nosocomial bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Acinetobacter baumannii* and *Citrobacter koseri*). Our results showed that the essential oil of

*L.mairei* has a significant antimicrobial and antioxidant activity but remains lower than the essential oil of *L.stoechas* and *L.dentata*. The highest antioxidant activity was observed in the EOs of *L.stoechas* as measured by DPPH (IC<sub>50</sub> = 3,11 ± 0,13 mg/ml), FRAP (EC<sub>50</sub> = 6,88 ± 0,8 mg/ml) and TAC (443,2 ± 38 mg AAE/g EO) assays. The essential oils of the Moroccan *Lavandula* genus could have potential as antimicrobial compounds for the treatment of many infectious bacterial diseases, including multi-resistant bacterial strains of nosocomial origin.

## CONSENT

It's not applicable.

## ETHICAL APPROVAL

It's not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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