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Antibacterial Activity of *Lavandula mairei* Humbert Essential Oil Against Carbapenem-resistant *Acinetobacter baumannii*

Lavandula mairei Humbert Esansiyel Yağının Karbapenem Dirençli *Acinetobacter baumannii*'ye Karşı Antibakteriyel Aktivitesi

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Abstract

Introduction: Nosocomial infections associated with a carbapenem-resistant *Acinetobacter baumannii* (CRAB) are considered as a significant challenge in the Intensive care units' (ICU) critically ill patients. Lack of new antimicrobial agents to treat infections caused by this multi-drug resistant microorganism reinforces the exploration of novel strategies to encounter and control this infection. Thus, the aim of the study was to evaluate, for the first time, the antibacterial effectiveness of the *Lavandula mairei* Humbert essential oil (EO) against a CRAB isolated from the hospital environment of the two ICUs.
Materials and Methods: Carbapenemase encoding genes were detected by the polymerase chain reactions and sequencing methods. The chemical composition of *Lavandula mairei* (EO) was determined by a gas chromatography-mass spectrometry analysis, and its antibacterial activity was evaluated by a disk diffusion and broth microdilution methods.
Results: The OXA-23 and NDM-1 variants were revealed in the selected strains. Twenty-six compounds were identified in the *Lavandula mairei* EO, in which carvacrol was the most dominant one (77.32%). All the tested CRAB strains showed a wide sensitivity to the oil, with the inhibition zones' diameters ranging from 28.67±1.15 mm to 40±2.00 mm, and a bactericidal effect proved by the similarity of minimal inhibitory and bactericidal concentrations values (0.39-3.125 µL/ml).
Conclusion: These findings remain very promising for the future use of *Lavandula mairei* EO as a source of the natural antibacterial agents.
Keywords: Antibacterial activity, carbapenem-resistant *Acinetobacter baumannii*, essential oils, *Lavandula mairei* Humbert, nosocomial infections

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Öz

Giriş: Karbapenem dirençli *Acinetobacter baumannii* ile ilişkili hastane enfeksiyonları, yoğun bakım ünitelerinde yatan kritik hastalarda önemli bir sorun olarak kabul edilmektedir. Bu çok ilaca dirençli mikroorganizmanın neden olduğu enfeksiyonları tedavi etmek için yeni antimikrobiyal ajanların olmaması, bu tür enfeksiyonlarla mücadele etmek için yeni stratejilerin araştırılmasını teşvik etmektedir. Bu nedenle, bu çalışmanın amacı, iki yoğun bakım ünitesinden izole edilen karbapenem dirençli *Acinetobacter baumannii*'ye karşı *Lavandula mairei* Humbert esansiyel yağının antibakteriyel etkinliğini ilk kez değerlendirmektir.

Gereç ve Yöntem: Karbapenemaz kodlayan genler, polimeraz zincir reaksiyonu ve dizileme yöntemleri ile tespit edildi. *Lavandula mairei* esansiyel yağının kimyasal bileşimi gaz kromatografisi-kütle spektrometrisi analizi ile belirlendi ve antibakteriyel aktivitesi disk difüzyon ve et suyu mikrodilüsyon yöntemleri ile değerlendirildi.

Bulgular: Seçilen suşlarda OXA-23 ve NDM-1 varyantları ortaya çıktı. *Lavandula mairei* esansiyel yağında, karvakrolün en baskın olduğu (%77,32) 26 bileşik tespit edildi. Test edilen tüm karbapenem dirençli *Acinetobacter baumannii* suşları, 28,67±1,15 mm ila 40±2,00 mm arasında değişen inhibisyon bölgelerinin çapları ile yağa karşı geniş bir duyarlılık gösterdi ve minimum inhibitör ve bakterisidal konsantrasyon değerlerinin benzerliği ile (0,39 ila 3,125 µL/ml) kanıtlanmış bir bakterisidal etki gösterdi.

Sonuç: Bu bulgular, doğal antibakteriyel ajanların kaynağı olarak *Lavandula mairei* esansiyel yağının gelecekteki kullanımı için çok umut verici olmaya devam etmektedir.

Anahtar Kelimeler: Antibakteriyel aktivite, karbapenem dirençli *Acinetobacter baumannii*, esansiyel yağlar, *Lavandula mairei* Humbert, hastane enfeksiyonları

Introduction

Acinetobacter baumannii (A. baumannii) is a Gram-negative non-motile and non-fermentative coccobacilli usually encountered in the hospital environment, especially in the intensive care units (ICUs). It is incriminated in serious nosocomial infections (NIs) including bacteremia, ventilator-associated pneumonia, central nervous system, and urinary tract infections[1].

Because of the anarchic and irrational antibiotic therapies, *A. baumannii* has rapidly emerged as multidrug-resistant (MDR) in healthcare facilities[2]. Multidrug-resistant *A. baumannii* (MRAB) is defined as a resistant to three or more groups of antibiotics (aminoglycosides, carbapenems, cephalosporins, and quinolones)[3]. Numerous studies proved its wild prevalence in the hospital environment[4-6], leading to the limited treatment options.

Carbapenems are known as the broad-spectrum antibiotics of a beta-lactam family, and the last resort to treat the antibiotic resistant infections. However, the worldwide dissemination of a carbapenem-resistant *A. baumannii* (CRAB) strains made them common and vulnerable[7,8]. Since carbapenems are the treatment of choice for *A. baumannii* infections, the carbapenem resistance, itself, is sufficient to consider CRAB as an extremely resistant[9]. In 2017, the World Health Organization underlined the threat of the CRAB to modern medicine as the first concern among a published list of 12 antibiotic resistant bacteria[10].

Considering the therapeutic failures associated with the emergence of MDR bacteria (e.g., CRAB), several studies have focused on the research of alternative drugs especially based on the phytomedicine[11-13]. Since ancient times, plants and their derivatives such as essential oils (EOs) have been used in the traditional medicine worldwide. The antibacterial activity of the plant's EOs against the MDR pathogens has been evaluated by many investigations[11,14-16].

Lavandula L. (*Lamiaceae*) is an old-world genus, and one of the most economically important members of the flowering plants of *Lamiaceae* family. It comprises about thirty-nine species, numerous hybrids, and about 400 registered cultivars[17]. Native to the Mediterranean region, *Lavandula* is largely distributed and cultivated in Europe, Canary Islands, Madeira, North Africa, Southwest Asia, Arabian Peninsula, India, North and South America[18]. The EOs of several *Lavandula* species are widely used in the aromatherapy with the significant antioxidant, antimicrobial, anti-inflammatory, spasmolytic, and carminative effects[18-20].

In Morocco, nine species and subspecies are identified in the genus of *Lavandula,* and in which, five are endemic[21]. *Lavandula mairei* (*L. mairei*) is one of these endemic species and it is considered as rare[22]. The wild plant is a perennial shrub with the spike violet flowers growing in Saharian, arid, and semi-arid bioclimates, from the southeast to the southwest mountains of Morocco[21]. This rare plant is mostly used in the traditional medicine for the treatment of gastrointestinal ailments, lung disorders, microbial infections, cough, asthma, headache, and fever[23].

Therefore, we have conducted a study to evaluate the antibacterial activity of *L. mairei* EO against the CRAB isolated from the hospital environment of two ICUs (adult ICU and neonatal ICU) of the Regional Hospital Center of Agadir-Morocco (RHCA).

To the best of our knowledge, there has been no previous report found investigating the antibacterial properties of *L. mairei* EO against the CRAB.

Materials and Methods

Molecular Identification of *A. baumannii* Strains

The studied *A. baumannii* strains were isolated from the surfaces and medical equipment of two ICUs of the RHCA. These latter were subjected to a molecular identification by the amplification and sequencing of the *16S ribosomal RNA* (*16S rRNA*) gene. The DNA extraction from the bacterial isolates was performed by a standard DNA extracted KIT (Isolate II Genomic DNA Kit, Bioline), according to the manufacturer's instructions. The amplification of the *16S rRNA* gene was carried out in a DNA thermal cycler (Veriti, Applied Biosystems), with the following cycling program: Initial denaturation at 95 °C for 2 min, and 35 cycles of denaturation at 95 °C for 30 sec annealing at 52 °C for 30 sec, extension at 72 °C for 30 sec, and a final extension at 72 °C for 3 min using the universal primer sequences[24,25]; fD1: 5'- *AGAGTTTGATCCTGGCTCAG*-3'and rP2: 5'-*TACGGCTACCTTGTTACGACTT*- 3. The sequencing of polymerase chain reactions (PCR) products was performed on an ABI 3130 XL sequencing apparatus (ABI 3130 XL Genetic Analyzer, Applied Biosystems). The acquired sequences were analyzed with the software available from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

Antibiotic Susceptibility Testing of *A. baumannii* Strains

The disc diffusion method on Mueller-Hinton Agar (MHA) was performed for an antibiotic susceptibility testing[26]. The following antibiotics were used for the test are: piperacillin-tazobactam (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (IMP) (10 µg), amikacin (AK) (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25 µg-23.75 µg), meropenem (MEM) (10 µg), tetracycline (T) (30 µg). The results were interpreted conforming to the guidelines of the Antibiogram Committee of the French Society for Microbiology[27].

Molecular Characterization of Carbapenem Resistance Genes

Preparation of DNA Template for PCR

Overnight cultures of CRAB strains on Luria Bertani agar (Bio Rad) were subjected to a DNA extraction using the PureLink® Genomic, DNA Mini Kit (Invitrogen, Carlsbad, USA) conforming to the manufacturer's instructions. DNA extracts were used as a template for the PCRs. These DNAs were stocked at -20 °C until the use.

Detection of Carbapenemase Encoding Genes

The carbapenemase genes were assessed by a single PCR using the specific primers as previously described[28-32] to identify: bla_{NDM}, bla_{OXA-23} bla_{OXA-24}, bla_{OXA-58} bla_{KPC}, $bla_{VIM,}$ and bla_{GES} (Table 1). Amplification reactions were performed in a final volume of 50 µL with 5 µL of 1× PCR buffer (Promega, USA), 2 U MyTaq DNA polymerase (New England BioLabs Inc., Beverly, MA, USA), 100 µM of deoxynucleoside triphosphates (dNTPs) (Invitrogen, USA), 2.5 mmol/µL of $MgCL_2$ (Promega, USA), 0.4 µM of each primer (Integrated DNA Technologies, USA), and 2 µL of extracted DNA. All the primers used in the PCRs are represented in Table 1.

The thermal cycling conditions of each reactions are the initial denaturation at 94 °C for 5 min, followed by the 30 cycles of denaturation (94 °C for 1 min), annealing (60 °C for 1 min for bla_{KPC}, bla_{VIM}, and $bla_{GES,}$ 57°C for 1 min for bla_{NDM} and 54 °C for 40 sec for bla_{OXA-23} bla_{OXA-24}, and bla_{OXA-58}), extension (72 °C for 1 min for bla_{KPC}, bla_{VIM}, $bla_{GES,}$ and $bla_{NDM,}$ 72°C for 50 sec for bla_{OXA-23} bla_{OXA-24}, and bla_{OXA-58}), and achieving with a final extension (72 °C for 7 min for bla_{KPC}, bla_{VIM}, $bla_{GES,}$ and $bla_{NDM,}$ 72°C for 6 min for bla_{OXA-23} bla_{OXA-24}, and bla_{OXA-58}).

The PCR amplicons were then analyzed by an electrophoresis in a 1% agarose gel containing ethidium bromide.

Sequencing of Resistance Genes

All the amplified products were sequenced to validate their identities. Both strands of the purified amplicons were sequenced with a genetic Analyzer 3130x1 sequencer (Applied Biosystems), using the same primers as used for a PCR amplification. Nucleotide sequences were analyzed with the software available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Plant Materials

Aerial parts of the wild *L. mairei* were collected at flowering stage, in the end of May 2014, from the Tafraout village in the southwest of Morocco. The 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. All the plants samples were air-dried in the shade and stored in dark at 4 °C until use.

Essential Oil's Extraction and Gas Chromatography/Mass Spectrometry Analysis

Dried aerial parts of the wild *L. mairei* were subjected to a hydro distillation for 3 h using a Clevenger apparatus. The obtained EO was dried over anhydrous sodium sulfate and stored in an amber bottle at 4 °C prior to the analysis. The chemical composition of the studied EO was revealed by a gas chromatography/mass spectrometry (GC/MS) analysis. It was carried out by using the Agilent GC/MSD system (Agilent Technologies 6890/5973) equipped with an Agilent DB_5 MS capillary column (30.0 m x 0.25 mm ID x 0.25 mm film thickness) programmed from 60-46 °C at 3 °C/min. The helium (high purity) was used as a carrier gas at a constant linear velocity of 37 cm/s. The transfer line,

ionization source and quadrupole temperatures were 280, 230, and 150 °C, respectively, operating at 70 eV ionization energy and scanning the m/z range 41-450. EO samples (60 ml) were diluted with the acetone (2 ml). The injection volume was 1 ml, the split ratio was 1:50 and the injector temperature was 260 °C. Identification of the individual components was based on: (i) comparison of their mass spectra with that of pure reference samples consulting the Wiley and NBS libraries (WILEY275 and NBS75K), and the compilation by Adams and Sparkman[33] (ii) comparison of their retention indices on a DB_5 (apolar, 5% phenyl polysilphenylenesiloxane), calculated relative to the retention times of a series of C-9-C-24 n-alkanes, with a linear interpolation, with those of authentic compounds or the literature data[33]. For semi-quantitative purposes, the normalized peak area of each compound was used without any correction factors to establish the abundances.

Antibacterial Activity Test

Disc Diffusion Method

The qualitative evaluation of the antibacterial activity of the wild *L. mairei* EO was carried out using the disc diffusion method on MHA[26]. The results were determined by measuring the diameters of inhibition zones after the incubation at 37 °C for 24 h. Amikacin discs (30 µg/disc) were used as the positive controls. All the tests were performed in triplicate.

The EO activity is classified into four levels: not sensitive for diameters <8 mm; sensitive for diameters 9-14 mm; very sensitive for diameters 15-19 mm, and extremely sensitive for diameters >20 mm[34].

Determination of Minimal Inhibitory and Minimal Bactericidal Concentrations

The minimal inhibitory concentration (MIC) analysis was performed in the MHB via broth microdilution technique using the 96-well microtiter plates[35], with some modifications. Briefly, the bacterial suspension was prepared from a fresh overnight culture and diluted in MHB into an approximately 10^6 CFU/ml. The EO was serially diluted in MHB with 4% (v/v) of dimethylsulfoxide (DMSO) to give a final EO concentration in the medium ranging from 25-0.04 µL/ml. Each plate was placed in a shaker and was incubated at 37 °C for 24 h. MHB added with the inoculum, and MHB with 4% DMSO were used as the positive and negative controls, respectively. Following the incubation, 20 µL of 20 mg/ml solution of TTC (2,3,5-triphenyl tetrazoliumchloride, Sigma) were added to each well and incubated at 37 °C for 15 min. A pink-red coloration of the wells indicated a bacterial growth. The MIC is defined as the lowest antimicrobial concentration which prevented the visible growth[36]. The MIC value was referred to the corresponding EO concentration of the well in which no pink-red coloration was observed.

The minimal bactericidal concentration (MBC) is the lowest concentration at which the inoculum viability is reduced up to 99.9% or no apparent growth occurred[37]. It was determined that by spreading 10 µL from the wells with no color change in Tryptic-Soy agar[38]. The plates were then incubated at 37 °C for 24 h. All the tests were performed in triplicate.

Statistical Analysis

The data were submitted to a statistical treatment by the analysis of variance using the R software, version 3.16 (RStudio, Inc).

Table 1. Amplicon sizes and primers used for the detection of carbapenemase encoding genes

Genes	Primers	Nucleotide sequences 5'-3'	Product size (pb)	References
bla_{NDM}	NDM (+)	*AATGGAATTGCCAATATTATGC*	489	[29]
	NDM (-)	*CGAAAGTCAGTTGCGCTGTG*		
bla_{KPC}	KPC (+)	*ATGTCACTGTATCGCCGTCT*	881	[28]
	KPC (-)	*TTACTGCCCGTTGACGCCCA*		
bla_{VIM}	VIM (+)	*AAAGTTATGCCGCACTCACC*	865	[30]
	VIM (-)	*TGCAACTTCATGTTATGCCG*		
bla_{GES}	GES (+)	*ATGCGCTTCATTCACGCAC*	863	[31]
	GES (-)	*CTATTTGTCCGTGCTCAGGA*		
bla_{oxa-23}	OXA-23like (+)	*GATCGGATTGGAGAACCAGA*	501	[32]
	OXA-23like (-)	*ATTTCTGACCGCATTTCCAT*		
bla_{oxa-24}	OXA-24like (+)	*GGTTAGTTGGCCCCCTTAAA*	246	[32]
	OXA-24like (-)	*AGTTGAGCGAAAAGGGGATT*		
bla_{oxa-58}	OXA-58like(+)	*AAGTATTGGGGCTTGTGCTG*	599	[32]
	OXA-58like(-)	*CCCCTCTGCGCTCTACATAC*		

Results

Identification and Antibiotic Susceptibility of *A. baumannii* Isolates

The molecular identity of the six *A. baumannii* strains was proved by the BLAST analysis of the obtained *16S rDNA* sequences, which showed a similarity of 99~100% to the *A. baumannii* strain AYO-241 (Accession No: MT559273.1), *A. baumannii* strain 2-2-1-10 (Accession No: JF919866.1), *A. baumannii* strain 7-2-10 (Accession No: JF919865.1).

The antibiotic susceptibility test revealed that the six studied strains of the *A. baumannii* exhibited a considerable resistance to ß-lactams especially to the carbapenems (IMP and MEM) with a sensitivity to the aminoglycosides (Table 2). Moreover, a reduced susceptibility to the other antibiotic families (fluoroquinolones and sulfonamides) was showed, proving the MDR phenotype of the strains.

Molecular Detection of Carbapenemase Encoding Genes

The molecular identification of the carbapenemase genes by PCR-sequencing revealed the predominance of a bla_{OXA-23} among all the six *A. baumannii* strains. On one out of the six CRAB strains, the coexistence of bla_{OXA-23} and bla_{NDM} was detected. The analysis of the DNA sequences identified OXA-23 and NDM-1 variants (Table 3).

Chemical Composition of *L. mairei* EO

Based on the dry weight, the yield of the EO extracted by a hydro distillation from the aerial parts of the plant was 1.00±0.26%. The GC-MS analysis of the *L. mairei* EO revealed twenty-six identified compounds, representing >98% of the oil composition (Table 4). The monoterpenes were the most omnipresent constituents (89.99%), with the oxygenated monoterpenes as the major compounds (82.36%). Sesquiterpenes were the less common ones with only 5.13%. Moreover, carvacrol was found to be the principal constituent of the *L. mairei* EO with the 77.32% followed by a terpinolene (2.73%), octen-3-ol (1.93%), p-cymen-8-ol (1.91%), cis-ß-ocimene (1.78%), carvacrol methyl ether (1.67%), and ß-caryophyllene (1.41%).

Antibacterial Activity Test Results

Conforming to the results summarized in the Tables 5, 6, all the targeted CRAB strains presented a wide sensitivity to the *L. mairei* EO, with a significant difference ($p<0.05$) between the

Table 2. Antibiotic susceptibility of the six *A. baumannii* strains isolated from adult and neonatal intensive care units

Strains	Antibiotic susceptibility										
	TZP	CTX	CIP	CAZ	TS	IMP	MEM	CN	AK	T	CRO
Adult intensive care unit											
AB 1	R	R	R	R	R	R	R	R	S	R	R
AB 2	R	R	R	R	R	R	R	R	S	R	R
AB 3	R	R	R	R	R	R	R	S	S	R	R
AB 4	R	R	R	R	R	R	R	R	S	R	R
AB 5	R	R	R	R	R	R	R	S	S	R	R
Neonatal intensive care unit											
AB 6	R	R	R	R	R	R	R	S	S	S	R

R: Resistant, S: Sensitive, I: Intermediate, AB: *Acinetobacter baumannii*, TZP: Piperacillin-Tazobactam, CTX: Cefotaxime, CRO: Ceftriaxone, CAZ: Ceftazidime, IMP: Imipenem, AK: Amikacin, GN: Gentamicin, CIP: Ciprofloxacin, TS: Trimethoprim-sulfamethoxazole, MEM: Meropenem, T: Tetracycline

Table 3. Carbapenemase encoding genes' detection of CRAB strains isolated from adult and neonatal intensive care units

Strains	Cabapenemase encoding genes							Detected variants
	bla_{OXA-23}	bla_{OXA-24}	bla_{OXA-58}	bla_{NDM}	bla_{GES}	bla_{KPC}	bla_{VIM}	
AB 1	+	-	-	-	-	-	-	OXA-23
AB 2	+	-	-	-	-	-	-	OXA-23
AB 3	+	-	-	-	-	-	-	OXA-23
AB 4	+	-	-	-	-	-	-	OXA-23
AB 5	+	-	-	-	-	-	-	OXA-23
AB 6	+	-	-	+	-	-	-	OXA-23/NDM-1

(+): Presence; (-): Absence.

CRAB: Carbapenem-resistant *Acinetobacter baumannii*

tested strains. Indeed, the studied EO showed an antibacterial effect with the inhibition zones' diameters ranging from 40±2.00 - 28.67±1.15 mm (Table 5). Moreover, *L. mairei* EO revealed higher antibacterial properties than the positive control (AK), considered as an extended spectrum antibiotic. For the broth microdilution assay, the results are given in the Table 6. The EO inhibited the CRAB strains at concentrations varying from 0.39-3.125 µL/ml. It is important to mention the MBC and MIC values were equivalent for all tested strains, proving the bactericidal effect of the *L. mairei* EO (Table 6).

Discussion

The rapid emergence of the *A. baumannii* infections is posing a serious public health threat in the ICUs' immune-compromised patients. This microorganism is intrinsically resistant to a wide array of the antibiotics and may acquire an armamentarium of resistance mechanisms (efflux pumps, drug-inactivating enzymes, and the drug target mutations) that allow this pathogen to become easily resistant to almost or even all the convenient antimicrobial agents[39]. Due to their valuable activity and low

Table 4. Chemical composition of *L. mairei* essential oil

Compounds*	M+ (Parent Ion) **	RI***	% of compound
α-pinene	136	931	0.35
Octen-3-ol	128	972	1.93
Octan-3-one	128	984	0.46
β-Myrcene	136	990	0.79
Octan 3-ol	136	996	0.33
α-Phellandrene	136	1008	0.15
d-3-Carene	136	1012	0.51
α-Terpinene	136	1016	0.14
p-Cymene	132	1021	0.75
Limonene	136	1025	0.29
Cis-β-Ocimene	136	1042	1.78
Trans-β-Ocimene	136	1051	0.14
Terpinolene	136	1088	2.73
Linalool	154	1096	0.33
Borneol	154	1165	0,04
Mentha-1.8-dien-4-ol	154	1177	0.29
Terpinen-4-ol	154	1178	0.37
p-Cymen-8-ol	150	1184	1.91
α-Terpineol	154	1191	0.43
Carvacrol methyl ether	164	1244	1.67
Bornyl acetate	150	1294	0.27
Carvacrol	150	1302	77.32
β-Caryophyllene	202	1424	1.41
Spathulenol	224	1581	1.55
Caryophyllene oxide	216	1587	2.17
Manoyloxide	290	2036	0.45

Monoterpene hydrocarbons: 7.63

Oxygenated monoterpens: 82.36

Sesquiterpene hydrocarbons: 1.41

Oxygenated sesquiterpens: 3.72

Oxygenated diterpenes: 0.45

Others: 2.99

Total: 98.56

*: Compounds listed in order of elution.

**: Ionization mode: electron impact at 70 eV.

***: Retention Indices measured relative to n-alkanes (C-9-C-24) on a nonpolar DB_5-MS column.

Table 5. Inhibition zone diameters of *L. mairei* essential oil and positive control against a carbapenem-resistant *A. baumannii* strains

Bacterial strains	Inhibition zone diameters (mm)	
	L. mairei EO	Amikacin (positive control)
AB 1	32.50±2.00[a]	22.00±0.00[c]
AB 2	40.00±2.00[c]	20.00±0.00[b]
AB 3	33.33±1.53[ab]	21.00±0.00[bc]
AB 4	31.60±3.05[a]	21.33±0.57[c]
AB 5	38.00±0.00[bc]	21.00±0.00[bc]
AB 6	28.67±1.15[a]	17.00±1.00[a]

[a,b,c]: Mean statistically significant differences with $p<0.05$.

EO: Essential oil

Table 6. Inhibition zone diameters of *L. mairei* essential oil and positive control against a carbapenem-resistant *A. baumannii* strains

Bacterial strains	CMI (µL/ml)	CMB (µL/ml)
AB 1	1.56±0.00[c]	1.56±0.00[c]
AB 2	0.78±0.00[b]	0.78±0.00[b]
AB 3	0.78±0.00[b]	0.78±0.00[b]
AB 4	3.125±0.00[d]	3.125±0.00[d]
AB 5	0.39±0.00[a]	0.39±0.00[a]
AB 6	0.78±0.00[b]	0.78±0.00[b]

[a,b,c,d]: Mean statistically significant differences with $p<0.05$.

EO: Essential oil

toxicity, carbapenems are recognized as the only appropriate treatment of infections caused by this microorganism. However, in the recent years, carbapenems resistance is increasingly common in the *A. baumannii* compromising this therapeutic option as well[40].

The six chosen strains for the current study were identified by the *16S rRNA* sequencing as *A. baumannii* with 99~100% of similarity. These identified strains revealed a great resistance to the several families of antibiotics including carbapenems (Table 2). Similarly, a study found that the *A. baumannii* strains isolated from a hospital environment in Iran, showed a considerable resistance to the IMP (94%)[41]. In another investigation carried out in the Mohammed V Military teaching hospital in Rabat-Morocco, a great resistant to the carbapenems (100%) was manifested by *A. baumannii* isolates[42]. It should be noted that this region of Morocco (Rabat-Salé-Zemmour-Zaer) has known as a significant spread of CRAB in the healthcare facilities ranging from 23.6% in 2001[43] to 76.19% in 2012-2014[44].

The molecular characterization of the carbapenemase encoding genes confirmed the resistance patterns of the isolated strains. Indeed, bla_{oxa-23} gene was detected in all the *A. baumannii* isolates (Table 3). The OXA-23 enzyme, a member of the Ambler class D group of the ß-lactamases, could hydrolyze carbapenems with a slow activity against oxyimino cephalosporins, aminopenicillins, and oxacillin[45,46]. The genes encoding for these enzymes are especially related with the mobile genetic elements prompting their transmission to the other species[47]. Worldwide outbreaks of the infections due to *A. baumannii* strains carrying bla_{oxa-23} have been documented by the previous investigations[48-51]. In a Moroccan study, most of the clinical and environmental CRAB (69.6%) isolates presented bla_{oxa-23} gene[51].

In addition to the bla_{oxa-23} gene, one strain (AB 6) of six of *A. baumannii* isolated in the current study harbored another carbapenemase gene: bla_{NDM} commonly reported among *Enterobacteriaceae*[52]. This coexistence maybe explained by the possible transmission of the resistance genes via a horizontal gene transfer[53]. This phenomenon was also found in the two isolated CRAB in Thailand[54].

Therefore, finding the new chemical substances with efficient therapeutic effects to the combat MDR infections has become a necessity worldwide. EOs, a complex mixture of the secondary metabolites of aromatic and medicinal plants is known to be a potential source of natural active compounds[55].

In this study, among the identified compound of the extracted *L. mairei* EO, the phenol monoterpene carvacrol was the most dominant constituent (77.32%) (Table 4). A study conducted to investigate the chemical composition of the *L. multifida* EO, revealed that the carvacrol was the main component with 61.73%[56]. Likewise, findings of a report affirmed the predominance of carvacrol (42.6%) in the *L. canariensis* (L.) Mill. EO[57]. On the contrary, other *Lavandula* species expressed lower concentrations of carvacrol or even the absence of the molecule[58,59].

L. mairei EO exhibited a great antibacterial activity against all the tested strains of CRAB (Tables 5, 6). Indeed, the obtained results showed that the MIC and MBC values were identical (MIC=MBC=0.39-3.125 µL/ml), proving the bactericidal effect of the oil. In a literature survey, no study related to the antibacterial activity of the *L. mairei* EO against a carbapenem-resistant nosocomial *A. baumannii* isolates has been reported. Thus, the obtained results could not be compared with the other studies. However, they were analyzed with some investigations conducted with the other aromatic and medicinal plants' species. It was underlined in a previous study that the considerable effect of the Moroccan *L. coronopifolia* on a clinical isolate of the MRAB with MIC and MBC values ranging between 1 and 2%[60]. On other hand, an investigation concluded the bactericidal action (MIC=MBC=0.31-0.62 mg/ml in most of the cases) of *Satureja khuzestanica* Jamzad EO on twenty-one MRAB strains[14]. The inhibition's growth of CRAB by *Origanum vulgare* L. essential has also been proved with a CMI of 0.015% (v/v)[61].

The strong activity of the *L. mairei* EO could be associated with the presence of high concentrations of carvacrol (Table 4). Due to its hydrophobic nature, and the hydroxyl group present in its chemical structure, this constituent has a great effect on the membrane of the bacterial cells[55]. It causes the disintegration of the outer membrane of Gram-negative bacteria, compromising a series of critical functions, especially the energy for the conversion processes, nutrient processing, synthesis of the structural macromolecules, and of many growth key enzymes, eventually leading to the bacterial death[62]. The antibacterial activity of the carvacrol against CRAB has been confirmed by a previous work[63]. The most promising antimicrobial activity results of the carvacrol were against three strains of *A. baumannii* that were MDR or extremely a drug resistant, positive for carbapenemase encoding genes[63]. However, considering the EO as a mixture of bioactive molecules, the main compound is not necessarily behind the whole antibacterial activity. The involvement of the other minor molecules should be considered[64,65]. These molecules could interact with each other or with the major active compounds. The inhibition effect may differ according to their interactive functions. Different mechanism of actions of one or several compounds could be involved, and thus acting at several sites of the cell[66].

The limitation of the study was the small number of *A. baumannii* isolates. It should be noted that among the *A. baumannii* strains isolated during the study conducted in the RHCA, the six chosen strains for the current study had the most relevant antibiotic patterns with a resistance to carbapenems.

Conclusion

For the first time, this study highlighted a significant antibacterial activity of *L. mairei* EO against the CRAB. All the tested bacteria presented OXA-23 variant with coexistence between this latter and NDM-1 variant in one strain, proving their MDR and capacity to induce NIs difficult to treat. Thus, the obtained results are very encouraging, and suggest that this EO, rich in the carvacrol (77.32%), is an eventual candidate for further studies to develop the alternative bioactive molecules or to include it in the formulations of some disinfectant and antiseptic solutions to reduce the cross contaminations in the hospital environment.

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Ethics

Ethics Committee Approval: The study were approved by the Bioethics Consultative Commission Faculty of Sciences Agadir (protocol number: ER-BS-02/2022-0001, date: 27.02.2022).

Informed Consent: The strains studied were isolated from solid surfaces found in the hospital environment such as silicone, ceramics.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.A.A., F.H., M.H., R.M., F.M., B.B., Design: A.A.A., F.H., M.H., R.M., M.T., Data Collection or Processing: A.L., K.N., F.M., M.T., M.El Y., A.El H., Analysis or Interpretation: M.B., A.L., B.B., K.N., M.T., F.M., A.El H., M.El Y., Literature Search: A.L., K.N., M.B., B.B., M.El Y., Writing: A.L., M.H., F.H., R.M., A.A.A., B.B., A.El H.

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