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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *Lavandula Multifida*

Abdellah Elmakaoui[1], Ilhame Bourais[2], Asmaa Oubihi[3], Asmae Nassif[2], Tatyana Bezhinar[4], Mohammad Ali Shariati[5], Andrey Vladimirovich Blinov[6], Lukas Hleba[7], and Souad El Hajjaji[1]

Address(es):
[1]Laboratory of Spectroscopy, Molecular Modeling, Materials, Nanomaterials, Water and Environment, CERNE2D, Faculty of Sciences, Mohammed V University in Rabat, Morocco.
[2]Laboratory of Human Pathologies Biology, Department of Biology, Genomic Center of Human Pathologies, Faculty of Sciences, Mohammed V University in Rabat, Morocco.
[3]Laboratory of Natural Resources and Sustainable Development, Department of biology, Faculty of Science, University Ibn Tofail, Kenitra, Morocco.
[4]Institute of Veterinary Medicine, South Ural State Agrarian University, 13 Gagarin St., Troitsk, 457100, Russian Federation.
[5]Department of Scientific Research, K. G. Razumovsky Moscow State University of Technologies and Management (The First Cossack University), 73 Zemlyanoy Val, Moscow, Russian Federation.
[6]North Caucasus Federal University, Stavropol, Russia.
[7]Institute of biotechnology, Faculty of Biotechnology and Food Sciences Tr. A. Hlinku 2, Slovak University of Agriculture in Nitra, 949 76 Nitra, Slovak republic.

*Corresponding author: IlhameBourais: i.bourais@um5r.ac.ma; Souad El Hajjaji: s.elhajjaji@um5r.ac.ma

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ABSTRACT

This work aims to determine the antioxidant, antibacterial properties and chemical profile of the essential oil (EO) of *Lavandula multifida* (LM) at the flowering stage collected in northwestern Morocco. So far, information on essential oils of *L. multifida* remains limited. The extraction yield of the essential oil was 0.46%. The composition was determined by GC-MS-FID. Twenty compounds were identified and the major phytochemicals were carvacrol, ß-bisabolene, and careophyllene oxide (44.3%, 31.9%, and 5.8% respectively). In addition, information on the essential oil of *L. multifida* collected in the Rabat region is reported for the first time in this study. DPPH and FRAP tests were used to evaluate its antioxidant activity. Antibacterial activities were also studied. Indeed, the essential oil of *L. multifida* exhibited inhibition effects on the growth of various microorganisms, including S. aureus where the MIC and MBC were equal to 0.5 and 2µg/ml, respectively. Therefore, the use of *Lavandula multifida* essential oil shows great promise in various fields.

Keywords: *Lavandula multifida;* essential oil; antioxidant; antibacterial effect; chemical composition

INTRODUCTION

Medicinal plants have always been a part of human societies' basic knowledge since ancient times **(Aquaron, 2005; Benabdelkader, 2012)**. As medical technology progresses, herbal treatments, generally known as "alternative or supplementary medicine", are becoming increasingly popular **(Qidwai and Ashfaq, 2013)**and the growing interest in their application has prompted increased research into plant-based chemicals **(Bagiu *et al.*, 2012)**.The industry is beginning to rethink the use of natural substances in formulations. In point of fact, aiming to improve their quality of life, the majority of consumers are turning to the use of natural substances as alternatives to synthetic substances that can be harmful. Phenolic compounds are the most well-known example; they have been widely used in folk medicine **(Haraoui *et al.*, 2020)**. In addition, various biological properties such as antiviral, antimicrobial, anti-inflammatory, anticancer, as well as prevention of cardiovascular and degenerative diseases have been attributed to phytochemicals extracted from medicinal plants **(Alain *et al.*, 2018)**. Lavender is a plant of the *Lamiaceae* family, rich in essential oils and mainly used in traditional medicine and cosmetics. In Morocco, medicinal plants represent various treatments for different diseases **(Zrira, 2017)**.The plants contain biological properties, such as antifungal, antibacterial and antioxidant properties. However, some species from Rabat, including *Lavandula multifida* (*LM*), have not been examined so far, *L. multifida* is a semi-persistent perennial plant in the Lamiaceae family with extremely multifides aerial parts formed by numerous tiny leaves ***(Douhri et al., 2014; Saadi et al.,* 2016)**. It thrives on rocky outcrops and on more or less drained limestone soils in the Mediterranean area and tropical Africa, where it is mostly found in pre-Saharan regions **(Zuzarte *et al.*, 2012)**. In Morocco, it often grows on the borders of rivers and between rose bushes to protect itself from aphids. *L. multifida* plays an important role in traditional Moroccan medicine. Indeed, it is used against various problems and pathologies among others gastric disorders, polyarthritis… **(Znini *et al.*, 2012).** Summary studies on the pharmacological properties of *L. multifida* revealed a broad spectrum of biological activities mainly antimicrobial **(Benbelaid *et al.*, 2012)**, antidiabetic, antiepileptic and sedative **(Cong *et al.*, 2008)**, antifungal, anti-inflammatory and antidepressant **(Zuzarte *et al.*, 2012)**.The essential oil of *L. multifida*, collected in northwestern Morocco, was subjected to GC-MS-FID analysis to identify its various chemical components. In addition, antioxidant and antibacterial activities were evaluated to fill the data gap for this species. The activities of *L. multifida* essential oil (EO) are discussed in more detail with the aim of highlighting their potential use for therapeutic purposes.

MATERIAL AND METHODS

Plant material origin

Lavandula multifida used in this study was collected from its native environment at the full bloom stage in March 2021 in Northwest Morocco in the region of Rommani, 81 km from Rabat (33° 32′ 00″ N and 6° 36′ 0″ W), and at an altitude of 306 meters (Figure 1). The plant was then authenticated by Professor Oufae Benkhnigue of the Scientific Institute of the Mohammed V University of Rabat, Morocco (example code: RAB113339). The samples were then transported to the laboratory. The plant was properly washed before being dried in the shade at room temperature. Afterwards, *L. multifida* was blended and ground into powder using a Pro-Stard 8200 blender.

Figure 1 *Lavandula multifda* collected in the norwest of Rabat region (Morocco)

Essential oil extraction

The essential oil of *Lavandula multifida* was extracted by hydrodistillation performed with a Clevenger type device for 5 hours. An amount of 250 g of plant (leaves and stems) was added to 350 ml of water. The yield of the EO was 0, 46%. The essential oil was dried with anhydrous sodium sulfate. The oil was then refrigerated at 4°C until use.
The yield of essential oil was determined using the formula below:

$$\textbf{Yield (\%)} = \frac{Wf}{Wi} \times 100 \qquad (1)$$

Where: **Wf** represents the mass of the extracted oil and **Wi** the initial mass of dried plant material.

Chemical composition of essential oil

Chromatographic analyses were performed on a Perkin Elmer TM GC-680 gas chromatograph equipped with an HP-5 MS (5%-phenyl)-methylpolysiloxane capillary column (60 m × 0.25 mm) and a film thickness of 0.25 µm. The column temperature was programmed from 60 to 300ºC/min and the ionization energy was equal to 70 eV. The carrier gas used was helium with a constant flow rate at 1 mL/min, where the sample injection was performed without fractionation. The identification of the constituents was carried out on the basis of their retention indices (IR) with a Chem-Station type computer system attached to the instrument. Indeed, it was based on the comparison of retention times with those of a series of n-alkanes (C_5-C_{24}) determined by **Van Den Dool and Kratz (1963)** equation, as well as on the comparison of the mass spectra of the sample with those described in the library (NIS-T98) **(Adams, 2007)**.

Antibacterial activity

Gelose diffusion method

Evaluation of the antibacterial activities of *L. multifida* essential oil was first performed by the disc diffusion method due to its simplicity and effectiveness in testing the susceptibility of strains **(Oubihi *et al.*, 2020)**. A 20 mL volume of chilled Muller Hinton super agar medium was poured into petri dishes. A microbial suspension with an optical density of 10^6 CFU/mL was dispersed on the surface of the culture medium after it solidified. Whatman absorbent paper discs of 6 mm diameter were autoclaved, they are injected with 15µl of essential oil and then deposited on the agar surface. The Petri dishes are kept at 4°C for one hour so that the essential oil can diffuse before germs growing **(Rozman and Jersek, 2009)**. The whole is incubated at 37°C for 24 hours. Upon application of the impregnated discs, the essential oil diffuses evenly, and after incubation, the absence of microbial growth is reflected by a translucent halo around the disc, identical to sterile agar. Amoxicillin (25µg) and penicillin (5µg) were used as positive controls.

Minimal inhibitory concentration (MIC) and bactericidal (MBC) concentrations

The influence of an essential oil on a bacterial strain is demonstrated in a simple and macroscopic manner. The minimum inhibitory (MIC) and bactericidal (MBC) concentrations are estimated by the agar dilution method **(Al-Jaber *et al.*, 2011**).The essential oil is emulsified with a 0.2 percent agar solution. Dilutions are prepared at 100, 40, 20, 10, 5, 3.3 and 2 µL/mL in this agar solution. To generate final concentrations of 10, 4, 2, 1, 0.5, 0.33, and 0.2 mL/mL, we aseptically add 1.5 mL of each dilution into test tubes containing 13.5 mL of solid medium and sterilized in an autoclave at 121°C. The contents of each tube are immediately placed in a sterile Petri dish. The contents of each tube are immediately placed in a sterile Petri dish. Controls consisted of culture medium and 0.2% agar solution. Colonies were isolated by the streak method using a calibrated platinum loop to collect the same volume of inoculum. The media were then incubated at 37°C for 24 hours. To minimize experimental error, each test is performed in triplicate. The diffusion method or blotting paper discs was chosen to determine the zones of inhibition. It consists in depositing blotting paper discs impregnated with essential oil at a certain concentration on the surface of the culture medium. An inhibitory zone of 6 mm in diameter appeared with the use of a concentration of 15µl of essential oil, 25 µg of amoxicillin and 5 µg of penicillin per disc.

Antioxidant Activity (AA)
DPPH assay for scavenging free radicals

A solution was prepared by mixing 2.5 mL of plant extract with 0.5 mL of DPPH methanolic solution (0.2mM). The mixture was homogenized with a vortex and then left at 25°C for 30 minutes and read at 517 nm. The evaluation of the antioxidant potential is proportional to the decolorization of the solution and was calculated by the following relationship:

$$\%RSA = \frac{A_D - A_E}{A_D} \text{x } 100 \qquad (2)$$

Where: $\mathbf{A_D}$refers to the blank value and $\mathbf{A_E}$to the test solution value.

Reducing ferric power determination

The objective of this technique is the reduction of Fe^{3+} ions to Fe^{2+} under the action of a reducing agent. The essential oil or standard (Ascorbic acid and Trolox) was mixed with 2.5 mL of phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was then incubated for 20 minutes at 50°C. This was then followed by the addition of 2.5 mL of trichloroacetic acid (10%) and centrifugation for 10 minutes. Equal amounts of 2.5 mL of this combination and purified water (0.1%) were added to 0.5 mL of iron chloride. The absorbance of the sample and positive controls were measured at 700 nm **(Jamali *et al.*, 2013)**. The test was repeated three times and the mean value of 50% reduction of Fe^{3+} ions (IC_{50}) was recorded in the means ± SD.

RESULTS AND DISCUSSION

Yield of essential oil

Hydrodistillation of *Lavandula multifida* produced a pale yellow essential oil with a fresh scent. The amount of EO produced is 0.46%. When this yield is compared to others described in the literature, it is clear that *LM* collected in Rabat region is relatively rich in essential oil. Indeed, our yield exceeds that of **Douhri *et al.* (2014)** which was 0.097%, and is lower than 2.4% found by **Laghchimi *et al.* (2014)**, for different regions of Morocco.From the point of view, this may be due to abiotic factors (air, water, soil, temperature) and vitality in ecosystems, part of the plant used, and age. Yields in essential oils that can be qualified as average were obtained, is 0,08 % , 2.6% , 2.5% and 1.83% respectively for *L.maroccana* **(Ouarhach *et al.*, 2020)**, *L. dentata*, *L. stoechas* , *and L. peduncultata* **(Bachiri *et al.*, 2017)**. The observed variations are due to morphological differentiations which appear during the phenological cycle, or even genetic factors **(Angioni *et al.*, 2006)**. **Kozlowski *et al.***, (2012) showed that photosynthetic and metabolic matter is changed to flowers and fruits following ontogenesis, based on the "source-to-sink transfer" concept. The increase in essential oil concentration in flowers and leaves had biological and ecological significance **(Bachiri *et al.*, 2016).**

Anti-radical activity using DPPH and FRAP assays

The antioxidant potential of *L. multifida* essential oil and the two controls (Ascorbic acid and trolox), which is based on the free radical scavenging capacity, is measured by DPPH and FRAP methods. The results obtained are presented in Table 1. The low IC_{50} value also indicates a strong protective activity. The antioxidant activities of *LM* essential oil, expressed as IC_{50} values, were 327, 24±10, 69 and 48, 08±1,71 µg/mL using DPPH and FRAP tests, respectively. The recorded antioxidant activity of the essential oil can be considered interesting compared to the antioxidant activities of the standards used. The standard IC_{50} values obtained by Ascorbic acid and Trolox were 13.49 ±0.48 and 20.72 ±0.51 µg/mL for DPPH radicals, as well as 19.89± 0.41 and 36.95± 1.08 µg/mL for iron reducing capacities. Based on the above results, *Lavandula multifida* appears to have a lower antioxidant activity than that found by **Messaoud *et al.* (2012)** with an IC_{50} = 201.6± 2.0 µg/mL for DPPH and 39.1 ±1.7 µmol $.g^{-1}$ for FRAP. This study is the first to investigate the antioxidant activity of *L. multifida* essential oil in the Rabat region. This plant has a higher antioxidant capacity than *Lavandula stoechas* collected in Rabat with IC_{50} was equal to 785.38±9.04 µg/mL and 107.53± 1 .74 µg/mL by DPPH and FRAP, respectively **(Bouyahya *et al.*, 2017).** The antioxidant properties of *L. multifida* are certainly due to the main phenolic components of the essential oil, especially carvacrol.

Table 1 Antioxidant activity of *Lavandula multifida* essential oil *vs* ascorbic acid and trolox standards

	DPPH	FRAP
Essential oil (µg/mL)	327,24±10,69	48,08±1,71
Ascorbic acid (µg/mL)	13,49±0,48	19,89±0,41
Trolox (µg/mL)	20,72±0,51	36,95±1,08

Antibacterial activity

The evaluation of the increase in antibacterial activity of *L. multifida* essential oil is determined by measuring the widths of the zones of inhibition Table 2. In general, we notice that the inhibitory power of essential oil is very important each time, the diameters of the inhibition zone ranging from 10 to 20 mm for each strain tested. The antibiogram test revealed that *LM* essential oil exhibit inhibitory effects on the development of different microorganisms. Significant antibacterial activity has been established against *Staphylococcus epidermidis, S. aureus, Acinetobacter baumannii* and *Escherichia coli all of clinical origin* strains when compared to the reference antibiotic.

Table 2 Comparison of the of inhibition zones diameters (mm) illustrating the antibacterial activity of the essential oils from the three lavenders: *L. dentata, L.pedunculata, L. steochas* and studied *L.multifida*.

	Inhibition zone diameter (mm)					Reference
	S. aureus	***S. epidermidis***	***K. pneumoniae***	***A. baumannii***	***E. coli***	
L. dentata	20 ± 0,32	-	9 ± 01	-	9 ± 0,20	**Bachiri *et al.*, 2017**
L. stoechas	-	-	10 ± 0,26	-	9 ± 0,00	
L. pedunculata	11,10±0,10	-	9,03±0,06	-	-	
L. multifida	20 ± 0.01	14.66 ± 0.44	11 ±0.01	10.16 ± 0.22	9 ± 0,01	
Ampicilin	12 ± 00	7 ± 00	14 ± 00	-	-	Our study
Penicilin	-	-	-	-	-	

The diameters of the zones of inhibition recorded vary between 10 and 20 mm and were lesser than those obtained antibiotics except for the case of the *Klebsiella pneumonia* strain where the synthetic antibiotic tested (Amoxycillin) to be given values greater than 14 mm essential oil for *Klebsiella pneumonia*. Similar results were reported by **Benbelaid *et al.* (2012)** and **Douhri *et al.* (2014)**. The screening of the antibacterial activity of the essential oil of *Lavandula multifida* highlights evidence of high antibacterial activity against the five strains of tested bacteria. The Staphylococcus aureus strain is more sensitive to the essential oil of Lavandula multifida (MIC = 0.5 µg / mL), while the MIC against Staphylococcus epidermidis, Escherichia coli and *Acinetobacter baumannii* was 2µg / mL. *Lavandula multifida* essential oil has a bactericidal effect (MIC = MBC) on *Staphylococcus epidermidis*, Klebsiella *pneumonia* and *Escherichia coli* (Table 3). However, it has a bacteriostatic power against *Staphylococcus aureus* and *Acinetobacter baumannii*. In addition, Gram-negative bacteria appear to be more resistant to Gram-positive microorganisms in *LM* essential oil. Much research has confirmed these findings **(Kaplan *et al.*,2007)**.

Table 3 Comparison of minimum inhibitory (MIC) and bactericidal (MBC) concentrations of essential oils from two essential oils of reported lavenders *L. dentata and L. stoechas* and studied *L. multifida* EO.

Bacterial strains	Lavandula specie	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Reference
S. aureus	*L. dentata*	0,08	0,1	1,25	**Bachiri *et al.*,2016**
	L. steochas	0,17	0,23	1,35	
	L. multifida	2	0,5	0,25	Our study
S. epidermidis	*L. dentata*	-	-	-	**Bachiri *et al.*,2016**
	L. steochas	-	-	-	
	L. multifida	2	2	1	Our study
K. pneumoniae	*L. dentata*	0,14	0,16	1,14	**Bachiri *et al.*,2016**
	L. steochas	0,14	0,16	1,14	
	L. multifida	4	4	1	Our study
A. baumannii	*L. dentata*	-	-	-	**Bachiri *et al.*,2016**
	L. steochas	-	-	-	
	L. multifida	4	2	0,5	Our study
E. coli	*L. dentata*	0,14	0,16	1,14	**Bachiri *et al.*,2016**
	L. steochas	0,14	0,16	1,14	
	L. multifida	2	2	1	Our study

The resistance mechanism of Gram (-) bacteria is due to the permeability of the outer membrane and the cytoplasmic membrane. As a matter of fact, these structures modulate the passive diffusion mechanism preventing hydrophobic substances from diffusing through the lipopolysaccharides. *Lavandula multifida* essential oil has been shown to possess antimicrobial properties that can be attributed to the chemical composition of this plant, including β-bisabolene and carvacrol, major components of this oil, with carvacrol being the most active antimicrobial component in some plants **(Djenane *et al.*, 2011; Douhri *et al.*, 2014)**. Carvacrol has a strong antimicrobial effect on many Gram-negative and Gram-positive bacteria **(El Asbahani *et al.*, 2015; Jaber *et al.*, 2021; Zayyad *et al.*, 2014)**. Due to differences in chemical composition, experimental methodologies, and strains studied, our results may not perfectly match those cited in the literature. The antimicrobial action of *Lavandula multifida* essential oil is still poorly elucidated. However, essential oils of species in the *Lamiaceae* family, such as *Origanum compactum*, have the ability to cross cell membranes, causing potassium leakage, disruption of the respiratory system, and minor changes in cell morphology **(*Bouyahya et al.*, 2017).** The antibacterial activities of essential oils are therefore due to their ability to deregulate quorum sensing signaling pathways resulting in decreased resistance to microorganisms **(Luís *et al.*, 2016; Myszka *et al.*, 2016)**.

Chemical composition of the essential oil

Table 4 presents the chemical components of *Lavandula multifida* essential oil. The latter is mainly composed of 48.7% oxygenated monoterpene and 35.6% sesquiterpene hydrocarbons. The monoterpene hydrocarbons and oxygenated sesquiterpenes were found to be low, 6.8% and 6.2%, respectively. Analysis of the EO showed that it contains major compounds including carvacrol (44.3%), β-bisabolene (31.9%), and careophylene oxide (5, 8%). 21.6% of the overall composition of the essential oil is represented by seventeen minor compounds.

Table 4 Chemical composition of the essential oil of *Lavandula multifida* aerial part collected in Northwest of Rabat region

No.	RT	RIa	RIb	Compounds	Contribution (%)
1	11.9	939	937	α-Pinene (M.H)	0.3
2	12.2	974	971	1-Octen-3-ol (O)	0.2
3	12.9	990	993	β-Myrcene (M.H)	0.2
4	13.5	1002	1000	α-Phellandrene (M.H)	1.8
5	13.9	1008	1010	3-Carene (M.H)	0.3
6	14.4	1024	1026	p-Cymene (M.H)	0.2
7	14.8	1032	1031	β-Ocimene (M.H)	1.8
8	15.5	1068	1070	1-Octanol (O)	0.3
9	15.5	1082	1090	Terpinolene (M.H)	2.2
10	15.6	1095	1091	Linalol (O.M)	0.5
11	16.2	1116	1115	Fenchol (O.M)	3.2
12	16.4	1130	1135	α-Terpineol (O.M)	0.4
13	16.6	1244	1241	Carvacrol methyl ether (O.M)	0.3
14	17.1	1298	1301	Carvacrol (O.M)	44.3
15	18.9	1408	1405	Caryophyllene (S.H)	2.1
16	19.1	1442	1441	β-Farnesene (S.H)	0.2
17	19.8	1484	1486	Germacrene-D (S.H)	1.4
18	21.1	1505	1501	β-Bisabolene (S.H)	31.9
19	21.3	1582	1579	Careophylene oxide (O.S)	5.8
20	21.6	1652	1650	α-Cadinol (O.S)	0.4
Monoterpenehydrocarbons (M.H)					6.8
Oxygenatedmonoterpenes (O.M)					48.7
Sesquiterpenehydrocarbons (S.H)					35.6
Oxygenatedsesquiterpenes (O.S)					6.2
Monoterpene alcohols (O)					0.5
Total identified compounds					97.8

RT: Retention time
RIa: Reported retention index
RIb: Calculated retention index

The predominance of carvacrol in the essential oil of *L.multifida* from the region of Rabat is in agreement with those reported in the literature (Table 5). According to **Khadir *et al.* (2016)**, the main components of *L. multifida* essential oil were carvacrol (27.5 to 57%), β-bisabolene (25.2 to 38.4%) and caryophyllene oxide (3.5 to 7.5%), depending on the developmental stage (pre-, full- and post-inflorescence stages). **Saadi *et al.* (2016)** reported that the essential oil of *Lavandula multifida* is carvacrol, with a percentage of 61.7% in the inflorescence stage and 50.9% in the post-flowering stage**. Douhri *et al.* (2014)** revealed that the essential oil of *Lavandula multifida* contains two main compounds carvacrol (47.6%) and β-bisabolene (9%).According to previous research studies, carvacrol is a major component of the tested *L. multifida* essential oil with significant differences in the percentage of phytochemicals. Indeed, the 44.3% of carvacrol that we obtained after GC/MS analysis is close to that obtained by tests performed on *Lavandula multifida* from Morocco **(Douhri *et al.*, 2014)**, Algeria **(Saadi *et al.*, 2016)** and Portugal **(Zuzarte *et al.*, 2012)** which are 47.62%, 50.92% and 42.8%, respectively. However, in Tunisia, the yield in HE does not exceed 1.33% **(Msaada *et al.*, 2012)**.

Table 5 Main phytochemicals found *L. multifida* in different countries comparing to obtained data.

	Portugal (Zuzarte *et al.*, 2012)	Algeria (Saadi *et al.*, 2016)	Tunisia (Msaada *et al.*, 2013)	Morocco (Douhri *et al.*, 2014)	Our Study
Compounds			Contribution (%)		
Monoterpene hydrocarbons					
α-Pinene	0,8	-	0,45	1,21	0.3
β-Myrcene	-	0,37	-	0,32	0.2
α-Phellandrene	-	-	-	0,09	1.8
3-Carene	0,5	-	-	-	0.3
p-Cymene	0,3	-	1,77	0,67	0.2
(Z)-b-Ocimene	1,7	0,63	-	1,02	1.8
Terpinolene	3,7	0,83	2,05	2,41	2.2
Oxygenated monoterpenes					
Linalol	0,3	5,69	50,05	7,42	0.5
Fenchol	-	0,24	-	-	3.2
α-Terpineol	-	0,69	0,26	0,02	0.4
Carvacrol methyl ether	0,1	-	-	-	0.3
Carvacrol	42,8	50,92	1,33	47,62	44.3
Monoterpene alcohols					
1-Octen-3-ol	0,6	0,3	0,25	-	0.2
1-Octanol	-	0,14	-	-	0.3
Sesquiterpene hydrocarbons					
Caryophylleneoxide	0,3	-	0,1	-	2.1
β-Farnesene	2,6	-	0,13	-	0.2
Germacrene-D	0,5	-	0,84	-	1.4
β-Bisabolene	5,6	5,81	-	9,01	31.9
Oxygenated sesquiterpenes					
Caryophylene	-	1,24	-	-	5.8
α-Cadinol	0,2	-	-	-	0.4

In a recent analysis of essential oils of two *L. multifida* samples collected in Portugal, 33 compounds were found, with the main phytochemical compounds corresponding to carvacrol (41.5 - 42.8%) and cis-ocimene (27.0 - 27.4%). Environmental conditions, part of the plant used, plant age and vegetative stage, and even genetic factors can all contribute to variations in the chemical profile of essential oils. The presence of a large amount of carvacrol in the essential oil of *Lavandula multifida* is characteristic of this species. In addition, other species of *Lavandula* express high levels of carvacrol such as the essential oil of *Lavandula canariensis* of Australian origin with 23.6%.

CONCLUSION

The essential oil of *Lavandula multifida* collected in Rabat region is composed mainly by carvacrol and beta-bisabolene. Antibacterial investigations showed a great potential of *L. multifida* essential oil. In addition, it exhibited interesting antioxidant activities. Based on these results, further experiments could be performed to identify the phytochemicals responsible for the various biological activities of *Lavandula multifida* essential oil.

Conflicts of interest : The authors declare that they have no competing interests.

Availability of data and materials: The data used in this study are included within the article.

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