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Lavandula stoechas essential oil from Morocco as novel source of antileishmanial, antibacterial and antioxidant activities

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ABSTRACT

This study aimed to reveal the chemical composition of *Lavandula stoechas* L. (*L. stoechas*) essential oils and to evaluate their antileishmanial, antibacterial and antioxidant properties. The essential oil was extracted by hydrodistillation using Clevenger apparatus. The chemical composition of L. *stoechas* essential oil was determined using GC-MS analysis. The antibacterial activity was tested against pathogenic strains using the diffusion method, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) by microtitration assay. The antioxidant activity was estimated by DPPH free radical scavenging ability and ferric-reducing power. The antiparasitic activity was tested against *Leishmania major, Leishmania tropica* and *Leishmania infantum* using MTT (3-(4.5-dimethylthiazol-2yl) − 2.5-diphenyltetrazolium bromide) assay. The major components of *L. stoechas* essential oils are represented by fenchone (31.81%), camphor (29.60%), terpineol (13.14%), menthone (8.96%) and eucalyptol (5.88%). The oil has revealed an important antioxidant capacity compared with ascorbic acid and Trolox. The highest inhibition was obtained against *Listeria monocytogenes* and *Staphylococcus aureus* MBLA by a diameter inhibition of 23 ± 0.85 and 21 ± 0.25 mm respectively. The lowest MIC and MBC values were obtained against *L. monocytogenes* (MIC = MBC = 0.25% (v/v)). The essential oil was most active against *L. major* with an IC_{50} value of 0.9 ± 0.45% (v/v). From these results, we conclude that *L. stoechas* essential oils could have potential applications in the food and pharmaceutical industries.

1. Introduction

The incidence of bacterial infectious diseases, leishmaniasis and oxidative stress related diseases has increased during the last decades. These diseases are related to difficulties encountered in their treatment, increase in drug resistance and side effects of conventional medication (Bouyahya et al., in press, 2017b). In this context, natural products have a key role in drug discovery as an alternative way (Essid et al., 2015; Khouchlaa et al., 2017). Secondary metabolites of medicinal and aromatic plants present key candidates for discovering antimicrobial agents to fight against numerous microbial diseases. Indeed, several studies have focused on pharmacological properties of medicinal and aromatic products and thus several original researches and reviews revealed their antimicrobial (Bakkali et al., 2008; Bouyahya et al., 2017e), antioxidant (Bouyahya et al., 2016), antileishmanial (Et-Touys et al., 2016), antitumor (Aneb et al., 2016) and anti-inflammatory (El Hachimi et al., 2017) activities.

Lavandula is an important medicinal and aromatic plant from the Lamiaceae family which produces essential oils. These plant species are largely used in traditional medicine to fight against diseases around the world. In the Ouezzane province, in the North-West of Morocco, medicinal plants have been used for a long time to treat several diseases (Ennabili et al., 2000; Merzouki et al., 2000, 2003; Bouyahya et al., 2017d). *In vitro* pharmacological screening activities have shown that some of these plant species possess several biological activities such as antibacterial, antioxidant and antileishmanial effects (Et-Touys et al., 2016; Khay et al., 2016; Bouyahya et al., 2017f, 2017g). However, some species from the province of Ouezzane have not been tested yet. *Lavandula stoechas* L. is an aromatic plant largely used in Moroccan traditional medicine. The essential oil of *L. stoechas* showed several pharmacological activities such as antibacterial (Dadalioglu and Evrendilek, 2004), antioxidant (Carrasco et al., 2015) and anti-inflammatory (Kaplan et al., 2007) activities. However, the antileishmanial property of *L. stoechas* essential oil has not been reported.

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In this study, we report the chemical composition and the antileishmanial, antibacterial and antioxidant activities of *L. stoechas* essential oil in order to assess their potential use for health and/or cosmetic purposes. As far as we know, this is the first report on the antileishmanial activity of *L. stoechas* essential oil against *Leishmania* promastigote species.

2. Material and methods

2.1. Plant material and essential oil extractions

The aerial parts of *L. stoechas* were collected from its wild habitat in province of Ouezzane (North-West of Morocco: 34° 47′ 50″ N and 5° 34′ 56″ W). The identification of the plant was carried out by Pr. Ennabili Abdessalam (PAMSN Laboratory, National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University of Fes, Morocco) (Voucher specimen: RAB02). The samples were air dried at room temperature in the shade. The extraction of essential oils was carried out by hydrodistillation using Clevenger-type apparatus. The obtained oils were dried with anhydrous sodium sulfate, weighed and stored at 4 °C until use.

2.2. GC-MS analysis of essential oils

The GC-MS analysis of essential oils (diluted in chloroform) was carried out as described by Talbaoui et al. (in press). It was performed on a TRACE GC ULTRA equipped with non-polar VB5 (5% phenyl, 95% Methylpolysiloxane), Capillary Column (30 m × 0.25 mm i.d., film thickness 0.25 μm), directly coupled to a mass spectrometer (Polaris Q) (EI 70 eV). The temperature of the injector and the detector was set at 250 and 300 °C, respectively. The oven temperature was programmed at 4 °C/min for temperatures from 40 to 180 °C, and at 20 °C/min for those for 180–300 °C. Helium was used as gas carrier with a flow rate of 1 mL/min; the sample (0.5 μL) was injected in splitless mode. Individual essential oil components were identified by comparing their relative retention times with those of authentic samples or by comparison of the relative retention indices (RRI) of the GC peaks to those of a homologous series of n-alkanes (series of C-9 to C-24n-alkanes) reported in the literature. Each compound was confirmed by comparison of its mass spectra with those of NIST02 library data of the GC/MS system and Adams libraries spectra (NIST/EPA/NIH, 2002; Adams, 2007) (Adams, 2007). For the percentage of individual components, we have established abundances by normalizing the GC peak areas of each compound without any correction factors.

2.3. Antibacterial activity

2.3.1. Bacteria strains and growth conditions

To evaluate the antibacterial activity of *L. stoechas* essential oil, we have used the following bacteria: *Escherichia coli* K12 and *Staphylococcus aureus* MBLA (Laboratory of Food Microbiology, UCL, Belgium: MBLA), *Staphylococcus aureus* CECT 976, *Staphylococcus aureus* CECT 994, *Listeria monocytogenes serovar* 4b CECT 4032 and *Proteus mirabilis* (Spanish Type Culture Collection: CECT), *Pseudomonas aeruginosa* IH (Institute of hygiene, Rabat, Morocco: IH) and *Bacillus subtilis* 6633 (German Collection of Microorganisms: DSM). Strains were maintained on an inclined agar medium LB (Lysogeny Broth 0.8% of agar) at 4 °C. Before use, the bacteria were revived by two subcultures in an appropriate culture medium: Lysogeny broth (LB) (Biokar Diagnostics, Beauvais, France) at 37 °C for 18–24 h. For the test, final inocula concentrations of 10^6 CFU/mL bacteria were used according to the National Committee for Clinical Laboratory Standards, USA (NCCLS 1999).

2.3.2. Agar-well diffusion assay

The principle of this technique is to estimate the bacteriostatic activity of the essential oils by measuring the growth inhibition zone of bacteria around wells. It is mostly used in a preliminary step to further study because it essentially provides access to qualitative results. Briefly, a basal layer was prepared by Muller-Hinton agar. After the agar plates were solidified, sterile 8 mm diameter cylinders were deposited. Six mL of LB medium in superfusion containing 0.8% agar were inoculated into a fresh culture of indicator bacterial strain (the final concentration was 10^6 CFU/mL). After solidification, the wells were filled with 50 μL of essential oil. After incubation at appropriate temperature (37 °C) for 24 h, all plates were examined for any zone of growth inhibition, and the diameter of these zones was measured in millimeters (Bouhdid et al., 2008). All the tests were performed in triplicate.

2.3.3. Minimal inhibitory concentration (MIC)

MICs were determined using the broth micro-dilution assay as described (Bouhdid et al., 2009). Agar at 0.15% (w/v) was used as a stabilizer of the extract-water mixture and resazurin as a bacterial growth indicator. 50 μL of Bacteriological Agar (0.15% w/v) were distributed from the 2nd to the 8th well of a 96-well polypropylene microtitre plate. A dilution of the essential oil was prepared in Mueller Hinton Broth supplemented with bacteriological agar (0.15% w/ v), to reach a final concentration of 2%; 100 μL of these suspensions were added to the first test well of each microtitre line, and then 50 μL of scalar dilution were transferred from the 2nd to the 8th well. The 8th well was considered as a growth control, because no essential oil was added. Then, 50 μL of a bacterial suspension were added to each well at a final concentration of approximately 10^6 CFU/mL. The final concentration of the essential oil was between 2% and 0.03% (v/v). Plates were incubated at 37 °C for 18 h. After incubation, 10 μL of resazurin were added to each well to assess bacterial growth. After further incubation at 37 °C for 2 h, the MIC was determined as the lowest essential oil concentration that prevented a change in resazurin colour. Bacterial growth was detected by reduction in blue dye resazurin to pink resorufin. A control was carried out to ensure that, at the concentrations tested, the essential oil did not cause a colour change in the resazurin (Bouyahya et al., 2017h). Experiments were performed in triplicate.

2.3.4. Determination of minimal bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) corresponded to the lowest concentration of the essential oil yielding negative subcultures after incubation at appropriate temperature (37 °C) for 24 h. It is determined in broth dilution tests by sub-culturing 10 μL from negative wells on plate count agar (PCA) medium. All the tests were performed in triplicate (Bouhdid et al., 2008).

2.4. Antioxidant activity

2.4.1. DPPH free radical-scavenging assay

The stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used for the determination of free radical-scavenging activity of essential oils (Almoui Jamali et al., 2013). Aliquots (0.2 mL) of various concentrations of the essential oils samples dissolved in methanol were added to 1.8 mL of a 0.004% methanolic solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm by using a UV spectrophotometer (Bouyahya et al., 2017i). The percentage (%) to scavenge DPPH radical was calculated using the following formula: DPPH scavenging activity (AA in %) = $[(A_c\text{-}A_t)/A_c] \times 100$. Where, A_c is the absorbance of the control (without oil) and A_t is the absorbance of the test (with oil). Trolox and ascorbic acid were used as positive control and essential oils concentration providing 50% inhibition (IC_{50}) was calculated by plotting the inhibition percentages against the concentrations of the sample (Almoui Jamali et al., 2013). The test was carried out in triplicate and the IC_{50} values were reported as means ± SD.

2.4.2. Reducing ferric power determination

Reductive ability was investigated by the Fe + 3to Fe + 2 transformations in the presence of the oils (Almoui Jamali et al., 2013). The essential oils and control (ascorbic acid and Trolox) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe (CN) 6] (2.5 mL, 1%). The mixture was then incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloro acetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1100 g. Finally, the upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance at 700 nm against the corresponding sample concentration. Trolox and ascorbic acid were used as positive controls. The test was carried out in triplicate and IC_{50}values were reported as means ± SD.

2.5. Antileishmanial Activity

2.5.1. Culture of leishmania species

Leishmania species tested in this study are: *Leishmania infantum* (MHOM/MA/1998/LVTA), *Leishmania tropica* (MHOM/MA/2010/LCTIOK-4) and *Leishmania major* (MHOM/MA/2009/LCER19-09). These three species were isolated and identified from Moroccan infected patients at the National Reference Laboratory of Leishmaniasis, National Institute of Health of Rabat, Morocco. The species were cultivated as described by Et-Touys et al. (2016). Briefly, parasite cultures of each *Leishmania* species were washed with phosphate buffered saline (PBS) and centrifuged at 280 g for 10 min. Cells were then re-suspended in RPMI 1640 (GIBCO) supplemented with 10% of heat-inactivated fetal calf serum and 1% of Penicillin-Streptomycin mixture. Cultures were maintained at 23 °C.

2.5.2. Antileishmanial activity

Before evaluating the antileishmanial activity, the cellular density of each species was calculated using light microscopy. When cellular density reached a threshold concentration of 10^6 cells/mL, *L. infantum*, *L. tropica* and *L. major* promastigotes were washed twice with phosphate buffered saline (PBS) and centrifuged at 800 g for 10 min. To evaluate the anti-promastigote activity, 100 µL of parasite cultures were resuspended in a 96-well tissue culture plate, in fresh culture medium according to Et-Touys et al. (2016). Briefly, parasites were incubated at 2.5×10^6 cells/well for 72 h at 23 °C in the presence of various concentrations of essential oil dissolved in 1% DMSO. DMSO was used at a final concentration never exceeding 1% which is not toxic to parasites (Oliveira et al., 2011; Essid et al., 2015). Sterile PBS and 1% DMSO (vehicle) were used as negative controls and Glucantime® was used as positive control.

2.5.3. Cell viability assay

The viability of *Leishmania* species was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl) − 2,5-diphenyl tetrazolium bromide) colorimetric assay as described by Essid et al. (2015). Briefly, 10 µL of MTT (5 mg/mL) were added to each micro-well and incubated for 3 h at 30 °C. The reaction was stopped by the addition of 100 µL of 50% (v/v) isopropanol-10% (w/v) sodium dodecyl sulfate (SDS) mixture to each well in order to dissolve insoluble formazen formed after tetrazolium dye reduction. After 30 min of incubation at room temperature, absorbance was measured at 560 nm using an ELISA plate reader. All assays were conducted in triplicate and compared to the negative control (parasites) and the reference drug (Glucantime). Cell viability was also evaluated by determination of the extract concentrations which inhibited half of the cell population (IC_{50}), obtained by plotting %age of inhibition against concentration of essential oil. Cytotoxicity percentage was calculated using the following formula (Et-Touys et al., 2016):

$$\text{Cytotoxicity}\,(\%) = 100 \times (\text{Absorbance of untreated cells} - \text{Absorbance of treated cells}) / \text{Absorbance of untreated cells.}$$

Table 1
Chemical composition of essential oil of *L. stoechas*.

RT	Compound	%	Identification
11.559	1,8-cineole	1.33	MS, I_R
14.04	Fenchone	31.81	MS, I_R
15.681	Lanalool	1.44	MS, I_R
16.612	Borneol	0.88	MS, I_R
16.922	ni	0.55	MS, I_R
19.384	Camphor	29.60	MS, I_R
25.034	Caryophyllene	1.51	MS, I_R
47.612	Eucalyptol	5.88	MS, I_R
51.098	Terpineol	13.1	MS, I_R
51.218	Menthone	8.96	MS, I_R
51.301	Menthol	2.68	MS, I_R
51.428	ni	0.71	MS, I_R
51.745	2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde	1.33	MS, I_R

RT = retention time on the DB-5 column. Compounds in less than 0.1% are not reported. ni: not identified.

3. Results

3.1. Chemical composition

The chemical composition of *L. stoechas* essential oil was determined by GC-MS. The identified molecules are presented in Table 1. It appears that the chemical composition of *L. stoechas* essential oil is diverse and dominated by five major compounds: fenchone (31.81%), camphor (29.60%), terpineol (13.14%), menthone (8.96%) and eucalyptol (5.88%).

3.2. Antibacterial activity

The antibacterial activity of *L. stoechas* essential oil was evaluated using the wall diffusion assay and the MIC values were determined by micro-dilution assay. The results of the qualitative antibacterial activities are summarised in Fig. 1. Gram-positive bacteria show more sensitivity toward essential oils than Gram-negative bacteria. The highest diameter of inhibition was found with ethanolic extract *L. monocytogenes* (23 ± 0.85 mm) and *S. aureus* MBLA (21 ± 0.25 mm). The MIC and MBC values of *L. stoechas* essential oil against tested bacteria are shown in Table 2. The lowest MIC value was 0.25% (v/v) and the highest MIC value was >2% (v/v). *L. monocytogenes* was the most sensitive strain to *L. stoechas* essential oil (MIC = MBC = 0.25% v/v), while *P. aeruginosa* was the most resistant strains to this oil (MIC = MBC>2% v/v). In all cases, the MIC values were equal to MBC values indicating the bactericidal action of the essential oil of *L. stoechas* at the MIC.

3.3. Antioxidant activity

The antioxidant activity of *L. stoechas* essential oil was estimated by two *in vitro* antioxidant methods: the ferric-reducing capacity assay and the DPPH free radical scavenging method. The concentrations that led to 50% inhibition or effectiveness (IC_{50}) are given in Table 3. The lowest IC_{50} values reflect a better protective action. The antioxidant capacity (IC_{50}) of *L. stoechas* essential oil is 785.38 ± 9.04 and 107.53 ± 1.74 µg/mL for DPPH scavenging and ferric-reducing power assay respectively. The results of antioxidant activity of *L. stoechas* essential oil could be considered interesting compared to the ascorbic acid and Trolox as standards antioxidants (IC_{50} values were 27.20 ± 0.17 and 43.72 ± 0.31 µg/mL for DPPH assay and 47.63 ± 0.58 and

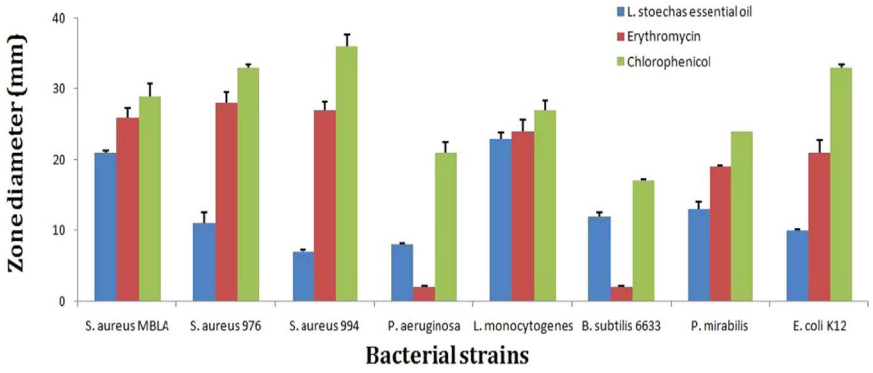

Fig. 1. Antibacterial activity of *L. stoechas* essential oil and commercial antibiotic (Erythromycin and Chlorophenicol) against eight bacterial strains. Final bacterial density was around 10^6 CFU/mL and the values of diameter inhibition are means ± standard deviation of three separate experiments.

Table 2
The MIC and MBC values (% (v/v)) of *L. stoechas* essential oil against 8 human pathogen bacteria strains tested in microdilution assay.

Essential oils	Microorganisms	MIC = MBC (% (v/v))	Effect
Lavandula stoechas	*S. aureus* MBLA	0.5	Bactericidal
	S. aureus 976	2	Bactericidal
	S. aureus 994	1	Bactericidal
	P. aeruginosa	> 2	nd
	L. monocytogenes	0.25	Bactericidal
	B. subtilis 6633	2	Bactericidal
	P. mirabilis	1	Bactericidal
	E. coli K12	0.5	Bactericidal

MIC: Minimum inhibitory concentration.
MBC: Minimum bactericidal concentration.
Final bacterial density was around 10^6 CFU/mL.

Table 3
IC_{50} values (μg/mL) of *L. stoechas* essential oils. Values represent means (standard deviations) for triplicate experiments.

Assays	Essential oil	Ascorbic acid	Trolox
DPPH	785.38 ± 9.04	27.20 ± 0.17	43.72 ± 0.31
Reducing power	107.53 ± 1.74	47.63 ± 0.58	85.45 ± 1.36

85.45 ± 1.36 μg/mL for ferric-reducing capacity).

3.4. Antileishmanial activity

The antileishmanial activity of *L. stoechas* essential oil against *L. major*, *L. tropica* and *L. infantum* was evaluated using MTT assay. The results obtained are summarised in Table 4. They were expressed as the concentration of the essential oil of *L. stoechas* that reduce 50% of parasite cells. The *L. stoechas* essential oil was most active against *Leishmania* species than the positive control. *L. major* was the most sensitive strain (IC_{50} = 0.9 ± 0.45 μg/mL), followed by *L. infantum* (IC_{50} = 7 ± 0.54 μg/mL) and L. *tropica* (IC_{50} > 10 μg/mL), while the IC_{50} value of Glucantime was > 10 μg/mL for the three tested species.

Table 4
IC_{50} values (μg/mL) for the antileishmanial activity of *L. stoechas* essential oil and positive control (Glucantime) against *L. major, L. infantum, L. tropica.*

Leishmania species	Essential oil	Glucantime
L. major	0.9 ± 0.45	>10
L. infantum	7 ± 0.54	>10
L. tropica	> 10	>10

4. Discussion

Because of the problem of resistance, microbial infections become a cause of several problems of public health. Bacterial strains and some *Leishmania* species have developed several mechanisms that allow them to counterbalance the antimicrobial agents. On the other hand, numerous studies have confirmed the direct or indirect implication of oxidative stress in the pathogenesis of several diseases such as diabetes, cancer and inflammation (Grimsrud et al., 2008). Furthermore, the searches of alternative bioactive molecules that possess antimicrobial and antioxidant effects are important and may participate as efficient alternative bioactive natural products. Medicinal and aromatic plants synthesizes a wide variety of secondary metabolites that possess several pharmacological properties including antibacterial, antioxidant and antileishmanial activities (Bouyahya et al., in press, 2017b, 2017e; Et-Touys et al., 2016; Talbaoui et al., in press). Essential oils are complex mixtures of volatile natural compounds of various organic structures. The word oil is attributed to its hydrophobic character and to its solubilizing properties in fats, whereas the essential word reflects the distinctive odor emitted by the producing plant (Bouyahya et al., in press). These compounds are synthesized by aromatic plants as secondary metabolites and possess several biological activities (Bakkali et al., 2008).

In this work, we studied the essential oil of *L. stoechas* as a novel source for antibacterial, antioxidant and antileishmanial activities. Firstly, the aerial part of *L. stoechas* has been collected from the North-West of Morocco based on ethnopharmacological study carried out by our laboratory (Bouyahya et al., 2017d). The essential oil showed a wide variety of chemical composition determined using the GC-MS analysis. It contains numerous terpenoid oxygenated and not oxygenated compounds such as terpineol, fenchone, camphor and menthone.

The obtained results are in consonance with others carried out on *L. stoechas* collected from other areas worldwide (Hassiotis, 2010; Zuzarte et al., 2013; Cherrat et al., 2014; Carrasco et al., 2015). Indeed, they corroborate with studies that have revealed the richness of *L. stoechas* essential oil in fenchone and camphor as the main compounds (Dadalioglu and Evrendilek, 2004; Angioni et al., 2006). However, other studies have found fenchone, camphor and eucalyptol as major compounds (Carrasco et al., 2015). Hassiotis (2010) who has analyzed the essential oil of *L. stoechas* collected from Greece found fenchone, camphor, p-Cymene and 1,8-Cineole as the main compounds. On the other hand, Cherrat et al. (2014) found that the main compounds of *L. stoechas* essential oils are Cubenol, 10s,11s-Himachala-3(12),4-diene, methyl eugenol and δ-Cadinene. The fluctuations observed in *L. stoechas* essential oil between our study and other ones are certainly due to the difference in the region of collection. This may be explained by the influence of the external environment on the regulation of secondary metabolism pathways in plants (Crisp et al., 2016). Indeed, the chemical composition of aromatic plants varies with phenological changes, collection regions, collected parts and methods of extraction (Bakkali et al., 2008; Aboukhalid et al., 2016). Moreover, the synthesis of the

chemical composition is regulated by plants in space and time via various epigenetic factors, such as DNA methylation, histones modifications and remodelage of chromatin in order to respond against specific physiological functions (Vriet et al., 2015; Avramova, 2015).

The antibacterial activity of the essential oil of *L. stoechas* was tested against eight bacterial strains. The oil has shown a more significant growth inhibition than the Erythromycin against *P. aeruginosa* and *B. subtilis* ($p < 0.05$). In addition, not a significant difference ($p > 0.05$) in the growth inhibition has been noted between *L. stoechas* essential oil and two commercialized antibiotics (Erythromycin and Chlorophenicol) against *S. aureus* MBLA and *L. monocytogenes*. However, the MIC and MBC values of *L. stoechas* essential oil against tested bacteria do not have the same effects found in the inhibition growth.

The antibacterial activity of essential oil may be due to the major compounds present in the essential oil, the synergistic effects between these components and the additive effects of the minor compounds which can reinforce the antibacterial action (Bouhdid et al., 2008). On the other hand, we note that the Gram-negative bacteria are more resistant against *L. stoechas* essential oil than the Gram-positive bacteria. These findings have been shown by numerous studies (Ozcelik et al., 2003; Kaplan et al., 2007). The resistance of Gram-negative bacteria is due to the outer membrane surrounding the cell wall which restricts the diffusion of hydrophobic compounds through the lipo-polysaccharide. In addition, the periplasmic space contains enzymes, which are able to break down foreign molecules introduced from outside (Bondet et al., 1997). The results of the antibacterial activity of *L. stoechas* essential oil are corroborated by those found in the literature (Dadaliogÿlu and Evrendilek, 2004; Cherrat et al., 2014). Indeed, Cherrat et al. (2014) showed bactericidal effects against *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Our results are not exactly similar to these studies because of the difference in the chemical components, the experimental methods used and the strains tested. The antibacterial mechanism of *L. stoechas* essential oil remains unresolved. However, the essential oil of *Origanum compactum* (endemic Moroccan medicinal plant belonging to Lamiaceae family) has demonstrated the capacity to cross the cell membrane thus inducing the leakage of potassium, the disturbance in the electron respiratory chain and the slight modifications in cellular morphology (Bouhdid et al., 2009; Bouyahya et al., 2017j). Other antibacterial mechanisms of essential oil have been investigated as related to the capacity of essential oil components to deregulate the quorum sensing signaling pathways thus leading to decrease the bacterial resistance (Luís et al., 2016; Myszka et al., 2016; Bouyahya et al., 2017a).

Furthermore, several studies have revealed that essential oils from medicinal plants possess antioxidant and thus prevent lipid peroxidation (Keshvari et al., 2013). The antioxidant activity of *L. stoechas* essential oil evaluated using DPPH scavenging activity and reducing ferric assay showed a significant antiradical effect. This activity is certainly related to the chemical compound of this oil which is rich in phenolic compound such as fenchone and camphor. Indeed, several studies have demonstrated the antioxidant effects of essential oils rich in such compounds. On the other hand, the capacity of the essential oil obtained from the aerial part of *L. stoechas* to scavenge the DPPH free radical is in agreement with what has been reported by other studies (Matos et al., 2009; Carrasco et al., 2015; Cherrat et al., 2014). Carrasco et al. (2015) found interesting antioxidant activity, while Cherrat et al. (2014) showed a moderate antioxidant effect of *L. stoechas* essential oil. The phenolic compounds of *L. stoechas* essential oils could be responsible for the antioxidant effects. However, the synergetic effects of various essential components could also contribute to the antioxidant activity. The fluctuations in the antioxidant capacities may be due to the difference in composition and the method used.

Leishmania species are responsible of high mortality and morbidity worldwide. *L. major, L. tropica and L. infantum* are the most causative leishmaniasis etiological agents found in Morocco. Because of the resistance developed by parasites; the screening of natural products which possess antileishmanial properties is necessary. Aromatic and medicinal plants could constitute a veritable candidate for identifying natural drugs which possess antileishmanial activities (Et-Touys et al., 2016; Essid et al., 2015). Actually, essential oils and their compounds are considered as potential antileishmanial drugs (Bakkali et al., 2008). The evaluation of antileishmanial activity of *L. stoechas* essential oil revealed that this oil exhibited a stronger effect than Glucantime. *L. major* promastigotes appear to be more sensitive than *L. infantum* and *L. tropica* toward tested oil. There are not data regarding the antileishmanial activity of *L. stoechas* essential oil. However, some studies have shown that essential oils extracted from medicinal plants belonging to the Lamiaceae family such as *Salvia officinalis*, *Rosmarinus officinalis* and *Thymus hirtus* have the antileishmanial effects (Essid et al., 2015).

The sensitivity of each parasitic strain depends on its ability to tolerate the action of essential oil compounds. So far, no studies have been carried out on the antileishmanial activity of the *L. stoechas* essential oil. Furthermore, some studies on essential oils of other medicinal plants belonging to the Lamiaceae family have shown the efficacy of these oils to inhibit the growth of *Leishmania* species at low concentrations (Essid et al., 2015). The antileishmanial activity is probably attributed to the main compounds of *L. stoechas* essential oil. Indeed, camphor has shown interesting antileishmanial activity against *L. major* (5.55 ± 1.27 μg/mL) and *L. infantum* (7.90 ± 0.42 μg/mL) and also showed good security against human macrophage cell lines (Essid et al., 2015). On the other hand, essential oils and their main compounds have several targets of action against *Leishmania* species. These targets include the morphological destruction, the induction of apoptosis, the disruption of the electron transport chain and the inhibition of DNA topoisomerase (Castro et al., 1992).

5. Conclusion

The essential oil of *L. stoechas* contains several major compounds. The findings of this study revealed the potential antibacterial, antileishmanial and antioxidant of *L. stoechas* essential oils. The essential oil of *L. stoechas* has exhibited an important antioxidant activity which indicates their phenolic compounds as potential source of antioxidant compounds. Furthermore, this oil showed a great capacity to inhibit the growth of bacteria and *Leishmania* species. However, future studies should be conducted to offer new comprehensive investigations on the elucidation, characterization and identification of molecules responsible for these biological effects.

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Author disclosure statement

The authors declare that there is no conflict of interests regarding the publication of this article.

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