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

Abdelhakim Bouyahya[a,b,*], Abdeslam Et-Touys[a,c], Jamal Abrini[b], Ahmed Talbaoui[a], Hajiba Fellah[c], Youssef Bakri[a], Nadia Dakka[a]

[a] *Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, and Genomic Center of Human Pathologies, Mohammed V University, Rabat, Morocco*

[b] *Biology and Health Laboratory, Department of Biology, Faculty of Science, Abdelmalek Essaadi University, Tetouan, Morocco*

[c] *National Reference Laboratory of Leishmaniasis, National Institute of Health, Rabat, Morocco*

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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.

* Corresponding author at: Laboratory of Human Pathologies Biology, Faculty of Sciences of Rabat, University Mohammed V of Rabat 4, Av. Ibn battouta, BP1014 Rabat, Morocco.
E-mail address: boyahyaa-90@hotmail.fr (A. Bouyahya).

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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.