

The Antimicrobial Activity of Moroccan Lavaender Esssential Oil Against Bacterial Pathogens Isolated Urinary Tract Infections

Ez Zoubi Y^{1,2*}, El Ouali Lalami A^{2,3}, Bousta D⁴, Polissiou M⁵, Daferera D⁵, Lachkar M⁶, El Khanchoufi A⁴, Farah A¹

¹Laboratory of Applied Organic Chemistry, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, Po. Box 2202 – Route d'Imouzzer, Fez, Morocco.

²Regional Diagnostic Laboratory of Epidemiological and Environmental Health, Regional Health Directorate, EL Ghassani Hospital, Fez 30000, Morocco.

³Institute of Nursing Professions and Health Techniques of Fez (Annex Meknes), Regional Health Directorate, EL Ghassani Hospital, Fez 30000, Morocco.

⁴Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

⁵Laboratory of Chemistry, Agricultural University of Athens, Department of Food Science and Nutrition, 75 Iera Odos, 118 55, Athens, Greece.

⁶Engineering Laboratory of Organometallic and Molecular Materials (CNRST, URAC 19), Faculty of Sciences, University Sidi Mohamed Ben Abdellah, Po. Box 1796 (Atlas), 30000 Fez, Morocco.

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ABSTRACT

Lavandula stoechas genus is an important member of family Lamiaceae, it is widely distributed in north Provinces of Morocco and is used in traditional medicine to treat various diseases. In order to evaluate the antimicrobial effect of *Lavandula stoechas* essential oil as well as to compare its inhibitory effect versus commercial Antibiotics, it was tested against urinary isolates bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The chemical compounds of essence of *Lavandula stoechas* were identified by GC-MS analysis. The paper disk agar diffusion method and the macrodilution assay for determination of Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC) were used. The main compounds of essential oil obtained were camphor, fenchone, camphene, borneol, α -pinene and 1,8-cineole. *Lavandula stoechas* essential oil was very active against *Staphylococcus aureus* with inhibition zone of 17.2 ± 0.8 mm and moderately active against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. MICs for *Lavandula stoechas* indicated that the best values were enregistered for *Staphylococcus aureus* and *Klebsiella pneumonia* with equal value of $2.5 \mu\text{g/mL}$, followed by *Escherichia coli* and *Pseudomonas aeruginosa* with MIC values of $5 \mu\text{g/mL}$ for each bacterium. Ofloxacin had the widest coverage against all bacteria's, followed by Chloromphenicol. Cephalosporin's third generation, Gentamicin and Amikacin have presented an average activity against pathogens. The bacteria of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* have been resistant for Penicillin antibiotics. The results showed that the essential oil of *Lavandula stoechas* was revealed highly inhibitory antimicrobial activity especially on gram positive bacteria (*Staphylococcus aureus*) and can be used instead of chemical drugs to treat bacterial infections.

Keywords: *Lavandula stoechas*, Essential oil, Chemical composition, Antibacterial activity, Urinary Tract Infections (UTI), Morocco.

INTRODUCTION

Urinary Tract Infection (UTI) represents one of the most common diseases occurring from the neonate to the geriatric age groups encounters in medical practice today¹. More than 95% of UTI are caused by single bacterial species *E. coli* which is the most frequently infecting organisms². However, many other bacteria can also meet and lead to infections for example, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Proteus*, *Staphylococcus*, *Mycoplasma*, *Chlamydia*, *Serratia* and *Neisseria* spp. It is reported that about 35% of healthy women suffer symptoms of urinary tract infection and about 5% of

women each year suffer with the problem of painful urination (dysuria) and frequency³. A study realized in at the Avicenne Teaching Hospital (Marrakech, Morocco) from 2010 to 2012 showed that *Escherichia coli* and *K. pneumoniae* have been reported to be the most common organisms causing UTI⁴. This is demonstrated by the prevalence of these two pathogens in the epidemiology of both nosocomial and community-acquired UTIs. Although *E. coli* is a more common cause of UTIs (63%) and the prevalence of *K. pneumoniae* species has been 22%, and it was isolated in 10% and 28% of the urine samples in the Meknes⁵ and Rabat⁶ regions respectively. Thus, in light of

the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance⁷. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy⁸. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against microbe strains with ultimate goal to provide efficient drugs to the patient. Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory⁹. The lavenders are a genus of about 25-30 species of flowering plant in the mint family, Lamiaceae, native of Mediterranean region south to tropical Africa and many regions of Asia and it has been used for centuries as an herbal remedy for many ailments¹⁰. One species of this genus is *Lavandula stoechas* (*L. stoechas*), which is one of the most explored and exploited lavenders in the world. Some studies focused on the antibacterial^{11,12}, anti-inflammatory¹³, antifungal¹⁴, insecticidal¹⁵ and antioxidant^{16,17} properties of *L. stoechas*. This plant is used traditionally in Morocco for the treatment of painful illnesses like inflammatory diseases, cystitis, nephritis and rheumatic arthritis¹⁸. The present study, realized in the first time in Morocco, made an attempt to find out the chemical composition and the antibacterial activity of essential oil of *L. stoechas* as well as to compare its inhibitory effect versus commercial antibiotics against five bacteria's urinary tract infections bacterial pathogens isolates.

MATERIAL AND METHODS

Plant material and extraction of the Essential Oil

The aerial parts (leaves, stems and wood) of *L. stoechas* are collected in Taounate Province/Morocco, between April and June 2015. The botanical identification and authenticated voucher specimens have been deposited in the Herbarium of the National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fez, Morocco. Samples of 100g of the fresh aerial parts of *L. stoechas* were subjected to hydrodistillation for 2 hours using a Clevenger apparatus; the obtained Essential Oil (EO) was stored at 4°C so that can be used in the upcoming experiments.

Chemical characterization of essential oil of L. stoechas

The analysis of the essential oils was carried out on a Hewlett Packard 5890 II GC coupled with a Hewlett Packard 5972 MSD operating in the EI mode at 70 eV. A non-polar OPTIMA-5 capillary column (30 m × 0.25 mm, film thickness 0.25 µm) used with a programmed temperature gradually increased from 60°C to 250°C by a rate of 3°C/min. Injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. Diluted samples (1/100 in acetone v/v) were injected in the splitless mode. Helium, the carrier gas, was at 2.5 psi. Identification of the compounds was based on the comparison of their relative retention indexes and mass spectra with those of NIST 98, Wiley 275 library data¹⁹. The percentages relative of the

compounds were obtained electronically from area percent data. Kovats index for each compound on OPTIMA-5 column was calculated in reference to n-alkanes.

Antimicrobial activity

Microorganisms

The antimicrobial activity of *L. stoechas* essential oil was tested against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Proteus mirabilis* (*P. mirabilis*), *Klebsiella pneumonia* (*K. pneumonia*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) isolated from patients having urinary infection in nephrology service at Hassan II University Hospital at Fez city (Morocco), these bacteria were identified and confirmed by classical biochemical gallery and the API (bioMérieux, France).

Disc diffusion assay

Antimicrobial susceptibility test of the essential oil and fractions was tested against the above mentioned Gram positive and Gram negative bacteria by disc diffusion method²⁰. The susceptibility tests were performed on Muller-Hinton Agar, 10 µL of essential oil was diluted with two volumes of 5% dimethylsulfoxide (DMSO) and impregnated on the filter paper discs and used for the study. Ampicillin, Amoxicillin, Chloromphenicol, Ofloxacin, Cefotaxim, Ceftriaxone, Gentamicin, Cefaclor and Amikacin were used as positive reference standards to determine the sensitivity of the tested strains and 5% DMSO was used as blind control. Finally, the petri dishes inoculated, were incubated at 37°C for 24 h and the inhibition zones were observed according to the guidelines of the Antibiogram Committee of the "Société Française de Microbiologie" (CA-SFM)²¹.

Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)

The test oils of *L. stoechas* and its fractions were dissolved in 5% DMSO to obtain 1000 µl/mL stock solution. 0.5 mL of stock solution was incorporated into 0.5 mL of Mueller Hinton broth to get the concentration of 500 µl/mL and serially diluted to achieve 0.62, 1.25, 2.5, 5, 10, 20, 40 and 80 µl/mL. Fifty microliter of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and not the essential oil and 5% DMSO was used as blind control. The culture tubes were incubated at 37°C for 24 hours. The lowest concentrations, which did not show any growth of tested organisms after macroscopic evaluation was determined as MIC²². Referring to results of the MIC assay, the MBC was determined. Fifty microliters from each dilution of essential oil and fractions, showing growth inhibition zone in disc diffusion method, were added to 5 mL of Trypticase Soy Agar (TSA) broth tubes then incubated at 37°C for 24 hours in an incubator shaker. From tubes without microbial growth, 0.1 mL of cells was spread on TSA agar plates. MBCs were determined as the highest dilution at which no growth occurred on the plates.

Statistical analysis

The values are presented as the mean \pm SEM of triplicate analysis using one-way analysis of variance (ANOVA).

RESULTS

Component analysis of essential oils of *L. stoechas*

GC/MS analysis of *L. stoechas* essential oil led to the identification of 27 components were characterized representing 98.2% of the total oil, of which camphor 47.2 %, fenchone 33.3 %, camphene 3.3 %, borneol 2.9 %, α -pinene 2.9 % and 1,8-cineole 1.4%, were the major components (Figure 1). The percentage compositions of remaining 21 compounds ranged from 0.1% to 1.2%. The constituents of *Lavandula stoechas* was identified as Oxygenated monoterpenes were the major constituents in this oil (91.2 %) followed by Monoterpene hydrocarbons (7.1%), Oxygenated sesquiterpenes (0.8%) and Sesquiterpene hydrocarbons (01%).

Antimicrobial activities

Disc diffusion study

The in vitro results of antibacterial activity of the EO of *L. stoechas* by the paper disk agar diffusion method against microorganisms are summarized in Table 1. The essential oil of *L. stoechas* was very active against *S. aureus* with inhibition zone 17.2 ± 0.8 and moderately active against *E. coli*, *K. pneumonia*, *P. mirabilis* and *P. aeruginosa* showed an inhibition zone between 12.5 ± 0.6 and 14.5 ± 1.3 mm. Among the antibiotics (Table 2), Ofloxacin had the widest coverage against all bacteria's (between 14.5 ± 1.5 and 25.4 ± 3.2 mm), followed by Chloromphenicol (between 14.4 ± 2.2 and 20.4 ± 2.3 mm). Cephalosporin's third generation (Cefotaxin and Ceftriaxon), Gentamicin and Amikacin have presented an average activity against pathogens with diameter of inhibitions zones between 12.5 ± 1.3 and 15.7 ± 1.6 mm, but Ceftriaxon and Gentamicin showed high activity against *P. mirabilis* and *S. aureus* with diameter inhibition at 18 ± 1.4 and 17.2 ± 2.2 mm respectively. Penicillin (Ampicillin and Amoxicillin) have showed poor activity against *P. mirabilis*, On the other hand *E. coli*, *S. aureus* and *K. pneumonia* bacteria were resistant to these antibiotics.

Determination of Minimum Inhibitory Concentration

(MIC) and Minimum Bactericide Concentration (MBC)

The antimicrobial activity of *L. stoechas* was confirmed by the macrodilution assay (Table 3). *L. stoechas* EO exhibited much higher antibacterial activity with the MIC values of 2.5 μ l/ml against *S. aureus* and *K. pneumonia*. The MICs of *E. coli* and *P. aeruginosa* values were 5 μ l/ml and MBC= 10 μ l/ml. *P. mirabilis* showed the lowest antibacterial activity in the macrodilution method, MIC at 10 μ l/ml and MBC at 20 μ l/ml.

DISCUSSION

In the present work, realized for the first time in the North east of Morocco, we are interested in examining the chemical composition and antimicrobial activity against pathogen bacteria as well as to compare its inhibitory effect versus commercial Antibiotics. Our chemical composition of EO results is in accordance with other studies related to *L. stoechas*, the total mass of sex major components (camphor, fenchone, camphene, borneol, α -

Table 1: Antimicrobial activities of *L. stoechas* essential oil using disc diffusion method

Microorganisms	Inhibition zone diameter of <i>L. stoechas</i> (10 μ l/disc)
<i>E. coli</i>	14.5 ± 1.3
<i>S. aureus</i>	17.2 ± 0.8
<i>K. pneumonia</i>	14 ± 1.4
<i>P. mirabilis</i>	13.3 ± 0.3
<i>P. aeruginosa</i>	12.5 ± 0.6

- Values represent averages \pm standard deviations for triplicate.

- Inhibition zone including disc diameter (6 mm).

pinene and 1,8-cineole) in Lavandin essential oil to be 91% of the total mass of essential oil. This plant is characterized by significant variations in the amounts of fenchone, camphor and 1,8-cineole, being the fenchone/camphor chemotype the most commonly identified¹¹. The analysis of *L. stoechas* oil from Greece oil found α -Cardinol (7.2 %) in addition to fenchone, camphor and 1,8 cineole compounds²³. The main components of Moroccan *L. stoechas* oil were -Fenchone (30.5%), Camphor (18.2%), 1,8 Cineole (8.6%) and Camphene (3.5%)²⁴. However, the absence of some of the above mentioned compounds makes the difference to distinguish among biochemotypes of *L. stoechas*. In this way, only the EO reported by Topal et al.²⁵ shows a lack of the most volatile compounds (α -pinene to limonene), myrtenyl acetate and viridiflorol. Ristocelli et al.²⁶ reported the chemical composition of the essential oils from 50 samples of *L. stoechas* from different areas of Corsica during the flowering stage; they found significant differences in the major compounds: fenchone (14.9-75.5%), camphor, (2.5-56.2%) and 1,8-cineole (0.17-8%). The different qualitative and quantitative chemical compositions of the EO could be explained by different environmental conditions, genetics (degree of hybridization), geographical origin, part of plant extract and harvest period²⁷⁻²⁹. Due to the emergence of antibiotic resistant pathogens in hospitals and homes, plants are being looked upon as an excellent alternate to combat the further spread of multidrug resistant microorganisms. In this study, the essential oil of *L. stoechas* showed good antimicrobial activity against *S. aureus*, *E. coli*, *K. pneumonia*, *P. mirabilis* and *P. aeruginosa* isolated from patients having urinary infection in Nephrology service at Hassan II University Hospital. The in vitro MIC value indicated that concentration 2.5 μ g/mL of essential oil is sufficient to inhibit *S. aureus* and *K. pneumonia*. The MIC and MBC values of *E. coli* and *P. aeruginosa* was found that 5 μ g/mL and 10 μ g/mL respectively for each bacteria. Cherrat et al.³⁰ were tested Moroccan *L. stoechas* EO towards five Gram-positive and four Gram-negative bacteria and showed great efficacy against Gram-positive bacteria but poorer against Gram-negative, especially against *E. coli* O157, *Listeria monocytogenes*, and *S. aureus* with inhibition zone of 16.2 ± 0.60 , 32.0 ± 2.00 and 28.0 ± 0.70 mm respectively. A very effective antibacterial capacity was noted and proven in vitro against *S. aureus*, *E. coli*, *K pneumoniae* and *P. aeruginosa* by Gören et al.³¹.

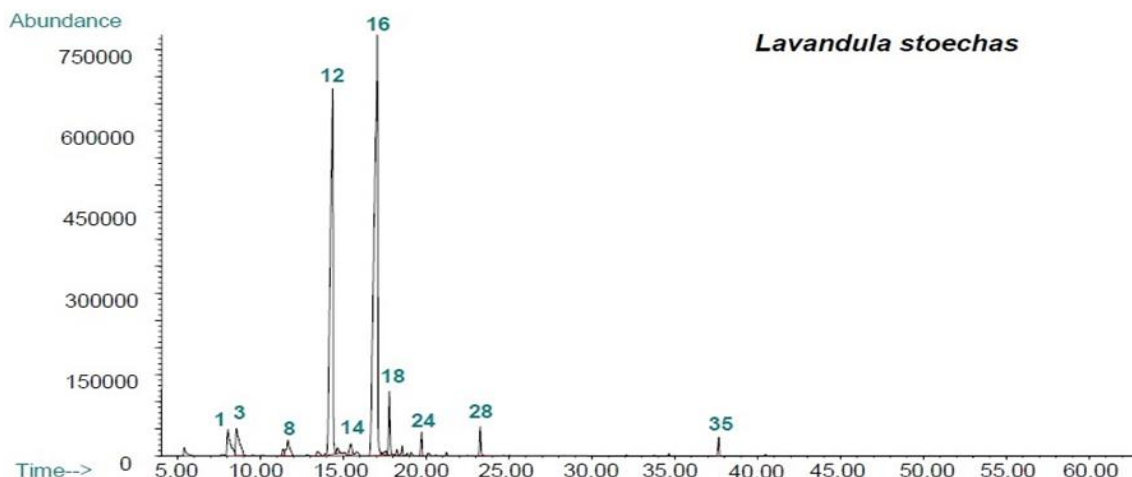


Figure 1: GC chromatogram of the essential oil from *L. stoechas*

Table 2: Antimicrobial activity of commercial antibiotics determined by the agar diffusion method

Bacteria	AMP (5µl/disc)	AMX (10 µl/disc)	CLR (30 µl/disc)	GMC (10 µl/disc)	AMK (10 µl/disc)	CEF (10µl/di s)	CFX (5 µl/disc)	CFT (30 µl/disc)	OFX (5 µl/disc)
<i>E. coli</i>	NI	NI	20.4±2.3	13.6±0.8	13.8±0.6	8.2±1.4	14.7±1.3	14±1.5	25.2±3.4
<i>S. aureus</i>	NI	NI	17.5±1.2	17.2±2.2	14.5±0.5	7.4±0.6	14.2±0.8	14.2±1.2	21±2.5
<i>K. pneumonia</i>	NI	NI	21±2.2	14.4±1.4	13.2±1.2	NI	15.5±0.7	14.5±1.5	21.6±2.2
<i>P. mirabilis</i>	8.5±1.5	8.5±1.2	14.5±1.5	13.4±0.8	12.4±1.6	NI	14±1.5	18±1.4	25.4±3.2
<i>P. aeruginosa</i>	NI	8±1.5	14.4±2.2	13±1.4	13.4±1.2	8.5±1.3	13.5±1.4	13.2±1.2	14.5±1.5

*AMP : Ampicillin ; AMX : Amoxicillin ; CLR : Chloromphenicol ; GMC : Gentamycin ; AMK : Amikacin ; CEF : Cefaclor ; CFX : Cefotaxim ; CFT : Ceftriaxon ; OFX : ofloxacin

- Values represent averages ± standard deviations for triplicate.

- NI: no inhibition.

Table 3: Antimicrobial activities of *L. stoechas* essential oil using macro-dilution method.

Bacteria	Essential oil of <i>L. stoechas</i>	
	MIC (µl/mL)	MBC (µl/mL)
<i>E. coli</i>	5	10
<i>S. aureus</i>	2.5	5
<i>K. pneumonia</i>	2.5	2.5
<i>P. mirabilis</i>	10	20
<i>P. aeruginosa</i>	5	10

The limited number of published studies about the antimicrobial activity of *L. stoechas* EO, such as those from Gören et al.³¹, Bouzouita et al.³² and Cherrat et al.³⁰, confirms its strong antimicrobial activity, similar to other sesquiterpenes-rich EOs. Camphor the major component of *L. stoechas* EO analyzed has antibacterial properties itself³³. Moreover, it has been demonstrated that 1,8 cineole presents antimicrobial activity against bacteria such as *S. aureus* and *E. coli*³⁴. Other study suggests the synergistic effect of minor components in the chemical composition of the EOs in relation to its antimicrobial activity³⁵. The gram positive bacterium is more susceptible to the antimicrobial properties of essential oil than gram negative bacteria and it is considered to be due to its outer membrane³². However, contrary to our study, according to Prabuseenivasa et al.³⁶ reported that *E. coli* and *S. aureus* were resistant to Lavender oil. The results of antibiotic

susceptibility test of the bacterial isolates indicated that *E. coli*, *S. aureus* and *K. pneumonia* were resistant to Ampicillin and Amoxicillin. The acquisition of resistance to Penicillin antibiotics is a global phenomenon showing widely varying occurrence rates. In the Marrakech region, antimicrobial resistance of urinary *K. pneumoniae* isolates to amoxicillin has been reported to be similar to the resistance rates reported in the Rabat region (Morocco)⁶ and in Algeria³⁷.

CONCLUSION

The essential oil of *L. stoechas* was chemically analyzed and antimicrobial activity *in vitro* against clinical bacteria was conducted. 27 components were identified, of which camphor, fenchone, camphene, borneol, α-pinene, and 1,8-cineole, were the major components. The essential oil of *L. stoechas* was revealed highly inhibitory antimicrobial activity against *S. aureus* and moderately active against *E. coli* and *K. pneumonia*, *P. mirabilis* and *P. aeruginosa*. Ofloxacin had the widest coverage against all bacteria's followed by chloromphenicol Cephalosporin's third generation; gentamicin and amikacin have presented an average activity against pathogens. *E. coli*, *S. aureus* and *K. pneumonia* bacteria were resistant to the Penicillin antibiotics. The oil of *L. stoechas* can have application in therapy of the infectious diseases is like substituent of certain antibiotics or like complementary agents used in

synergy with the synthesis substances. Further advance research on toxicological and clinical studies are required to prove the safety of the oil.

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