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Research Article

Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil and its Fractions of *Lavandula stoechas* L. From Morocco

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ABSTRACT
The emerging multi-drug resistance in food borne pathogens and consumers demand for minimally processed fresh natural foods has paved the path for natural antimicrobials and antioxidants to be used in food industry. This work investigates the chemical composition, antimicrobial and antioxidant properties of essential oils of *Lavandula stoechas* and its fractions which were obtained from the *Lavandula stoechas* using apparatus Glass Oven B-585. GC/MS analysis of *Lavandula stoechas* essential oil has led to the identification of 27 components, of which camphor, fenchone, camphene, borneol, α-Pinene and 1,8-cineole, were the major components. Fractions 1 and 2 have displayed qualitative similarities; fraction 3 has showed a different chemical profile characterized by the presence of various oxygenated sesquiterpenes. In DPPH assay, the IC_{50} value of *Lavandula stoechas* and its fractions have varied between 0.8 and 1.6 µg/ml, while phosphomolybdenum assay of essential oils of *Lavandula steochas*, fraction 1, 2 and 3 have showed values in the order of 79 ± 4.4, 73.7 ± 3.7, 76.2 ± 5.5 and 148.2 ± 6.2 µg/ml respectively. The essential oil of *Lavandula steochas* was very active against *Staphylococcus aureus* and *Listeria spp*. Fractions 1 and 2 were moderately active, however fraction 3 recorded a weak effect against all bacteria's. The Chloromphenicol antibiotic was very active against all bacteria's, Gentamycin has expressed average activity against pathogens but the Penicillin has showed poor activity. Our results suggest that essential oils of *Lavandula stoechas* and fraction 3 demonstrated interesting biological properties that suggest its use as a new potential source of natural antioxidant and antimicrobial agents.

Keywords: Antioxidant activity, Antimicrobial activity, Food borne, Fraction, *Lavandula stoechas*, pathogens.

INTRODUCTION

Food contamination by microorganisms is a global issue with serious consequences for human health. The World Health Organization reports that, annually, unsafe food results in the illnesses of at least 2 billion people worldwide and can be deadly[1]. The industrials and numerous researchers, are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage pathogenic bacteria in foods. In industrialized countries, the percentage of the population suffering from foodborne diseases each year has been reported to be up to 30%. In the United States, for example, around 76 million cases of foodborne diseases, resulting in 325000 hospitalizations and 5000 deaths, are estimated to occur each year[2].
In Morocco, during 1980 to 2007, 78.374 cases of food borne diseases have been reported[3]. In region of Fez-Meknès, 1508 cases of food borne diseases were reported during 2007 to 2011[4].
On the other hand, the increasing antibiotic resistance of some pathogens that are associated with foodborne illness is another problem[5]. Adds to that, consumers today are increasingly concerned about chemical preservatives used in preservation of food and tend to choose food products that are natural, safe and with multi-health benefits[6,7]. Many plants, their essential oils and extracts have potential

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in medical procedures and applications in the pharmaceutical, cosmetic and food industry[8]. Numerous scientists showed interest for biologically active components isolated from plants and for their influence on the proliferation of pathogenic microorganisms[9]. The resistance, which certain microorganisms have developed against antibiotics, initiated antimicrobial investigations and different applications of essentials oils or plants against a wide range of bacteria (Gram-negative and Gram-positive) including antibiotic resistant species[10]. There are several studies regarding the use of essential oils as natural compounds because they show preservative properties[11,12]. The activity of the oils would be expected to relate to the respective composition of the plant volatile oils, the structural configuration of the components of the volatile oils and their functional groups as well as the possible synergistic interactions between the components[13].

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is some preference for antioxidants from natural rather than from synthetic sources[14,15].

Lavandula species comprise an important natural resource for cosmetics, perfumes, food processing, aromatherapy and similar applications world-wide. The usage of *Lavandula* sp. as flavoring and condiments in foods, such as salads and soups and in herbal teas has been documented[16]. It is reported that the volatile aroma components of individual *Lavandula* species have characteristic organoleptic effects on honeys produced from them[17]. Previous researches on the essential oil composition of *L. stoechas* collected from different Mediterranean countries have been reported[18,19]. This species is one of the most explored lavenders in the world, some studies have considered the antibacterial[20,21], antifungal[18,20], anti-inflammatory[9] and antioxidant[20,22,23] properties of the oils.

The objective of this study was to evaluate the chemical characterization and the determination of the antioxidant properties of essential oil and some fractions of *L. stoechas* collected in north east of Morocco as well as to test its antimicrobial activity against some food borne pathogenic bacteria. This is the first report on the antimicrobial and antioxidant properties of of *L. stoechas* essential oil and its fractions collected in north east of Morocco against food borne pathogens.

Experimental Section

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 EO was stored at 4°C so that can be used in the upcoming experiments.

Fractionation of essential oil

The fractional distillation was realized by using apparatus Glass Oven B-585. All of the glass balls were placed in the oven except for the glass ball farthest right. A volume of 10 ml was introduced in a glass ball and was exposed at temperature of boiling of fenchone and camphor with values of 193.5°C (Fraction 1) and 204°C (Fraction 2) respectively for 3 min for each fraction. Fraction 3 (residual) was recuperated in the first ball after fractionation.

Chemical characterization of essential oil of Lavandula stoechas and its fractions

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 data[24]. 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.

Antioxidant tests

Determination of free radical scavenging activity by DPPH method

The ability of the *L. stoechas* and fractions essential oils to scavenge Diphenylpicrylhydrazyl (DPPH) radicals was determined according to the procedure described by Soldier et al[25] with slight modifications. Briefly, 100 µl of various concentrations of the each essential oil were added to 10 mL of a methanol/DPPH solution (1.01×10^{-4} M). The mixture was vigorously shaken and then allowed to stand at a room temperature for 30 min in the dark. The absorbance of the mixture was measured at 517 nm by using a double-beam UV–visible Camspec M550 spectrophotometer. A mixture of 100 µL of methanol and 10 mL of DPPH solution is used as control and Butylated Hydroxytoluene (BHT) used as positive control. The IC_{50} values were calculated by nonlinear regression of plots[26].

Evaluation of total antioxidant capacity by Phosphomolybdenum method

The antioxidant activity of the essential oils was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al[27]. The tubes containing the reaction solution were capped and incubated in boiling water at 95°C for 90 min. The control consisted on 0.3 mL of methanol the absorbance of the solution is measured at 695 nm using a spectrophotometer. The antioxidant capacity of each sample expressed as ascorbic acid equivalent using the following linear equation established using ascorbic acid as standard:

[$A = 0.0037C + 0.0343$; $R^2 = 0.991$].
A: is the absorbance at 695 nm,
C: is the concentration as ascorbic acid equivalent (µg/mL).
The values are presented as the means of triplicate analysis.

Antimicrobial activity

Microorganisms

The antimicrobial activity of *L. stoechas* essential oil was tested against Gram-positive bacterial strains *Staphylococcus aureus* (*S. aureus*), and Gram-negative bacterial strains *Escherichia coli* (*E. coli*) and *Listeria spp*. These bacteria have been isolated from food samples having caused poisoning. They have been identified and confirmed by classical biochemical gallery and the API (bioMérieux, France). These microorganisms were obtained from the Microbiology Unit at the Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene (RDLEH) falling within Regional Health Directorate of Fez. This laboratory follows the requirements of the NM ISO 17025 since 2008.

Disc diffusion assay

Antimicrobial susceptibility test of the essential oil and fractions was tested against the above mentioned Gram positive, Gram negative bacteria by disc diffusion method[28]. 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, Chloromphenicol and Gentamycin 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)[29].

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 MIC[30]. 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 Tripticase 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 ± SEM of triplicate analysis using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Component analysis of essential oils of L. stoechas and its fractions.

The percent yield of the hydrodistilled volatile oil from aerial parts of *L. stoechas* was 1.8±0.3 %. GC/MS analysis of *L. stoechas* essential oil led to the identification of 27 components, 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 (Table 1).

Although *L. stoechas*, Fraction 1 and fraction 2 essential oils displayed qualitative similarities with the EO sample, quantitative differences among the relative percentages of three compounds were observed; for camphor (47.2, 46.4 and 50 % respectively), α-pinene (2.9, 1.5 and 0.7 % respectively) and camphene (3.3, 2.5 and 0.6 % respectively). The degrees of temperature used in the fractionation of essential oil, such as fractions 1 and 2 were very close (193.5 and 204 ° C respectively), in fact the chemical composition of these oils were not very different on *L. steochas* EO.

Fraction 3 (Residues) displayed a different chemical profile which characterized by the presence of various oxygenated monoterpenes and oxygenated sesquiterpenes with 90.9 and 4.8 % respectively, the slightly decrease of fenchone and the increase of camphor. Thus, the percentage of 1,10-di-epi cubenol was gradually increased from 0.8% to 4.6% (Table 1), and decrease of *α*-pinene (from 2.9 to 0.1%), camphene (from 3.3 to 0.1%) and 1,8-cineole (from 1.4 to 0.3%). Some compounds not detected in the essential oil of *L. stoechas* and present in the fractions could be formed by thermal transformation.

L. stoechas have been objected of several phytochemical studies that have pointed out a high chemical variability allowing the establishment of several chemotypes. *L. stoechas* oil is characterized by significant variations in the amounts of fenchone, camphor and 1.8-cineole, being the fenchone/camphor chemotype the most commonly identified[20,23]. In the EO extracted from north of Morocco (Tangier), whose composition was dominated by 10s, 11s-himachala-3(12),4-diene (23.62%), cubenol (16.19%), methyl eugenol (6.19%), δ-cadinene (5.31%) and myrtenyl acetate (4.96%)[31]. The comparison of the present results with the chemical composition of *L. stoechas* EO from other countries has shown qualitative and quantitative differences. For example, Malika et al[32] have studied the chemical composition of the essential oil of flowers of *L. stoechas* collected from Algeria and found 49 components with a prevalence of linalyl acetate (15.26%), linalool (10.68%), 1,8-Cineol (10.25%), γ-terpinene (11.2%) and camphor (11.25%). A study realized by Dadalioglu et al[21] in south of Turkey where α-fenchone (55.8%), camphor (18.2%), 1,8-cineole (8%), and myrtenyl acetate (9.5%)

Table 1: Chemical composition of essential oil of *Lavandula stoechas* and its fractions analyzed by GC-MS.

Peak N°	K.I.	Component	Relative percentage of *L. stoechas* oil and its fractions			
			L. stoechas	Fraction 1	Fraction 2	Fraction 3
1	927	Tricyclene	0.2	*tr* [&]	-	-
2	939	*α*-Pinene	2.9	1.5	0.7	0.1
3	954	Camphene	3.3	2.5	0.6	0.1
4	957	Thuja-2,4(10)-diene	-	-	0.9	0.2
5	995	3-Octanol	-	-	-	-
6	1026	*p*-Cymene	0.4	-	0.4	0.2
7	1032	Limonene	0.3	0.3	0.1	*tr*
8	1035	Eucalyptol	1.4	1.6	1.2	0.3
9	1062	γ-Terpinene	-	*tr*	-	-
10	1077	*cis*-Linalool oxide (furanoid)	0.4	0.6	0.4	0.3
11	1086	Camphenilone				0.1
12	1088	Fenchone	33.3	34.8	34.1	24.3
13	1101	Linalool	0.7	0.9	1.1	0.5
14	1121	Fenchol[**]	1.1	1.4	1.3	1.2
15	1130	*α*-Campholenal	0.4	0.3	0.3	0.2
16	1147	Camphor	47.2	46.4	50.0	51.7
17	1168	Pinocarvone	0.4	0.5	-	-
18	1172	Borneol	2.9	3.0	3.0	4.0
19	1177	*trans*-Linalool oxide (pyranoid)	-	-	-	0.1
20	1181	Terpinen-4-ol	0.3	0.4	0.4	0.5
21	1187	p-Cymen-8-ol	0.4	0.5	0.6	1.1
22	1193	*α*-Terpineol	0.1	0.1	0.1	0.2
23	1198	Myrtenal	0.2	0.2	0.2	0.4
24	1212	Verbenone	1.0	1.0	1.1	2.4
25	1221	*trans*-Carveol	0.1	0.1	0.1	0.4
26	1224	Fenchyl acetate *(endo)*	*tr*	0.1	0.1	-
27	1246	Carvone	0.1	0.1	0.1	0.3
28	1289	Bornyl acetate	1.2	1.2	1.4	2.7
29	1292	Thymol	-	-	-	0.1
30	1327	Myrtenyl acetate	-	-	-	0.1
31	1343	Piperitenone	-	-	-	*tr*
32	1487	Germacrene D	*tr*	-	-	-
33	1527	*trans*-Calamenene	-	-	-	0.3
34	1549	Selina-3,7(11)-diene	0.1	-	-	0.4
35	1623	1,10-di-epi Cubenol	0.8	0.2	0.2	4.6
36	1647	epi-*α*-Cadinol	-	-	-	0.2
37	1658	*β*-Eudesmol	-	-	-	*tr*
38	1661	*α*-Cadinol	-	-	-	*tr*
39	1680	Cadalene	-	-	-	*tr*
40	1694	*cis*-14-nor-Muurol-5-en-4-one	*tr*	-	-	0.6
41	1706	δ-Dodecalactone	-	-	-	0.1
Total identified (%)			99.2	97.7	98.4	97.7
Oxygenated monoterpenes			91.2	93.2	95.5	90.9
Monoterpene hydrocarbons			7.1	4.3	2.7	0.6
Oxygenated sesquiterpenes			0.8	0.2	0.2	4.8
Sesquiterpene hydrocarbons			0 .1	-	-	0.7
Others			-	-	-	0.7

* K.I.: Kovats Index determined on OPTIMA-5 non-polar column in reference to n-alkanes.
[&] *tr* : Trace for percentages ≤ 0.07%
**Correct isomer not identified

were the major essential oil constituents. From these results, we noticed that the chemical composition of the essential oil of the species *L. stoechas* cultivated in north east of Morocco was different from those obtained from many experiments on the same species, with a prevalence of the oxygenated monoterpenes (91.2 %) compounds in the majority of the cases. The different qualitative and quantitative chemical compositions of the EO could be related to different environmental conditions, degree of

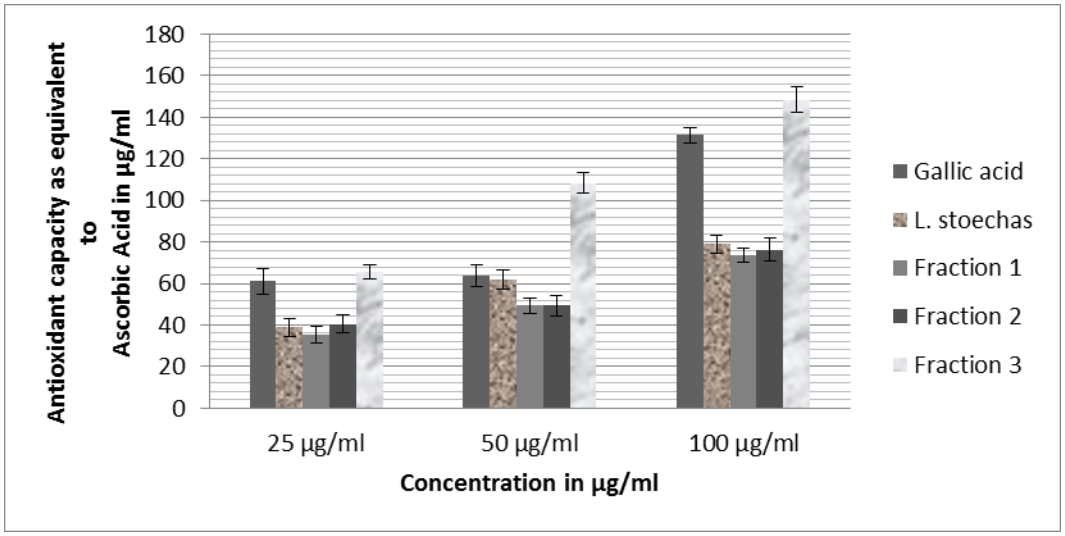

Figure 1: Antioxidant capacity of *L. stoechas* essential oil and its fractions by phosphomolybdate method.

Table 2: IC_{50} of *L. stoechas* essential oil, its fractions and BHT by DPPH method.

Antioxidant	IC_{50} [a] (µg/mL)
L. steochas	1.1
Fraction 1	1.6
Fraction 2	1.3
Fraction 3	0.8
Control (BHT)[b]	0.2

[a]: Concentration (µg/mL) for a 50% inhibition.
[b]: Synthetic antioxidant: Butylated hydroxytoluene

hybridization, geographical origin, harvest period and method of extraction[33].

Antioxidant capacity

In the DPPH assay quantitative, antioxidants are typically characterized by their IC_{50} value, concentration necessary to reduce 50% of DPPH radical, which is a simple, rapid and highly reproducible method widely used in antioxidant screening[34]. In general, the oil of *L. stoechas* and its fractions were able to reduce the stable radical DPPH to the yellow colored DPPH-H with an IC_{50} value between 0.8 and 1.6µg/mL. BHT positive control exhibited high antioxidant activity with IC_{50} value of 0.2 µg/mL (Table 2).

The DPPH scavenging effect of essential oil and fractions of *L. stoechas* and standard on the DPPH radical decreased in the order of BHT > Fraction 3> *L. stoechas* > fraction 2 > fraction 1. Figure 1 reveals the phosphomolybdate reducing power of *L. stoechas* and its fractions.

Essential oils of *L. steochas*, fraction 1, 2 and 3 showed values in the order of 79±4.4, 73.7±3.7, 76.2±5.5 and 148.2±6.2 µg/mL respectively of equivalent of ascorbic acid in comparison to gallic acid 131.2±3.4 µg/mL equivalents to ascorbic acid at 100 µg/mL concentration (Figure 1). In our study fraction 3 of *L. stoechas* and *L. stoechas* essential oils showed an important radical scavenging activity (RSA) for IC_{50} values of 0.8 µg/ml and 1.4 µg/mL respectively, but lesser than BHT (IC_{50} = 0.2 µg/mL). A study realized by Sebai et al[35] in Tunisia have reported that RSA of areal parts of *L. stoechas* (IC_{50}=241.23 µg/mL) is lesser than our results. Lis-Balchin et al[36] were reported that there are no correlation between the percentage of the principal components and the antioxidant activity of essential oils extracted from lavender. In our study fraction 3 characterized by reducing power in the phosphomolybdate assay more than all other EOs and standard. This activity can be attributed to the richness of this fraction by oxygenated monoterpenes and oxygenated sesquiterpenes.

Antimicrobial activities

The in vitro results of antibacterial activity of the EO of *L. stoechas* and its fractions by the paper disk agar diffusion method against microorganisms are summarized in Table 3. The essential oil and its fractions of *L. stoechas* were evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria. The essential oil of *L. stoechas* was very active against *S. aureus* and *Listeria spp* (17.5±3.1 and 18.5±1.3 mm respectively), moderately active against *E. coli*, showed an inhibition zone between 13±0.6 and 14.3±0.55 mm. Fractions 1 and 2 were moderately active against all pathogen's (between 10.8±1.4 and 14± 1.2 mm). The fraction 3 did not inhibit *E. coli* recuperate in Dairy and her activity was very weak against all bacteria's (between 7±0.5 and 8±1.2 mm). Among the antibiotics (Table 3), Chloromphenicol had the widest coverage against all bacteria's (between 14±0.8 and 21±1.6 mm), Gentamicin has presented an average activity against pathogens with diameter of inhibitions zones between 13.2±1.2 and 15.5±1.5 mm, but the Penicillin antibiotic has showed poor activity against all bacteria's.

The antimicrobial activity of *L. stoechas* and its fraction was confirmed by the macrodilution assay (Table 4). The essential oil of *L. stoechas* inhibited *Listeria spp* and *S. aureus* with value of MIC=2.5 µl/mL and MIC = 5 µl/mL was recorded against two species of *E. coli*. The MIC values of fractions 1 and 2 ranging from 5 to 10µl/mL, it is noticeable the MIC of fraction 3 is very higher value of 40 µl/mL for all bacteria's. The essential oil of *L. stoechas* exhibited a great antibacterial activity against all tested bacteria. This activity is probably reported by the presence

Table 3: Antimicrobial activities of *L. stoechas* essential oil and its fractions using disc diffusion method.

Microorganisms	Origin of bacteria	Inhibition zone diameter (mm) of essential oils				Inhibition zone diameter (mm) of antibiotics		
		L. stoechas (10 µl/disc)	Fraction 1 (10 µl/disc)	Fraction 2 (10 µl/disc)	Fraction 3 (10 µl/disc)	AMP [a] (5µl/disc)	CLP [b] (30 µl/disc)	GMC [C] (10µl/disc)
E. coli	Cooked ground meat	14.3±0.55	14± 1.2	12±0.8	7±0.6	7.2±0.4	19±2.4	13.8±2.4
E. coli	Dairy (milk product)	13±0.6	13.6±2.2	12.5±1.2	NI	8.2±0.8	21±1.6	14.5±1.5
Listeria spp	Cooked meat	18.5±1.3	12±1.6	12.5±1.2	8±1.2	7±0.6	15.2±1.2	13.2±1.2
S. aureus	Cheese	17.5±3.1	11±1.5	10.8±1.4	7±0.5	NI	14±0.8	15.5±1.5

* a: Ampiccilin; b: Chloromphenicol; c: Gentamycin
- Values represent averages ± standard deviations for triplicate.
- Inhibition zone including disc diameter (6 mm).
- NI: No Inhibition was determine

Table 4: Antimicrobial activities of *L. stoechas* essential oil and its fractions using macro- dilution method.

Bacteria	Origin of bacteria	MIC (µl/mL)				MBC (µl/mL)			
		L. stoechas	fraction 1	fraction 2	fraction 3	*L. stoechas*	fraction 1	fraction 2	fraction 3
E. coli	Cooked ground meat	5	10	10	40	10	20	10	80
E. coli	Dairy (milk product)	5	10	10	40	10	20	10	80
Listeria spp	Cooked meat	2.5	10	5	40	5	20	10	80
S. aureus	Cheese	2.5	5	5	40	5	40	10	80

of *α*-pinene, camphene and 1,8-cineole in the EO of *L. stoechas* and fractions 1 and 2.

The antimicrobial activity of this EO is comparable with data reported in previous studies[31,37]. Camphor the major component of *L. stoechas* EO analyzed has antibacterial properties itself[38]. Moreover, it has been demonstrated that 1,8-cineole, of the components of the *L. stoechas* essential oil, presents antimicrobial activity against bacteria such as *S. aureus*, *E. coli* and *L. monocytogenes* [39]. Burt[40] also suggested that the minor components of EOs are more critical to the activity than mixtures of the main EO components, and may have either an additive or synergistic effect. Therefore, we can suggest that good antimicrobial activity of *L. stoechas* EO may be due to the wealth of α-Pinene (2.9%), Camphene (2.3%) and 1,8-cineol (1, 4%), their combined presence may cause a synergistic effect giving essential oil a significant antimicrobial property. Viljoen et al[41] demonstrated that the synergistic effect can be active in an association between 1,8-cineol and camphor. Synergies similar could be involved in the activities *L. stoechas* essential oil.

CONCLUSION

The objective of this work was to analyze the chemical constituents and to evaluate the antioxidant and antibacterial activity of the essential oil and some fractions of *L. stoechas* from north east of Morocco. GC/MS analysis of *L. stoechas* essential oil led to the identification of 27 components, of which camphor, fenchone, camphene, borneol, α-Pinene, and 1,8-cineole, were the major components. Fractions 1 and 2 displayed qualitative similarities with the EO sample, although, quantitative differences among the relative percentages were observed. Fraction 3 displayed a different chemical profile which characterized by the presence of various oxygenated sesquiterpenes (4.8 %). Moreover, the essential oil has an antioxidant activity and the higher antioxidant effect was observed in fraction 3 when compared to other essential oils but the antimicrobial activity of this fraction was very weak against all bacteria's. The essential oil of *L. steochas* was very active against *S. aureus* and *Listeria spp*, moderately active against *E. coli*. Fractions 1 and 2 were moderately active against all bacteria's. We concluded that the slight difference in compounds of the same essential oil can affect the biological activities of essential oils. These results also indicate the possible use of the essential oil on food system as an effective inhibitor of food borne

pathogens. However, it is necessary to determine the level of toxicity of the essential oil of *L. stoechas* and its fractions.

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