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LAVANDULA STOECHAS ESSENTIAL OIL FROM ALGERIA: AROMATIC PROFILE DETERMINED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND BIOLOGICAL ACTIVITIES

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Abstract

Description of the subject: *Lavandula stoechas* L. (Lamiaceae) is an attractive shrub native to the Mediterranean regions, this species presents an increasing interest in medicine, cosmetics and for preserving and improving the flavor of foods.
Objective : This study aims to investigate the essential oil (EO) composition and biological activities (antibacterial, antifungal and antioxidant) of the essential oil of *Lavandula stoechas* cultivars in Algeria.
Methods : *Lavandula stoechas* essential oil, obtained from plants grown in the North of Algeria, was gathered, dried, hydrodistilled and their essential oil analyzed by gas chromatography coupled with mass spectrometry (GC-MS). allowing to obtain qualitative and quantitative results of *Lavandula stoechas*. This characterization was completed with different biological activities.
Results : Fifty compounds were identified, four of them (fenchone, camphor, lavandulyl acetate and cineole) accounting for more than 60% of the total oil in the analyzed samples. Antioxidant activity was evaluated positively by DPPH method. The antimicrobial activity revealed that *Lavandula stoechas* essential oils are inhibitory against the tested bacteria and fungal strains.
Conclusion : These properties support the potential use of L. stoechas EO as natural cosmetic and natural pharmaceutical ingredients for several skin diseases.
Keywords : *Lavandula stoechas*; composition; GC/MS; Antioxidant activity; Antibacterial and antifungal activities.

HUILE ESSENTIELLE DE *LAVANDULA STOECHAS* D'ALGÉRIE : PROFIL AROMATIQUE DÉTERMINÉ PAR CHROMATOGRAPHIE EN GAZ-SPECTROMÉTRIE DE MASSE ET ACTIVITÉS BIOLOGIQUES

Résumé

Description du sujet : *Lavandula stoechas* L. (Lamiaceae) est un arbuste attrayant originaire des régions méditerranéennes, cette espèce présente un intérêt croissant pour la médecine, les cosmétiques et pour la préservation et l'amélioration de la saveur des aliments.
Objectifs : Cette étude vise à examiner la composition de l'huile essentielle (HE) et les activités biologiques (antibactériennes, antifongiques et antioxydantes) de l'huile essentielle de Lavandula stoechas.
Méthodes : L'huile essentielle de *Lavandula stoechas*, obtenue à partir de plantes cultivées dans le Nord de l'Algérie. Cette plante a été récoltée, séchée et hydro distillée. L'HE obtenue a été analysée par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS), permettant d'obtenir des résultats qualitatifs et quantitatifs de *Lavandula stoechas*. Cette caractérisation a été complétée par différentes activités biologiques,
Résultats : Cinquante composés ont été identifiés, dont quatre (fenchone, camphre, acétate de lavande et cinéole) représentant plus de 60 % de l'huile totale dans les échantillons analysés. L'activité antioxydante a été évaluée positivement par la méthode DPPH. L'activité antimicrobienne a révélé que les huiles essentielles de *Lavandula stoechas* sont inhibitrices contre les bactéries et les souches fongiques testées.
Conclusion : Ces propriétés encouragent l'utilisation potentielle de l'HE de L. stoechas comme ingrédients cosmétiques naturels et pharmaceutiques naturels pour plusieurs maladies de la peau
Mots clés : *Lavandula stoechas* ; composition ; GC/MS ; Activité antioxidante ; Activités antibactériennes et antifongiques.

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INTRODUCTION

Aromatic plants are one of the most important plant categories growing in the Mediterranean region. Aromatic plants have been widely used from ancient times in medicine, cosmetics and for preserving and improving the flavor of foods. Essential oils are well known for their various beneficial effects on human health. The use of herbs in phytotherapy is mostly due to the essential oils and their various biological activities [1-6]. *Lavandula stoechas* is a species of aromatic flowering plant of the Lamiaceae family. The genus Lavandula, of the Lamiaceae family, consists of approximately 20 species with more than 100 varieties of lavender [1-6]. *L. stoechas* is an evergreen shrub, it usually grows up to one-meter-high with spike violet flowers. It occurs naturally in Mediterranean countries. It has been used as cooking spices and fragrance, and its essential oil (EO) is one of the aromatic ingredients in the production of food, drinks, soaps, perfumes, cosmetics and pharmaceuticals. These applications have been related with their bioactivities as natural antibacterial, antifungal, insecticide, antioxidant and anti-inflammatory agents, with low toxicity for human skin cells [1-6]. Therefore, the present study aimed to investigate the essential oil composition and biological activities (antibacterial, antifungal and antioxidant) of the essential oil of Lavandula stoechas cultivars in Algeria.

MATERIALS AND METHODS

1. Plant material and extraction procedure

The Lavandula stoechas was collected in North Algeria during the flowering season (April 2021). Afterwards, the flowers of Lavandula stoechas were dried at room temperature (22–28 °C) for 2 weeks in darkness and then stored in sealed paper bags until their use for analyses. The essential oils were extracted by hydrodistillation (HD) for 2 h using a Clevenger-type apparatus.

2. Essential oil analysis

Essential oil was analysed by GC-MS using a gas-chromatograph (model CLARUS 500-Perkin Elmer) coupled to a mass detector (Clarus 560S Perkin Elmer) and equipped with a Elite-5MS (1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane) 30-m capillary column, 0.25 mm i.d. and 0.25 µm thickness of the stationary-phase layer. The oven temperature was programmed as follows: from 70 to 220°C at 4°C/min and isothermally held for 15 min. Helium was used as carrier gas at 1 ml/min and 1µL of sample was injected for analysis. El mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the NIST spectra library. Quantitative data were obtained from the TIC peak areas without the use of response factors.

3. Test microorganisms and inocula preparation

The antibacterial activity of Lavandula essential oil was tested against the following bacteria: *Bacillus subtilis* (STCC4071), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853). In addition, antifungal activity was tested against yeast strains *Candida albica*ns (ATCC 10231).

4. Antibacterial and antifungal activities

The antimicrobial activities of the essential oil were screened using an agar disc diffusion method [1, 2]. Essential oil was diluted 1 :1 with ethanol containing 0.5% Tween 80 and sterilized by filtration through 0.22-µm-pore-size filters (Millipore, Bedford, USA). Standard suspension from a fresh culture of each test microorganism (~10^6 cfu/mL) was used to seed melted Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi, and poured into sterilized plates under aseptic conditions. These were allowed to solidify. Wells of 6mm diameter were then aseptically punched in the agar using a sterile metal cylinder, and aliquots of 50µL of the samples were placed into each of these wells. The plates were left 1h at 4°C to allow the diffusion of the sample and then incubated under aerobic conditions 24h at 37°C for bacteria and 48h at 24°C for fungi.

The antibacterial activity was determined by measuring the diameter of the inhibition zone in millimeters with a digital caliper, and the results were expressed as mean ± SD. Three plates were used for each treatment as replications and the experiment was repeated twice.

5. Antioxidant activity

The free radical scavenging activities of the investigated Lavandula essential oils and their main constituents were evaluated using the stable DPPH radicals according to the previously reported method [7]. Briefly, 0.1mM solution of DPPH in ethanol was prepared and 1mL of this solution was added to 3mL of the ethanol sample solution. Two-fold serial dilutions of the samples were assayed in the range of 1.6–400mg/mL.

After incubation for 30min in the dark at room temperature, the absorbance was measured spectrophotometrically at 517nm. Butylated hydroxyl toluene (BHT) was used as a positive control. The assay was carried out in triplicate. The capability to scavenge the DPPH radicals was calculated using the following equation: $(\%) = \frac{1-A_1}{A_0} \times 100$. Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the sample. The concentrations of samples that provide 50% inhibition (IC50) were obtained by interpolation from linear regression analysis.

RESULTS AND DISCUSSION

1. Chemical composition

The chemical composition of this essential oil was determined by GC–MS analyses. The identified volatile components and their percentage contents are listed in the Table 1.

Table 1. Chemical composition of essential oil of Lavandula stoechas

N°	Identified Compounds	Abundance (%)	IR	IR Adams (2017)
1	α-pinene	0,09	963	932
2	camphene	0,19	967	946
3	Benzaldehyde	0,11	969	952
4	β-pinene	0,14	979	974
5	myrcene	0,08	997	988
6	α-phellandrene	0,07	1 000	1 002
7	α-terpinene	0,05	1 006	1 014
8	**p - cymene**	**3,68**	1 015	1 020
9	**1,8-cineole (Eucalyptol)**	**8,87**	1 022	1 026
10	γ-terpinene	0,47	1 034	1 054
11	**Fenchone**	**29,42**	1 084	1 083
12	terpinolene	trace	1 091	1 086
13	ρ-Cymenene	0,40	1 096	1 089
14	**linalool**	**1,67**	1 100	1 095
15	**Camphor**	**13,41**	1 139	1 141
16	Menthone	0,37	1 153	1 148
17	borneol	0,80	1 156	1 165
18	lavandulol	0,64	1 159	1 165
19	**Terpinen-4-ol**	**2,29**	1 164	1 174
20	alpha-terpineol	0,18	1 178	1 186
21	**Myrtenol**	**4,05**	1 194	1 194
22	**verbenone**	**4,15**	1 210	1 204
23	endo-Fenchyl acetate (α-fenchyl acetate)	0,32	1 218	1 218
24	Carvone	0,63	1 228	1 239
25	**bornyl acetate**	**3,34**	1 256	1 284
26	**lavandulyl acetate**	**13,03**	1 291	1 288
27	carvacrol	0,19	1 299	1 298
28	iso-Menthyl acetate	0,18	1 307	1 304
29	iso-Verbanol acetate	0,11	1 310	1 308
30	cis-dihydro-α-Terpinyl acetate	0,48	1 317	1 316
31	myrtenyl acetate	0,62	1 321	1 324
32	hexyl tiglate	0,18	1 326	1 330
33	inconu	0,25	1 337	-
34	α-Cubebene	trace	1 347	1 345
35	inconnu	trace	1 368	-
36	α-Copaene	0,10	1 369	1 374
37	Geranyl acetate	0,11	1 383	1 379
38	methyl Eugenol	0,23	1 393	1 403
39	**alpha-gurjunene**	**1,25**	1 401	1 409
40	Aromadendrene	0,46	1 439	1 439
41	(E)-β-Farnesene	0,62	1 449	1 454
42	aromadendrene, dehydro	0,68	1 463	1 460
43	**germacrene D**	**1,72**	1 472	1 480
44	**viridiflorene (ledene)**	**1,83**	1 481	1 496

N°	Identified Compounds		Abundance (%)	IR	IR Adams (2017)
45	inconnu	0,25	1 493		-
46	δ-Cadinene	0,23	1 520		1 522
47	γ-Cuprenene	0,74	1 528		1 532
48	germacrene B	0,55	1 532		1 559
49	Caryophyllene oxide	0,18	1 564		1 582
50	α-Cadinol	0,56	1 653		1 652

RT = Retention Indices. Compounds with more than 1% are highlighted.

The chemical fractions of the essential oils obtained from flowers is listed in the table 2.

Table 2. Chemical fractions of the essential oils obtained from flowers

Chemical class	**%**
Monoterpene hydrocarbons	5.18
Oxygenated monoterpenes	85.20
Sesquiterpene hydrocarbons	8.19
Oxygenated sesquiterpenes	0.75
Others	0.49
Total identified (%)	**99.51**

2. Determination of antimicrobial activity

The essential oil *Lavandula stoechas* was evaluated for antimicrobial activity (bacteria and fungi). The zone of inhibition (mm) of the essential oil of *L. stoechas* is showed in the Table 3.

Table 3. The zone of inhibition (mm) of the essential oil of *L. stoechas*

Microorganism	**Inhibition zone diameter (mm)**
Bacillus subtilis	12.0
Pseudomonas aeruginosa	14.0
Escherichia coli	14.0
Candida albicans	32.0

3. Determination of antioxidant activitiy

Antioxidant effectiveness of the Lavandula essential oils and their main components were evaluated using the DPPH radical scavenging assay and compared with the activity of reference antioxidant ascorbic acid. Abilities of the tested samples to scavenge DPPH% are assessed on the basis of their IC50 values which were inversely related to their antioxidant capacities, as they express the amount of antioxidant needed to decrease the radical concentration by 50%. This simple and rapid spectrophotometric method involves the use of the commercially available, stable DPPH radicals to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors. The values of antioxydant activity are showed in the table 4.

Table 4. Values of Antioxidant activity

Samples	**IC 50 (mg/mL)**
Ascorbic acid	0.023 ± 0.008
L. stoechas	0.028 ± 0.007

DISCUSSION

Fifty compounds were identified. The major compounds were fenchone (29.42 %), camphor (13.41%), lavandulyl acetate (13.03 %) and cineole (8.87 %) accounting for more than 60% of the total oil in the analyzed samples (table 1). Previous studies on the same plant [1-8] reported that the major components of the essential oils are mostly in agreement, but in different proportions

Essential oil of Lavandula stoechas was characterized by a large amount of monoterpenes (90.38%) including oxygenated monoterpenes as major compounds (85.20%). Sesquiterpene hydrocarbons represented only 8.94% (Table 2).

The essential oil lavandula stoechas was evaluated for antimicrobial activity (bacteria and fungi). It was found to be active against all the microbes used for the activity. The essential oil was very active against *Candida albicans*. Moderately active against *Eschrichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, with an inhibition zone diameter varying from 12.0 to 32.0 mm. (see Table 3).

The antibacterial activity of EOs must be ascribed to their chemical composition, and normally the oxygenated monoterpenes rich oils show a higher antibacterial activity than the oils rich in monoterpene hydrocarbons [9].

Concerning the EO of *Lavandula stoechas* the antibacterial activity can be associated to the high percentage of oxygenated monoterpenes (Table 2). Our results were in accordance with results previously reported on *Lavandula stoechas* [1-3].

The DPPH radical method is applied to determine the antiradical power of several products including essential oils. Lower IC50 value means higher radical scavenging activity. The determination of the antioxidant activity of the EO of *L. stoechas* and the reference antioxidant, ascorbic acid, showed weak antioxidant activity with IC50 = 0.028 ± 0.007 mg/mL and 0.023 ± 0.008 mg/mL (Table 4).

The difference of antioxidant capacities between our results and the other reported in literature may be attributed to the differences in chemical compositions, which depend on the areas of the plant collection, plant parts used and the extraction method [1].

CONCLUSION

The chemical composition of this essential oils was determined by GC–MS analyses. Essential oils of *L. stoechas* have effective pharmacological activities and can be a very important source of plant protection components. Since this oil has a non-negligible anti-fungal power, it can be used as an antioxidant supplement.

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