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Volatile Constituents and Antimicrobial Activity of *Lavandula stoechas* L. Oil from Tunisia

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Abstract

An oil obtained from the dried leaves of *Lavandula stoechas* L. in 0.77% yield was analyzed by capillary GC and GC/MS. Fenchone (68.2%) and camphor (11.2%) were the main components of the 28 identified molecules. This oil has been tested for antimicrobial activity against six bacteria, and two fungi. The results showed that this oil was active against all of the tested strains; *Staphylococcus aureus* was the more sensitive strain.

Key Word Index

Lavandula stoechas, Lamiaceae, essential oil composition, fenchone, camphor, antimicrobial activity.

Introduction

Lavandula stoechas is an herbaceous wild plant, indigenous to the mountain regions of the countries bordering the western Mediterranean. The chemical composition of lavender's oils depends largely on the species from which it was obtained (1,2). The species *L. stoechas* present chemical composition quite different from other species like *L. dentata, L. angustifolia, L. latifolia* and *L. hybrida* (3-6).

Antimicrobial activities of essential oils have been reported by numerous workers (7-12) but, to our knowledge the properties of *L. stoechas* have never been reported. We report here the chemical composition and the antimicrobial properties of the oil of *Lavandula stoechas* growing in Tunisia using a submerged broth culture method.

Experimental

Lavandula stoechas was collected in April 2002 from Kairouan, Tunisia. Voucher specimens were identified and deposited at the Department of Botany, Faculty of Sciences, Tunis. Dried ground *Lavandula* leaves were subjected to hydrodistillation using Dean-Stark apparatus (until there was no significant increase in the volume of oil collection) to give the following yield (w/w): 0.77%. The oil was dried over anhydrous sodium sulfate and stored under N_2 at 4°C. The determination of retention data and the area percentage of the identified constituents were carried out on a Hewlett-Packard HP 6890 gas chromatograph equipped with a splitless injector (250°C) and a 30 m x 0.32 mm HP-5 column (1 µm film thickness). The temperature program was 40°-250°C at 5°C/min. The FID-detector was kept at 250°C and the carrier gas was helium (1.3 mL/min). Quantitative data were obtained from FID area counts without the use of correction factors. The mass spectra were recorded in the electron impact mode at 70 eV on a HP 5973 GC/MS system using the aforementioned chromatographic conditions. Individual components of *L. stoechas* oil were identified by their retention indices compared with literature values (13,14) and their mass spectra were interpreted on the basis of the WILEY 275.L computer library. The fragmentations of sesquiterpene hydrocarbons were systematically compared with those of Joulain and König atlas of spectral data (15).

Antibacterial activity was assayed against six bacteria. Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Streptococcus A* (ATCC 11700). Gram-negative bacteria: *Escherichia coli* (obtained from stock cultures of the Faculty of Sciences, Tunis), *Salmonella enteritidis* (ATCC 14028), *Klebsiella pneumoniae* (ATCC 13833) and *Pseudomonas aeruginosa* (ATCC 9027). Antifungal activity was assayed against

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Table I. Chemical composition of *Lavandula stoechas* oil from Tunisia

Compound	RI	%	Compound	RI	%
α-pinene	977	0.4	α-terpineol	1243	0.2
camphene	995	0.8	myrtenol	1252	0.6
oct-1-en-3-ol	1018	0.2	α-fenchyl acetate	1271	0.8
p-cymene	1070	0.4	carvone	1297	0.3
1,8-cineole + limonene	1079	4.9	carvone oxide°	1331	t
cis-linalool oxide†	1119	t	bornyl acetate	1339	1.4
fenchone	1145	68.2	myrtenyl acetate	1378	1.2
linalool	1152	0.3	cyclosativene	1434	0.2
α-fenchol	1166	1.9	α-copaene	1439	0.2
α-campholenol	1176	t	allo-aromadendrene	1533	0.2
camphor	1202	11.2	BHT*	1570	1.5
borneol	1218	0.6	δ-cadinene	1587	0.4
terpinen-4-ol	1230	0.2	selina-3,7(11)-diene	1616	0.2
p-cymen-8-ol	1235	0.4	viridiflorol	1671	1.4

*BHT = antioxidant from diethyl ether; † = furanoid form; t = trace (<0.05%); RI = Retention index on HP-5 capillary column; °correct isomer not identified

two fungi: *Geotrichum candidum* and *Candida albicans*. All bacteria (except *Escherichia coli* and *Candida albicans*) were obtained from the Institut Pasteur of Tunis collections. *Geotrichum candidum* was isolated from an aerated pilot scale bubble column fed continuously with Oil Mill Wastewater (16). It was identified as being the white-rot fungus *Geotrichum candidum* by Centraalbureau Voor Schimmelcultures. The fungus was conserved in Petri dishes containing potato dextrose agar (PDA) at 4°C.

The effects of different concentrations of the oil (0-0.05-0.075-0.1-0.5-0.75-1.0-1.5-2.5-3.5% v/v) on the tested strains were evaluated by submerged broth culture method: *Streptococcus A* in Todd-Hewitt broth, *Staphylococcus aureus* in special Staphylococcus broth, the other test bacteria in nutrient broth, *Geotrichum candidum* and *Candida albicans* in Sabouraud dextrose agar. The different solutions of oils were mixed with Tween 80 at final concentration of 0.5% (v/v) in broth medium. The surviving bacteria and *C. albicans* were determined by enumeration and reported as colony forming units per mL medium (CFU/mL) after incubation for 24 h at 37°C. The growth of *G. candidum* was evaluated directly on the submerged culture by measuring optical density at 600 nm with a 6505 UV/Vis JENWAY spectrophotometer after incubation for 48 h at 30°C. A control prepared for each strain containing 1.75% of Tween 80 give initial cell concentration of each bacteria and the initial A_{600} of the inoculum *G. candidum*. Each assay was replicated three times.

Results and Discussion

The 28 components identified in the oil, which can be seen in Table I, correspond to 96.6% of the total oil, with the major components being fenchone (68.2%), camphor (11.2%) and a mixture of 1,8 cineole-limonene (4.9%). According to the existing literature, the oil of *L. stoechas* consists of linalyl acetate, 1,8-cineole, fenchone and camphene (17,18). In a study of the plant from Greece its oil seemed to consist almost exclusively of fenchone, camphor, 1,8-cineole, pinocarvyl acetate and myrtenol (3). GC analysis showed that the Tunisian oil consisted of 1.6% monoterpene hydrocarbons, 4.9% monoterpene ether, 4.4% monoterpene alcohols, 3.4% monoterpene esters, 79.7% monoterpene ketones and 2.6% sesquiterpenes. The Tunisian *L. stoechas* differs from the Greek one (3) in the major components. The relative quantities of fenchone and camphor are noticeably smaller in the Greek plant (40.4%) but 1,8-cineole and myrtenol were higher.

The results obtained by submerged broth culture method are shown in Table II. The oil of *L. stoechas* exhibited a great antibacterial activity against all the tested bacteria. The Gram-positive bacteria were more sensitive than the Gram-negative bacteria examined. The most susceptible bacterium was *Staphylococcus aureus*, the number of viable cells were decreased from 3.6 10^8 CFU/mL to 2.2 10^3 CFU/mL after 24 h incubation in the presence of 3.5% oil (v/v). However this number increased in control containing 1.75% of Tween 80 from 3.6 10^8 CFU/mL to 2.2 10^9 CFU/mL. Our tests with Tween 80 confirmed that the presence of Tween 80 at a final concentration of 0.5% (v/v) in the watery media did not inhibit the growth of any bacteria. The detergent was used successfully to enhance the solubility of essential oil in the test medium (19). This result obtained with *Staphylococcus aureus* is interesting since this pathogen bacterium causes purulent and generalized septic infections. While *Pseudomonas aeruginosa* was the most susceptible of all Gram-negative bacteria tested, as shown in Table II. Thus, the oil caused a decrease of *P. aeruginosa* cell number of almost three log values, while the decrease of cell number of the other three Gram-negative bacteria was almost two log values. This number increased in control with one log value. The antifungal activity results showed that a 1% (v/v) of *L. stoechas* oil completely inhibited *Geotrichum candidum* and *Candida albicans*.

Most oils have more than one constituent. The nature and proportion of the individual constituents of the oils could influence their antimicrobial activity (20). Previous reports attribute the antibacterial activity of some *Lavandula* oils to α-terpineol and terpinen-4-ol (found in *L. dentata*, *L. latifolia* and *L. hybrida*) (21-23). α-Terpineol has been found to possess significant microbiostatic activity against *S. auereus*, *E. coli*, *P. aeruginosa* and *C. albicans* (24). Camphor, which is present in

Table II. Final cell concentration (CFU/mL) after 24 h growth in submerged culture at different concentration of *L. stoechas* oil

Microorganisms		Essential oil concentration % v/v									
		0	0.05	0.075	0.1	0.5	0.75	1.0	1.5	2.5	3.5
Staphylococcus aureus	$3.6\ 10^{8*}$	$2.2\ 10^{9}$	$2.8\ 10^{8}$	$2.6\ 10^{8}$	$2.3\ 10^{8}$	$3.4\ 10^{5}$	$1.1\ 10^{5}$	$6\ 10^{4}$	$5.5\ 10^{4}$	$4.8\ 10^{4}$	$2.2\ 10^{3}$
Streptococcus A	$4\ 10^{7*}$	$2.3\ 10^{8}$	$9.6\ 10^{7}$	$3.1\ 10^{7}$	$1.5\ 10^{7}$	$4.6\ 10^{6}$	$1.5\ 10^{6}$	$6.9\ 10^{5}$	$1.5\ 10^{4}$	$7\ 10^{3}$	$6.9\ 10^{3}$
Escherichia coli	6.10^{7*}	$2.6\ 10^{8}$	$2.9\ 10^{7}$	$1.6\ 10^{7}$	$1.2\ 10^{7}$	$1.6\ 10^{6}$	$1.3\ 10^{6}$	$9\ 10^{5}$	$6.9\ 10^{5}$	$3.1\ 10^{5}$	$1.3\ 10^{5}$
Salmonella enteritidis	$1.4\ 10^{7*}$	$2.6\ 10^{8}$	$3.2\ 10^{7}$	$1.2\ 10^{7}$	$4.1\ 10^{6}$	$3.2\ 10^{6}$	$1.2\ 10^{6}$	$9.4\ 10^{5}$	$7.1\ 10^{5}$	$4.9\ 10^{5}$	$1.2\ 10^{5}$
Klebsiella pneumoniae	$3.3\ 10^{8*}$	$3.4\ 10^{9}$	$9.1\ 10^{8}$	$1.6\ 10^{8}$	$2.3\ 10^{7}$	$6.2\ 10^{6}$	$6.2\ 10^{6}$	$6.1\ 10^{6}$	$5.2\ 10^{6}$	$2.1\ 10^{6}$	$1.5\ 10^{6}$
Pseudomonas aeruginosa	$1.4\ 10^{8*}$	$1.0\ 10^{9}$	$8.3\ 10^{8}$	$2.5\ 10^{8}$	$2.3\ 10^{8}$	$2.1\ 10^{8}$	$1.1\ 10^{8}$	$1.1\ 10^{8}$	$1.6\ 10^{7}$	$1\ 10^{7}$	$2.0\ 10^{5}$
Candida albicans	$1.5\ 10^{8*}$	$6.0\ 10^{10}$	$6.4\ 10^{6}$	$5.2\ 10^{6}$	$4.2\ 10^{6}$	$3.2\ 10^{6}$	$2.2\ 10^{6}$	$<10^{3}$	$<10^{3}$	$<10^{3}$	$<10^{3}$
Geotrichum candidum°	0.097**	0.359	0.127	0.100	0.092	0.060	0	0	0	0	0

°48 h at 30°C; **initial optical density at 600 nm; *initial cell concentration

each of the *Lavandula* oils, was found in high concentrations in *L. latifolia* (25, 26), and possesses antibacterial activity. α-Pinene, 1,8-cineole and p-cymene have some antifungal activity (27,28). Fenchone, the main component in our oil, was not considered a potent antimicrobial agent (29). An essential oil always represents a complex mixture of different chemical components. For that reason, it is very difficult to reduce the antimicrobial effect of total oil to one or few active principles.

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