The Chemical Constituents and Biological Activity of Essential Oil of *Lavandula stoechas* ssp. *stoechas*

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Lavandula stoechas ssp. *stoechas*, Essential Oil of *Lavandula*, Biological Activity

The composition of essential oil of the leaves of *Lavandula stoechas* ssp. *stoechas*, was analyzed by means of capillary GC-MS. The main components of *L. stoechas* ssp. *stoechas* oil were pulegone (40.4%), menthol (18.1%), menthone (12.6%). The essential oil of the plant was evaluated for antibacterial and a panel cytotoxic activities.

Lavandula genus is an important member of family Labiatae (Lamiaceae). *Lavandula* species are widely distributed in the Mediterranean region and cultivated in France, Spain and Italy. In Turkey, mainly two species, *Lavandula stoechas* and *Lavandula angustifolia* and their subspecies and hybrid forms grow wildly or are cultivated (Mill, 1982). The medicinal importance of the plant is well documented (Poucher, 1974; Hartwell, 1971a, 1971b) and the drugs prepared from this plant are registered in many Pharmacopeia (Leclerc, 1966). The plant is used as expectorant, antispasmodic, carminative, a good stimulan, deobstruent, resoluent and wound healing. The essential oil obtained from its flowering twigs has been used as a remedy against colic and chest affections, to relieve nervous headache, bliousness and for cleansing wounds (Saaed, 1970; Baytop, 1967; Hussain *et al.*, 1988). There are some essential oil studies on *Lavandula species* which grow in Turkey, the recent ones were performed by supercritical fluid extraction (Adaşoğlu *et al.*, 1994, Akgün *et al.*, 2001). The essential oil of the Greek *Lavandula stoechas* was reported by Kokkalou. (Kokkalou, 1988). Our previous study on *Lavandula stoechas* ssp. *stoechas* was its about nonvolatile compounds, afforded triterpenoids (Topçu *et al.*, 2001). We now present herein a study on the essential oil of *L. stoechas* ssp. *stoechas* obtained by hydrodistillation, investigated by GC-MS analysis and evaluated for its antibacterial and cytotoxic activities.

Materials and Methods

Plant material

Lavandula stoechas ssp. *stoechas* was collected from Ayvalık-Cunda Island, western Turkey, May 2001. A voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy, University of İstanbul.

Isolation of the essential oil

The essential oil of leaves of *L. stoechas* ssp. *stoechas* (150 g) was obtained by hydrodistillation for 3 h in a Clevenger-type apparatus, 2 ml essential oil was obtained (1.33% w/w).

Gas chromatography/mass spectrometry

GC-MS analysis was carried out with a HP 5890 GC, Micromass Zabspec (double focusing magnetic sector) for essential oil DB-5 fused silica column (L: 60 m × I. D.: 0.25 mm, Ft.: 0.5μm). The GC-Mass was operated under the following conditions: Initial temperature: 40 to 280 °C at 5 °C/min, carrier gas: He at 1 ml/min, injection: 0.1 μl, transfer line temperature: 250 °C; ion source temperature: 200 °C; splitting ratio: 1:50; ionization

energy 70 eV; trap current 200 μA; scan range 50–700 amu; scan time 2 s.

Identification of components

The identification of each compound was carried out by comparison of RRT (relative retention time) and mass spectral data obtained with literature and a computerized MS-data bank (NIST and NISTREP Library).

Antimicrobial activity test

The disk-diffusion method (NNCLS, 1997a, 1997b) was used to determine the inhibition zones of the oil. The standard bacterial strains used were *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilus* ATCC 6633 and a yeast *Candida albicans* ATCC 10231.

Cytotoxic activity test

The essential oil was evaluated against cultured KB (human epidermoid carcinoma), BC1 (human breast cancer), LU1 (human lung cancer), COL-2 (human colon cancer), KB-V (+VLB) (drug-resistant KB), P-388 (mouse leukemia), LNCaP (hormone-dependent human prostate cancer), and ASK (rat glioma) cell lines (Likhitwitayawuid *et al.*, 1993).

Results and Discussion

The essential oil of *L. stoechas* ssp *stoechas* showed a very diverse composition with 42 constituents reported in Table I. The oil from our plant is dominated by pulegone (40.37%), hexahydrothymol (menthol) (18.09%), menthone (12.57%) while the essential oil from the Greece was dominated by fenchone (30.85%) and pinocarvyl acetate (10.20) (Kokkalou *et al.*, 1988).

The essential oil of *L. stoechas* ssp *stoechas* was evaluated for cytotoxicity against a number of cell lines (see Table II). The essential oil was found to be active against COL-2 (9.8 μg/ml) and weakly active against LNCaP (17.6 μg/ml) while the chloroform extract of the same plant was found to be highly active against P-388 (1.4 μg/ml) . None of them showed any activity against the ASK cell line.

The essential oil was tested against standard bacterial strains (see Methods), and showed antibacterial activity against most of the tested standard bacterial strains except, *S. epidermidis, E. faecalis, and C. albicans* (Table III).

Table I. The percentage composition of the total oil from *Lavandula stoechas* ssp. *stoechas.*

RT [min]	Compound	Percentage %
35.24	α-thujene	0.1
36.13	α-pinene	1.2
37.46	camphene	0.4
39.48	sabinen	0.3
40.22	β-pinene	3.2
40.53	myrcene	0.3
43.37	α-terpinene	0.1
44.19	*p*-cymene	1.4
44.46	D-limonene	1.3
44.55	β-phellandrene	0.1
45.08	eucalyptol	3.9
45.56	3-carene	0.3
47.17	γ-terpinene	0.4
48.16	isolimonene	0.1
49.51	isoterpinolene	0.1
50.28	β-terpineol	2.3
52.49	*cis*-verbenol	0.2
54.02	*trans*-p-2,8-menthadien-1-ol	0.1
55.17	*trans*-dihydrocarvone	0.9
55.53	menthone	12.6
56.08	isopulegol	0.4
56.54	menthol	18.1
57.14	borneol	0.5
57.48	2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde	3.2
58.43	unidentified	0.2
58.51	α-terpineol	0.4
59.37	*cis*-carveol	0.1
1.00.06	piperitenone	0.1
1.01.03	unidentified	0.2
1.01.13	unidentified	0.2
1.03.08	pulegone	40.4
1.03.54	piperitone	0.2
1.04.15	α-citral	0.1
1.05.43	thymol	0.2
1.06.05	bornyl acetate	0.1
1.06.36	carvacrol	0.6
1.09.33	*p*-mentha-1(7), 8(10)-dien-9-ol	0.6
1.16.42	β-caryophyllene	0.1
1.24.41	nerolidol	0.1
1.27.15	spathulenol	0.4
1.27.47	caryophyllene oxide	0.1
1.30.46	β-cadinene	0.1

RT = retention time on the DB-5 column. Compounds in less than 0.1% are not reported.

Table II. Evaluation of cytotoxic potential[a].

	Cell lines (ED_{50})							
Sample	BC1	LU1	COL-2	KB	KB-V	P-388	LNCaP	ASK
Essential oil	>20	>20	9.8	>20	>20	>5	17.6	–
Chloroform extract	>20	>20	>20	>20	>20	1.4	>20	–
Ellipticine	0.2	0.02	0.3	0.04	0.3	0.1	0.8	–

[a] Compounds were initially tested at a concentration of 20 μg/ml, and this was followed by dose-response studies, as required, to yield ED_{50} values (μg/ml). With cultured ASK cells, tests were performed at a concentration of 20 μg/ml, ellipticine was used as a positive control.

Table III. Antimicrobial activity test results of essential oil of *L. stoechas* ssp. *stoechas.*

Strains	Essential oil	Hexane
B. subtilis	18	0
S. aureus	22	0
S. epidermidis	NA	0
P. mirabilis	25	0
E. coli	23	0
Kl. pneumonia	25	0
Ps. aeruginosa	24	0
E. feacalis	NA	0
C. albicans	NA	0

[a] The doses at 232.5 μg/ml, the results are given in mm as zone diameter by disc diffusion method.
[b] Since the essential oil was dissolved in hexane, it was used as control.

Mass spectral data of seven major compounds are as follows

β-*Pinene*: (RT 40.22) EI-MS *m/z* (rel. int.): 136 $[M]^+$ (12), 121 $[M\text{-}CH_3]^+$ (20), 107 (4), 95 (3), 94 (18), 93 $[M\text{-}43]^+$ (100), 92 (12), 91 (21), 80 (16), 79 (25), 77 (23).

Eucalyptol: (RT 45.08) EI-MS *m/z* (rel. int.): 154 [M, not observed]$^+$, 136 $[M\text{-}H_2O]^+$ (13), 126 (17), 125 (15), 121 (10), 112 (6), 111 (82), 109 (9), 108 (100), 107 (6), 97 (19), 96 (60), 95 (36), 93 (57), 85 (8), 84 (98), 83 (46).

β-*Terpineol*: (RT 50.28) EI-MS *m/z* (rel. int.): 154 $[M]^+$, 136 $[M\text{-}H_2O]^+$ (11), 121 $[M\text{-}H_2O\text{-}CH_3]^+$ (28), 109 (9), 107 (9), 105 (5), 96 (13), 94 (15), 93 (100), 92 (19), 83 (28).

Menthone: (RT 55.53) EI-MS *m/z* (rel. int.): 155 $[M+1]^+$ (3), 154 $[M]^+$ (48), 140(5), 139 $[M\text{-}CH_3]^+$ (54), 125 (13), 121 (4), 113 (15), 112 [M-isopropyl]$^+$ (100), 111 (32), 110 (5), 98 (14), 97 (31), 95 (23), 84 (18), 83 (34), 81 (7).

Menthol (Hexahydrothymol): (RT 56.54) EI-MS *m/z* (rel. int.): 155 $[M\text{-}1]^+$ (2), 154 (18), 138 $[M\text{-}H_2O]^+$ (74), 123 (59), 113 (9), 112 (68), 111 (12), 113 (11), 109 (27), 99 (12), 97 (21), 96 (52), 95 [M- H_2O-isopropyl]$^+$ (100), 85 (23), 83 (34), 82 (62), 81 (89).

Pulegone: (RT 1.0308) EI-MS *m/z* (rel. int.): 152 $[M]^+$ (97), 137 $[M\text{-}CH_3]^+$ (42), 109 $[M\text{-}CH_3\text{-}CO]^+$ (58), 95 (29), 82 (51), 81 (100), 80 (13), 79 (12), 77 (10), 69 (30), 68 (28), 67 (64).

2, 6, 6-Trimethyl-1-cyclohexen-1-carboxaldehyde: (RT 57.48) EI-MS *m/z* (rel. int.): 152 $[M]^+$ (36), 137 $[M\text{-}CH_3]^+$ (26), 124 (8), 123 $[M\text{-}CHO]^+$ (90), 119 (1), 110 (4), 109 $[M\text{-}CHO\text{-}2CH_3]^+$ (100), 108 (29), 93 (77), 82 (12), 79 (12).

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