

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

Ahmet C. Gören<sup>a</sup>, Gülaçtı Topçu<sup>\*a,b</sup>, Gökhan Bilsel<sup>a</sup>, Mine Bilsel<sup>a</sup>, Zeynep Aydoğmuş<sup>c</sup> and John M. Pezzuto<sup>c</sup>

<sup>a</sup> TÜBİTAK, Marmara Research Center, Materials and Chemical Technologies Research Institute, P. O. Box 21, 41470, Gebze-Kocaeli, Turkey. Fax: +902626412309. E-mail: gulacti-topcu@hotmail.com

<sup>b</sup> Istanbul University, Faculty of Pharmacy, 34452, Beyazıt-Istanbul, Turkey

<sup>c</sup> Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA

\* Author for correspondence and reprints requests

Z. Naturforsch. **57c**, 797–800 (2002); received March 15/June 14, 2002

*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 stimulant, deobstruent, resolvent 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 hydrodistilla-

tion, 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.: 0.5 µm). 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  $\mu$ A; 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 subtilis* 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  $\mu$ g/ml) and weakly active against LNCaP (17.6  $\mu$ g/ml) while the chloroform extract of the same plant was found to be highly active against P-388 (1.4  $\mu$ g/ml). None of

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

RT [min]	Compound	Percentage %
35.24	$\alpha$ -thujene	0.1
36.13	$\alpha$ -pinene	1.2
37.46	camphene	0.4
39.48	sabinen	0.3
40.22	$\beta$ -pinene	3.2
40.53	myrcene	0.3
43.37	$\alpha$ -terpinene	0.1
44.19	<i>p</i> -cymene	1.4
44.46	D-limonene	1.3
44.55	$\beta$ -phellandrene	0.1
45.08	eucalyptol	3.9
45.56	3-carene	0.3
47.17	$\gamma$ -terpinene	0.4
48.16	isolimonene	0.1
49.51	isoterpinolene	0.1
50.28	$\beta$ -terpineol	2.3
52.49	<i>cis</i> -verbenol	0.2
54.02	<i>trans</i> -p-2,8-menthadien-1-ol	0.1
55.17	<i>trans</i> -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	$\alpha$ -terpineol	0.4
59.37	<i>cis</i> -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	$\alpha$ -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	<i>p</i> -mentha-1(7), 8(10)-dien-9-ol	0.6
1.16.42	$\beta$ -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	$\beta$ -cadinene	0.1

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

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 II. Evaluation of cytotoxic potential<sup>a</sup>.

Sample	Cell lines (ED <sub>50</sub> )							
	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	–

<sup>a</sup> Compounds were initially tested at a concentration of 20 µg/ml, and this was followed by dose-response studies, as required, to yield ED<sub>50</sub> 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
<i>B. subtilis</i>	18	0
<i>S. aureus</i>	22	0
<i>S. epidermidis</i>	NA	0
<i>P. mirabilis</i>	25	0
<i>E. coli</i>	23	0
<i>Kl. pneumonia</i>	25	0
<i>Ps. aeruginosa</i>	24	0
<i>E. faecalis</i>	NA	0
<i>C. albicans</i>	NA	0

<sup>a</sup> The doses at 232.5 µg/ml, the results are given in mm as zone diameter by disc diffusion method.

<sup>b</sup> 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]<sup>+</sup> (12), 121 [M-CH<sub>3</sub>]<sup>+</sup> (20), 107 (4), 95 (3), 94 (18), 93 [M-43]<sup>+</sup> (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]<sup>+</sup>, 136 [M-H<sub>2</sub>O]<sup>+</sup> (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]<sup>+</sup>, 136 [M-H<sub>2</sub>O]<sup>+</sup> (11), 121 [M-H<sub>2</sub>O-CH<sub>3</sub>]<sup>+</sup> (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]<sup>+</sup> (3), 154 [M]<sup>+</sup> (48), 140(5), 139 [M-CH<sub>3</sub>]<sup>+</sup> (54), 125 (13), 121 (4), 113 (15), 112 [M-isopropyl]<sup>+</sup> (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-1]<sup>+</sup> (2), 154 (18), 138 [M-H<sub>2</sub>O]<sup>+</sup> (74), 123 (59), 113 (9), 112 (68), 111 (12), 113 (11), 109 (27), 99 (12), 97 (21), 96 (52), 95 [M-H<sub>2</sub>O-isopropyl]<sup>+</sup> (100), 85 (23), 83 (34), 82 (62), 81 (89).

*Pulegone*: (RT 1.0308) EI-MS *m/z* (rel. int.): 152 [M]<sup>+</sup> (97), 137 [M-CH<sub>3</sub>]<sup>+</sup> (42), 109 [M-CH<sub>3</sub>-CO]<sup>+</sup> (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]<sup>+</sup> (36), 137 [M-CH<sub>3</sub>]<sup>+</sup> (26), 124 (8), 123 [M-CHO]<sup>+</sup> (90), 119 (1), 110 (4), 109 [M-CHO-2CH<sub>3</sub>]<sup>+</sup> (100), 108 (29), 93 (77), 82 (12), 79 (12).

#### Acknowledgement

Authors thank Prof. Dr. C. B. Johansson for antimicrobial activity test performed in the Medicine Faculty, Marmara University, İstanbul, Turkey.

- Adaşođlu D., Dinçer S. and Bolat E. (1994), Supercritical-fluid extraction of essential oil from Turkish Lavender Flowers. *J. Supercrit. Fluids* **7**, 93–99.
- Akgün N.-A., Akgün M., Dinçer S. and Akgerman A. (2001), Supercritical fluid extraction of *Lavandula stoechas* L. ssp. *cariensis* (Boiss.) Rozeira. *J. Ess. Oil Res.* **13**, 143–148.
- Baytop T. (1984), *Medicinal and Toxic Plants of Turkey*. İsmail Akgün Press, İstanbul, p. 316–317.
- Hartwell J. L. (1971a), Plants Used against Cancer-Survey. *Lloydia* **34**, 204–255.
- Hartwell J. L. (1971b), Plants Used against Cancer-Survey. *Lloydia* **34**, 386–425.
- Hussain A., Virmani O. P., Sherma A., Kumar A. and Misra L. N. (1988), Major Essential Oil-Bearing Plants of India. Central Institute of Medicinal and Aromatic Plants, Lucknow, India.
- Kokkalou E. (1988), The Constituents of the essential oil from *Lavandula stoechas* growing wild in Greece. *Planta Med.* **54**, 58–59.
- Likhitwitayawuid K., Angerhofer C. K., Ruangrunsi N., Cordell G. A., Pezzuto J. M. (1993), Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Stephania erecta*. *J. Nat. Prod.* **56**, 30–38.
- Mill RR. (1982), *Flora of Turkey and the East Aegean Islands* (Davis, PH, ed.). Vol. 7. University Press, Edinburgh, p. 77–79.
- NNCLS (1997a), Performance Standards for Antimicrobial Disk Susceptibility Tests, 1<sup>st</sup> ed. Approved Standard. NCCLS document M2-A6, Wayne, Pennsylvania.
- NNCLS (1997b), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 4<sup>th</sup> ed. Approved Standard. NCCLS document M7-A4, Wayne, Pennsylvania.
- Pochers W. A. (1974), *Perfumes, Cosmetic and Soap*, Vol. 1. 8th ed. Chapman and Hall, London.
- Saad M. (1970), *Hamdard Pharmacopia of Eastern Medicine*, Hamdard National Foundation Karachi, Pakistan.
- Topçu G., Ayril M. N., Aydin A., Gören A. C., Chai H. B. and Pezzuto J. M. (2001), Triterpenoids of the roots of *Lavandula stoechas* ssp. *stoechas*. *Pharmazie* **56**, 892–895.