NPC Natural Product Communications

2009
Vol. 4
No. 7
1001 - 1006

Chemical Composition and Antimicrobial Activity of the Essential Oils of *Lavandula stoechas* L. ssp. *stoechas* Growing Wild in Turkey

Hasan Kırmızıbekmez[a], Betül Demirci[b], Erdem Yeşilada[a], K. Hüsnü Can Başer[b] and Fatih Demirci[b*]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, TR-34755, Kayışdağı, İstanbul, Turkey*

[b]*Department of Pharmacognosy, Faculty of Pharmacy Anadolu University, 26470, Eskişehir, Turkey*

fdemirci@anadolu.edu.tr

Received: May 13th, 2009; Accepted: June 18th, 2009

The chemical compositions of the essential oils obtained by hydrodistillation from the dried leaves and flowers of *Lavandula stoechas* L. ssp. *stoechas* were separately identified by GC-FID and GC-MS analyses. The main components were α-fenchone (41.9±1.2%), 1,8-cineole (15.6±0.8%), camphor (12.1±0.5%), and viridiflorol (4.1±0.4%) in the leaves; and α-fenchone (39.2±0.9%), myrtenyl acetate (9.5±0.4%), α-pinene (6.1±0.09%), camphor (5.9±0.05%) and 1,8-cineole (3.8±0.1%) in the flowers. Overall, 55 and 66 constituents were identified in the leaf and flower essential oils representing more than 90% and 94% of the total, respectively. In addition, the essential oils were evaluated for their antibacterial and anticandidal activities by broth microdilution. The flower essential oil was found to be relatively more active than the leaf oil towards the tested pathogenic microorganisms. Methicillin-resistant *Staphylococcus aureus* was more susceptible to the flower oil (MIC= 31.2 μg/mL). The oils, evaluated for their free radical scavenging activity using a TLC-DPPH assay, were inactive at a concentration of 2 mg/mL.

Keywords: *Lavandula stoechas* ssp. *stoechas*, Lamiaceae, essential oil, GC, GC-MS, α-fenchone, 1,8-cineole, camphor, antibacterial and anticandidal activity.

The genus *Lavandula* (Lamiaceae) is comprised of small evergreen aromatic species widely distributed in the Mediterranean region, but is not limited to this region. In Turkey, two subspecies of *L. stoechas* L., namely ssp. *stoechas* and ssp. *cariensis*, grow in the wild, whereas *L. angustifolia* is only cultivated ornamentally in gardens, as in other parts of the world [1]. In Anatolian folk medicine, the flowering tops of the aromatic *L. stoechas* L. ssp. *stoechas* (well-known as 'karabaş otu' in Turkish) is utilized mainly as an analgesic, antiseptic, sedative, and expectorant, as well as for the treatment of hyperlipidemia and cancer, in various forms and preparations [2,3]. *Lavandula* species and lavender oil comprise an important natural resource for cosmetics, perfumes, food processing, aromatherapy and similar applications world-wide [4-6]. Previous research on the essential oil composition of *L. stoechas* and *L. stoechas* ssp. *stoechas* collected from different Mediterranean countries has been reported [7-12]. Two studies of the chemical components of the essential oil of *L. stoechas* ssp. *stoechas* collected from different regions of Turkey have been reported. In one, the major constituents of the oil obtained from plants collected from the southern parts of Turkey were the monoterpenes fenchone (55.6 %), camphor (18.2%) and 1,8-cineole (8.0%) [11]. In the other report, pulegone (40.4%), menthol (18.1%), and menthone (12.6%) were the major compounds of the essential oil obtained from the aerial parts of the same subspecies collected from western Turkey [13]. These major differences prompted us to reinvestigate the chemical composition of the essential oils of *L. stoechas* ssp. *stoechas*. In this study, we analyzed the hydrodistilled essential oils obtained separately from the leaves and flowers, as well as their *in vitro* antibacterial and anticandidal properties using a broth microdilution technique. Also a DPPH TLC-

Table 1: Chemical composition of *L. stoechas* ssp. *stoechas* essential oils.

RRI [a]	Compound	Flower (%)	Leaf (%)	MI
1032	α-Pinene	6.1 ± 0.09[b]	0.7 ± 0.08	d,e
1072	α-Fenchene	0.2 ± 0	0.1 ± 0	d,e
1076	Camphene	1.0 ± 0.05	0.6 ± 0.05	d,e
1118	β-Pinene	0.2 ± 0	0.1 ± 0	d,e
1132	Sabinene	0.1 ± 0	Tr[c]	d,e
1135	Thuja-2,4(10)-diene	Nd	0.2 ± 0	d
1174	Myrcene	0.2 ± 0	Nd	d,e
1203	Limonene	3.0 ± 0.1	0.2 ± 0	d,e
1213	1,8-Cineole	3.8 ± 0.1	15.6 ± 0.8	d,e
1225	(*Z*)-3-Hexenal	Nd	0.1 ± 0	d
1255	γ-Terpinene	Nd	0.1 ± 0	d,e
1280	*p*-Cymene	0.4 ± 0	0.4 ± 0	d,e
1386	1-Octenyl acetate	0.2 ± 0	Nd	d
1406	α-Fenchone	39.2 ± 0.9	41.9 ± 1.1	d,e
1450	*trans*-Linalool oxide (Furanoid)	0.2 ± 0	0.4 ± 0.05	d
1452	1-Octen-3-ol	0.6 ± 0.05	0.3 ± 0	d
1474	*trans*-Sabinene hydrate	0.2 ± 0.05	Nd	d
1478	*cis*-Linalool oxide (Furanoid)	0.2 ± 0.05	0.3 ± 0	d
1482	Fenchyl acetate	0.5 ± 0	0.2 ± 0.05	d,e
1492	Cyclosativene	0.2 ± 0	0.3 ± 0	d,e
1497	α-Copaene	0.4 ± 0	0.1 ± 0	d,e
1499	α-Campholene aldehyde	0.2 ± 0.05	0.1 ± 0.05	d,e
1532	Camphor	5.9 ± 0.05	12.1 ± 0.5	d,e
1553	Linalool	2.1 ± 0.05	0.7 ± 0	d,e
1586	Pinocarvone	Nd	0.1 ± 0	d,e
1591	Bornyl acetate	1.5 ± 0	0.8 ± 0.05	d,e
1591	Fenchyl alcohol	0.8 ± 0.05	0.4 ± 0.05	d,e
1611	Terpinen-4-ol	0.2 ± 0.05	0.3 ± 0	d,e
1617	Lavandulyl acetate	0.2 ± 0.05	0.2 ± 0.05	d
1648	Myrtenal	0.8 ± 0	0.2 ± 0	d
1661	Alloaromadendren	0.2 ± 0	Nd	d
1663	*cis*-Verbenol	0.1 ± 0	0.3 ± 0	d
1670	*trans*-Pinocarveol	0.2 ± 0	0.2 ± 0	d
1682	δ-Terpineol	0.1 ± 0	0.2 ± 0	d
1683	*trans*-Verbenol	1.5 ± 0.05	1.0 ± 0.05	d
1704	Myrtenyl acetate	9.5 ± 0.4	1.9 ± 0.16	d
1706	α-Terpineol	0.4 ± 0	0.2 ± 0.05	d,e
1719	Borneol	0.1 ± 0	0.2 ± 0	d,e
1725	Verbenone	0.4 ± 0	0.5 ± 0.05	d
1740	α-Muurolene	0.2 ± 0.05	0.2 ± 0.05	d
1744	α-Selinene	Nd	0.1 + 0	d
1751	Carvone	0.3 ± 0	0.3 ± 0	d,e
1765	Geranyl acetate	0.4 ± 0	0.3 ± 0.05	d,e
1773	δ-Cadinene	0.3 ± 0	0.7 ± 0.08	d
1797	*p*-Methyl acetophenone	Nd	0.1 ± 0	d
1804	Myrtenol	1.6 ± 0.05	0.3 ± 0.05	d,e
1838	2-Phenylethyl acetate	Nd	0.2 ± 0	d
1845	*trans*-Carveol	0.3 ± 0	0.2 ± 0	d,e
1849	Calamenene	0.2 ±0	0.2 ± 0.05	d
1864	*p*-Cymen-8-ol	0.3 ± 0.05	0.5 ± 0.05	d,e
1898	1,11-Oxido-calamenene	0.1 ± 0	0.1 ± 0.05	d
1900	*epi*-Cubebol	1.1 ± 0.08	0.4 ± 0.05	d
1912	*p*-Cymen-9-ol	Nd	0.1 ± 0	d,e
1941	α-Calacorene	Nd	0.1 ± 0	d
1953	Palustrol	Nd	0.1 ± 0	d
1957	Cubebol	0.8±0 .09	0.4 ± 0.05	d
1984	γ-Calacorene	Nd	0.1 ± 0	d
1988	2-Phenylethyl-2-methylbutyrate	Nd	0.1 ± 0	d
1992	2-Phenylethyl-3-methylbutyrate	Nd	0.1 ± 0	d
2008	Caryophyllene oxide	0.4 ± 0	0.5 ± 0.08	d,e
2057	Ledol	0.2 ± 0	0.7 ± 0.05	d
2080	Cubenol	0.2 ± 0	0.2 ± 0.05	d
2088	1-*epi*-Cubenol	0.5 ± 0.05	0.4 ± 0.05	d
2098	Globulol	Tr	0.1 ± 0	d
2104	Viridiflorol	2.1 ± 0.14	4.0 ± 0.4	d
2210	Copaborneol	0.6 ± 0.05	1.5 ± 0.1	d
2144	Spathulenol	Nd	0.1 ± 0	d,e
2209	T-Muurolol	0.2 ± 0	0.2 ± 0	d
2219	δ-Cadinol	0.1 ± 0	0.1 ± 0	d
2256	Cadalene	Nd	0.2 ± 0	d
2289	Oxo-α-Ylangene	0.1 ± 0	0.3 ± 0	d
Identified compounds:		55	66	
	Total:	90.7	94.1	

[a] RRI Relative retention indices calculated against *n*-alkanes on the HP Innowax column; [b] mean % calculated from flame ionization detector (FID) data ± SD (*n*= 3); [c] Tr, trace (< 0.1 %); Nd, not detected; d, comparison of MS with the Wiley and Mass Finder libraries and retention times; MI: Method of Identification; e, comparison with genuine compounds on the HP Innowax column.

bioautography assay was used to detect free radical scavenging compounds, if present. Furthermore, the popular usage as a herbal tea of this particular plant stimulated the research for its flavour and aroma profile, as well as for its biological activities.

The essential oils from the air dried leaves and flowers were isolated separately by hydrodistillation for two hours giving yields of 0.8 and 1.3%, respectively. Analysis of each essential oils was performed both by GC-FID and GC-MS, quantitatively and qualitatively. In total, 71 compounds were detected in the investigated essential oils. In the oil obtained from the leaves, 66 compounds were identified representing 94.1% of the total, and from the flowers, 55 components representing 90.8 % of the total (Table 1).

The main constituents of the leaf oil were α-fenchone, 1,8-cineole, camphor, and viridiflorol, and of the flower oil α-fenchone, myrtenyl acetate, α-pinene, camphor, and 1,8-cineole. α-Fenchone was the major compound in both leaf and flower essential oils. The 1,8-cineole and camphor contents were higher in the leaf oil than in that of the flowers. *L. stoechas* ssp. *stoechas* collected from İstanbul can be considered as an intermediate between the 1,8-cineole/α-fenchone, and camphor/ α-fenchone chemotypes. Our results were in good agreement with those obtained from a collection in south Turkey where α-fenchone (55.8%), camphor (18.2%), 1,8-cineole (8%), and myrtenyl acetate (9.5%) were the major essential oil constituents [11]. On the other hand, the essential oil of *L. stoechas* ssp. *stoechas* collected from western Turkey contained menthol (18.1%), menthone (12.6%), and pulegone (40.4%) [13], and showed major differences from our results. Previous published data for *L. stoechas* ssp. *stoechas* essential oil obtained from plants growing in other Mediterranean countries [8,10,12] were similar to our

Table 2: Antibacterial minimum inhibitory concentrations (MIC) (μg/mL) of *Lavandula stoechas* ssp. *stoechas* essential oils.

Pathogens	Leaf	Flower	St1	St2
Escherichia coli NRRL B-3008	250	250	3.9	15.6
Pseudomonas aeruginosa NRRL B-23	500	250	7.8	15.6
Enterobacter aerogenes NRRL 3567	250	250	1.9	7.8
Salmonella typhimurium NRRL B-4420	500	250	7.8	nt
Staphylococcus epidermidis ATCC 12228	250	250	0.9	nt
Staphylococcus aureus (MRSA) Clin. isol.	125	31.25	31.25	250

St1: Chloramphenicol, St2: Ampicillin, nt: not tested,
MRSA: Methicillin resistant *S. aureus* Clin. isol.: Clinical isolate

results (camphor-fenchone-type). It can be deduced from all these data that *L. stoechas* ssp. *stoechas* is represented by α-fenchone, camphor and 1,8-cineole as major compounds, but not menthol, menthone or pulegone. The essential oil obtained in this study from leaves of *L. stoechas* ssp. *stoechas* growing in Istanbul contained quite high amounts of 1,8-cineole, compared with literature data [13]. Contrary to the commercially important *L. angustifolia*, our samples contained only small amounts of linalool (0.7-2.1±0.05%) and lavandulyl acetate (0.2±0.05%), and ocimenes and linalyl acetate were absent [4].

In this present study, both essential oils were subjected to *in vitro* antibacterial and anticandidal evaluation by broth microdilution methods compared with antibacterials and antifungal standards, respectively. A panel of Gram-positive and Gram-negative human pathogenic bacteria, as listed in Table 2, were tested with the leaf and flower oils resulting in a wide range of inhibition (MIC = 125-500 μg/mL). The most susceptible microorganism was MRSA, which when treated with both the leaf and flower oils, produced MICs of 125 and 31.2 μg/mL, respectively. Table 3 shows that the flower oil was more effective than the leaf oil against the eight tested *Candida* strains, especially *C. albicans* (clinical isolate) and *C. tropicalis* with MICs of 125 and 250 μg/mL, respectively.

At the tested concentration of 2 mg/mL, although the standard control, ascorbic acid, showed good radical scavenging activity, the oils were rather inactive, suggesting that the volatiles of *L. stoechas* ssp. *stoechas* have no radical scavenging activity.

Antimicrobial studies of *Lavandula* sp. have been previously performed by various research groups [11-13,15]. The antifungal activity of *L. stoechas* ssp. *stoechas* oil against several plant and human

Table 3: Anticandidal evaluation results (MIC) (μg/mL).

Candida Strain	Leaf	Flower	St3	St4
C. albicans Clin. isol.	500	125	31.25	250
C. albicans ATCC 90028	500	1000>	125	250
C. glabrata Clin. isol.	500	1000>	125	250
C. utilis NRRL Y-900	500	1000>	125	500
C. tropicalis NRRL Y-12968	500	250	125	125
C. krusei NRRL Y-7179	500	1000>	125	250
C. zeylanoides NRRL Y-1774	500	1000	250	250
C. parapsilosis NRRL Y-12696	500	500	7.8	250

St3: Ketoconazole, St4: Griseofulvin

pathogens has also been investigated [12,16]. The results obtained in these studies suggested that the investigated *Lavandula* essential oils had a relatively fast (15 h) and good inhibitory effect at low doses (0.1%, 650-1000 ppm). The proposed mechanism of the inhibitory activity for fungi and yeasts was cell membrane damage and lysis, even at the vapor phase, as observed by scanning electron microscopy (SEM) [15].

It is reported that the volatile aroma components of individual *Lavandula* species have characteristic organoleptic effects on honeys produced from them [9]. The usage of *Lavandula* sp. as flavoring and condiments in foods, such as salads and soups and in herbal teas, is documented [2-4]. The seasonal and plant part variability of the essential oil of *L. stoechas* ssp. *stoechas* is also important, which was shown by a study by Angioni and co-workers [12], affecting the biological activities, including plant pathogenic fungi such as *Rhizoctonia solani*, *Fusarium oxysporum* and *Aspergillus flavus*. Cultivation of aromatic plants of industrial interest, such as *L. latifolia*, was elaborated and evaluated for the essential oil finger print contributing to its commercial importance [17].

Candidiasis has become a major problem in the last decades and essential oils and their components have been evaluated with partial success [18,19]. Essential oils are known to possess antifungal and anticandidal properties, which can be employed as antimycotic and antimicrobial agents in various forms [20]. Depending on the constituents, essential oils can exhibit several pharmacological and biological activities at one time, such as antimicrobial and antioxidant effects [21]. The antimicrobial effect may also be associated with the antioxidant properties of the constituents. *Lavandula* essential oil containing preparations can be utilized externally as an economic and feasible natural resource as alternative medicines, especially in microbial resistance cases. Taking into consideration that people use it commonly as a herbal tea, standard production and field studies should be performed as the plant has

potential as an industrial crop, not only as a herbal tea but also as an essential oil source for pharmaceutical and perfumery use. Further biological and pharmacological investigation both on the volatile and non-volatile fractions of *L. stoechas* would be worthwhile.

Experimental

Plant material: *Lavandula stoechas* L. ssp. *stoechas* was collected from Kayisdagi, Istanbul, northwest Anatolia, Turkey in April 2007. A voucher specimen (Akaydın, 10601) was deposited at the Herbarium of the Department of Biology Education, University of Hacettepe, Ankara, Turkey.

Isolation procedure: The air dried leaves and flowers were separately hydrodistilled for 2 h, using a Clevenger-type apparatus. After distillation, the essential oils were obtained in yields of 0.8%, v/w, dry weight for the leaves, and 1.3%, v/w, dry weight, for the flowers. The oils were dried over anhydrous Na_2SO_4 and stored at 4°C until use.

Gas chromatography (GC-FID): The GC analysis was carried out using an Agilent 6890N GC system. The flame ionization detector (FID) temperature was set to 300°C. To obtain the same elution order on the GC-MS, simultaneous auto-injection was performed on the same type of column (Innowax FSC column, 60 m x 0.25 mm, 0.25 μm film thickness) applying identical operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analytical results are expressed as mean percentage ± standard deviation (SD) (n= 3), as listed in Table 1.

Gas chromatography-mass spectrometry (GC–MS): The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. An Innowax FSC column, as above, was used with helium as carrier gas (0.8 mL/min gas flow). GC oven temperature was kept at 60°C for 10 min and adjusted to 220°C at a rate of 4°C/min, and then kept constant at 220°C for 10 min and finally programmed to 240°C at a rate of 1°C/min. Split ratio was set at 40:1. The injection port temperature was 250°C. Mass spectra (MS) were recorded at 70 eV and the selected mass range was from m/z 35 to 450.

Identification of the essential oil components was carried out by comparison of individual relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Computer matching against commercial (Wiley and MassFinder 2.1) [22,23] and in-house "Baser Library of Essential Oil Constituents" libraries made up from genuine compounds and components of known oils, as well as MS literature data [24,25] were used for the identification of individual components.

Antibacterial and antifungal activity: All microorganisms were obtained from different culture collections (ATCC, NRRL) or from clinical sources (Eskişehir Osmangazi University, Faculty of Medicine) and stored at -85°C in 15% glycerol, which were revived from storage before the experiments. Bacteria were refreshed in Nutrient Broth (Merck, Germany), and afterwards inoculated on Nutrient Agar (NA, Merck, Germany). *Candida* species were refreshed on Mueller Hinton Agar (MHA, Mast Diagnostics, UK) plates for microbial purity check, also at 35-37^{o}C. Sufficiently grown microorganisms were inoculated in Mueller Hinton Broth (MHB, Merck, Germany) 24 h before the antimicrobial screens. Strain numbers and sources of the acquired microorganisms are listed in Tables 2 and 3. The minimal inhibition concentration (MIC) values were evaluated according to previous published procedures [26,27]. Stock solutions of essential oils, pure substances and the antimicrobial standards were prepared in diluted dimethyl sulfoxide (20%, DMSO, Carlo-Erba, Milan, Italy). Dilution series were prepared initially from 2000 μg/mL, using 96-well microtiter plates containing 100 μL MHB in each well. Bacterial suspensions were standardized to 1 x 10^8 CFU/mL (McFarland No: 0.5) using the pre-grown inoculates. Freshly prepared bacterial suspensions were then pipetted into each well in equal volumes. The last-well row containing sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18-24 h, the first well without turbidity was assigned as the minimum inhibitory concentration (MIC) in μg/mL. Chloramphenicol and ampicillin were used as antibacterial standards against all pathogens. Experiments were repeated at three different times and the results were expressed as the average of the three values (Table 2).

The broth microdilution assay, as described above, was used for the anticandidal evaluation. Overnight grown *Candida* suspensions in Mueller Hinton Broth were standardized to approximately 1 x 10^6 CFU/mL using the McFarland No: 0.5 standard. Stock

solutions of the samples and standard antifungal agents ketoconazole and griseofulvin were prepared as above. From these *Candida* suspensions, 100 µL was then added to each well. The last row containing only the serial dilutions of antifungal agent without microorganisms was used as a negative control. Sterile distilled water and inoculated medium served as a positive control in the last column on each plate. After incubation at 37°C for 24-36 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC, µg/mL) and a tetrazolium salt (TTC, Sigma) was added for confirmation (Table 3).

TLC-bioautographic DPPH assay: The free radical scavening activity of the samples was evaluated using the method of Burits and Bucar [28]. The essential oils and the standard, ascorbic acid, were applied at 2 mg/mL concentration (MeOH), in two duplicates, onto silica gel coated GF_{254} aluminum plates (Merck, Darmstadt, Germany). *n*-Hexane:ethyl acetate (5:2, v/v) was used as the development solvent. After development, one plate was visualized by UV and anisaldehyde/H_2SO_4 reagent, followed by heating, and the second one with 0.2% DPPH in methanol. The second plate, having a purple background with yellow spots for the active components, such as ascorbic acid as positive control, was compared.

Acknowledgments - The authors would like to thank NRRL for supplying some of the microbial strains. The authors also wish to thank Dr Galip Akaydın (Hacettepe University, Department of Biology Education, Ankara, Turkey) for authentication of the plant material.

References

[1] Mill RR. (**1982**) *Lavandula* L. In *Flora of Turkey and East Aegean Islands.* Davis PH. (Ed). University Press, Edinburgh, Vol. ***7***, 76-79.

[2] Baytop T. (**1999**) *Therapy with Medicinal Plants in Turkey (past and present).* 2nd ed, Nobel Tıp Kitapevleri, Istanbul.

[3] Emre G, Tuzlacı E. (**2006**) *Plants used in folk medicine in Ezine (Canakkale). In XV*th *International Symposium on Plant Originated Crude Drugs (Proceedings book)* Sezik E, Yeşilada E, Orhan D (Eds), Ankara, 270-278.

[4] Lis-Balchin M. (**2002**) *Lavender: The genus Lavandula*, Taylor and Francis, London.

[5] Cavanagh HM, Wilkinson JM. (**2002**) Biological activities of lavender essential oil. *Phytotherapy Research*, ***16***, 301-308; (b) Surburg H, Panten J. (**2006**) *Common Fragrance and Flavor Materials Preparation, Properties and Uses*. Wiley-VCH, Weinheim.

[6] (a) Barrero AF, Herrador MM, Arteaga P, Arteaga JF, Burillo J. (**2008**) Cultivars of *Lavandula lanata* Boiss., a good source of lavandulol. *Natural Product Communications*, ***3***, 379-381; (b) Lane WA, Biswas K, Mahmoud SS. (**2008**) Composition of essential oil from *Lavandula angustifolia* and *L. intermedia* varieties grown in British Columbia, Canada. *Natural Product Communications*, ***3***, 1361-1366.

[7] Kokkalou E. (**1988**) The constituents of the essential oil from *Lavandula stoechas* growing wild in Greece. *Planta Medica*, ***54***, 58-59.

[8] Skoula M, Abidi C, Kokkalou E. (**1996**) Essential oil variation of *Lavandula stoechas* L. ssp. *stoechas* growing wild in Crete (Greece). *Biochemical Systematics and Ecology*, ***24***, 255-260.

[9] Guyot-Declerck C, Renson S, Bouseta A, Collin S. (**2002**) Floral quality and discrimination of *Lavandula stoechas*, *Lavandula angustifolia*, and *Lavandula angustifolia*×*latifolia* honeys. *Food Chemistry*, ***79***, 453-459.

[10] Zrira S, Benjilali B. (**2003**) The constituents of the oils of *Lavandula stoechas* L. ssp. *atlantica* Br.-Bl. and *L. stoechas* ssp. *stoechas* from Morocco. *Journal of Essential Oil Research*, ***15***, 68-69.

[11] Dadalioglu I, Evrendilek GA. (**2004**) Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas* L.) and fennel (*Foeniculum vulgare*) on common foodborne pathogens. *Journal of Agricultural and Food Chemistry*, ***52***, 8255-8260.

[12] Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. (**2006**) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. *stoechas* essential oils from stem/leaves and flowers. *Journal of Agricultural and Food Chemistry*, ***54***, 4364-4370.

[13] Goren CA, Topcu G, Bilsel G, Bilsel M, Aydogmus Z. Pezzuto M. (**2002**) The chemical constituents and biological activity of essential oil of *Lavandula stoechas* ssp. *stoechas*. *Zeitschrift für Naturforschung*, ***57c***, 797-800.

[14] Moon T, Wilkinson JM, Cavanagh HM. (**2006**) Antiparasitic activity of two *Lavandula* essential oils against *Giardia duodenalis, Trichomonas vaginalis* and *Hexamita inflata*. *Parasitology Research*, ***99***, 722-728.

[15] Inouye S, Nishiyama Y, Uchida K, Hasumi Y, Yamaguchi H, Abe S. (**2006**) The vapor activity of oregano, perilla, tea tree, lavender, clove, and geranium oils against a *Trichophyton mentagrophytes* in a closed box. *Journal of Infection and Chemotherapy*, ***12***, 349-354.

[16] Soylu EM, Soylu S, Kurt S. (**2006**) Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*. *Mycopathologia*, ***161***, 119-128.

[17] Guillén MD, Cabo N. (**1996**) Characterisation of the essential oils of some cultivated aromatic plants of industrial interest. *Journal of the Science of Food and Agriculture*, ***70***, 359-363.

[18] D'Auria FD, Tecca M, Strippoli V, Salvatore G, Battinelli L, Mazzanti G. (**2005**) Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form. *Medical Mycology*, ***43***, 391-396.

[19] Dalleau S, Cateau E, Bergès T, Berjeaud JM, Imbert C. (**2008**) *In vitro* activity of terpenes against Candida biofilms. *International Journal of Antimicrobial Agents*, ***31***, 572-576.

[20] Kalemba D, Kunicka A. (**2003**) Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, ***10***, 813-829.

[21] Bakkali F, Averbeck S, Averbeck D, Idaomar M. (2008) Biological effects of essential oils-a review. *Food and Chemical Toxicology*, ***46***, 446-475.

[22] McLafferty FW, Stauffer DB. (**1989**) *The Wiley/NBS Registry of Mass Spectral Data*. John Wiley and Sons, New York.

[23] Joulain D, König WA, Hochmuth DH. (**2001**) *Terpenoids and related constituents of essential oils*. Library of Mass Finder 2.1. Hamburg.

[24] Joulain D, Konig WA. (**1998**) *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*. Hamburg, E.B.-Verlag.

[25] Jennings WG, Shibamoto T. (**1980**) *Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC*. New York, Academic Press.

[26] Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. (**1997**) Color Atlas and Textbook of Diagnostic Microbiology. Lippincott-Raven Publ., Philadelphia, p.785.

[27] Iscan G, Kirimer N, Kürkçüoğlu M, Başer KHC, Demirci F. (**2002**) Antimicrobial screening of *Mentha piperita* essential oils. *Journal of Agricultural and Food Chemistry*, ***50***, 3943-3946.

[28] Burits M, Bucar F. (**2000**) Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research*, ***16***, 323–328.