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Original Article

Biological investigations of antioxidant-antimicrobial properties and chemical composition of essential oil from *Lavandula multifida*

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Abstract
Essential oils obtained from plants have a number of potential uses owing to their notable antimicrobial and antioxidant properties. The genus *Lavandula* is widely distributed in the archipelagoes of the Atlantic Ocean and the Mediterranean. Essential oils and plant extracts derived from the genus Lavandula have been used therapeutically for centuries. In the present study, the essential oil of *Lavandula multifida* collected from the Tafilalet region in South-East Morocco was obtained by hydrodistillation method from leaves. The gas chromatography-mass spectrometry analysis of the oil identified a total of 34 compounds (96.5%); the major compounds were carvacrol (66.2%), spathulenol (4.9%), p-cymene-8-ol (4.2%), caryophyllene oxide (2.7%) and terpinolene (2.6%). The essential oil exhibited significant antimicrobial activity against tested representative human disease bacteria, *i.e. Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and antifungal activity against phytopathogenic strains such as *Alternaria sp*, *Pencillium expansum*, *Rhizopus stolonifer*, *Botrytis cinerea*. Furthermore, the *Lavandula multifida* essential oil showed an important antioxidant property via radical scavenging ability, and antioxidant activity determined by 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay and β-carotene bleaching test.

INTRODUCTION

Essential oils obtained from plants have a number of potential uses, including food additive, as preservative from spoilage, and pharmaceuticals, owing to their notable antimicrobial and antioxidant properties [1-2]. The above is in line with the increasing demand from consumers to limit the use of synthetic additives, since those chemicals have sometimes demonstrated to be a source of potential health hazard, owing to toxic impurities deriving from synthetic pathway. Essential oils are much more acceptable by the consumers than synthetic substances, and they do not cause bacterial resistance, mainly because they are constituted by a wide spectrum of compounds.

The genus *Lavandula* (common name: Lavender; family: Labiatae or Lamiaceae), consisting of about 28 species, is widely distributed in the archipelagoes of the Atlantic Ocean and the Mediterranean [3]. Essential oils and plant extracts derived from the genus *Lavandula* have been used therapeutically for centuries and there are some reports on the biological effects [4-5]. In addition, the essential oil of Lavender is important for the perfume, cosmetic, flavoring, and pharmaceutical industries [6].

Lavandula multifida is a member of the Pterostoechas section (Lamiaceae) mainly distributed in pre-saharan zones, growing on the rocky outcrops and more or less drained calcareous soils, the borders of rivers of temporary drainage, between 800-2000 m altitude [7]. Its leaves and stems are used in the Moroccan folk medicine to prepare decoctions against rheumatism, chill and as digestive system benefit agent [8].

In the present study, the antimicrobial and antioxidant capacities of the essential oil of *L.multifida* were

investigated. The antimicrobial activities were determined by using agar disc diffusion and broth dilution methods. The antioxidant activities were determined by using three complementary *in vitro* assays, *i.e.* inhibition of DPPH radical and the ferric reducing power, and β-carotene bleaching assay. The chemical composition of the essential oil was studied by using gas chromatography/mass spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Plant material

The aerial part of *L.multifida* was collected in Errachidia (Morocco) during the flowering period (April-June). A duplicate specimen is held at the Faculty of Sciences & Techniques, Errachidia. The dried plant material is stored in the laboratory at room temperature (25°C) and in the shade before extraction.

Hydrodistillation apparatus and procedure

The essential oil of the aerial part of *L.multifida* was extracted by hydrodistillation. The essential oil obtained was dried under anhydrous sodium sulfate and stored at 4°C in the dark before analysis.

Essential oil analysis

Components were identified on the basis of GC-MS library and confirmed by calculation of retention indices from GC-FID (flame ionization detector). GC-MS was performed on an Agilent 6890N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-INNOWax capillary column (50 m x 0.2 mm; film thickness 0.5 µm); carrier gas: helium 1.6 ml/min, split 1/100; injector temperature: 280°C; oven temperature: 60°C (2 min isothermal), then 3°C/min to 180°C, then 8°C/min to 245°C (10 min isothermal). Agilent 5973N MS; source temperature: 230°C; mass range: 35 to 350 amu; scan speed: 1 scan/sec. GC-FID: Agilent HP 6850 fast GC equipped with a DB-Wax capillary column (20 m x 0.1 mm, 0.2 µm); carrier gas hydrogen at 0.7 ml/min, split 60 ml/min; injector temperature: 275°C; detector temperature: 275°C; oven temperature: 60°C (2 min isothermal), then 12°C/min to 248°C (5 min isothermal). Detected compounds concentrations are relative percentages (ISO 7609) with a threshold of 0.05%.

Antibacterial activity

Microorganisms

The antimicrobial activity was evaluated by paper disc diffusion and dilution methods against four selected species: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus cereus* (ATCC 29213). Microorganisms were obtained from the culture collection of the Institute of Hygiene, Rabat, Morocco.

Diffusion method

The qualitative antimicrobial essay of the volatile fraction of *L.multifida* was carried out by the disc diffusion method [9]. It was performed using culture growth at 37°C for 18 h and adjusted to approximately 10^8 colony forming units per milliliter (CFU/ml). The culture medium used for the bacteria was Mueller Hinton agar (MHA). Five hundred microliters of the inoculums were spread over plates containing MHA and a Whatman paper disc (6 mm) impregnated with 5, 10 and 15 µl of the volatile fraction was placed on the surface of the media. The plates were left 30 min at room temperature to allow the diffusion of the oil. They were incubated 24 h at 37°C for the bacteria. After incubation period, the inhibition zone obtained around the disc was measured. Two controls were also included in the test, the first was involving the presence of microorganisms without test material and the second was standard antibiotic: Ampicillin used to control the sensitivity of the tested bacteria. The experiments were run in triplicate, and the developing inhibition zones were compared with those of reference discs.

Dilution method

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of tested volatile fractions were determined using the Mueller Hinton broth (MHB) dilution method [9]. All tests were performed in MHB supplemented with Tween 80 (1%) [10]. Bacterial strains were cultured overnight in MHB at 37°C. Tubes of MHB containing various concentrations of volatile fractions were inoculated with 10 µl of 10^8 CFU/ml of standardized microorganism's suspensions. Control tubes without tested samples were essayed simultaneously. All samples were tested in triplicate. The MIC was defined as the lowest concentration preventing visible growth [11-12].

Antifungal activity

Fungal strains

Four agricultural pathogenic fungi were obtained from the culture collection at Faculty of Sciences & Techniques, Errachidia. The different fungi were cultivated on potato dextrose agar (PDA) and were stored at 4°C. The fungal species used in the experiments are *Alternaria sp, Pencillium expansum, Rhizopus stolonifer, Botrytis cinerea.*

Antifungal activity tests

The antifungal activity is evaluated as described by Chang *et al* [13]. The quantities of essential oil (100 and 200 µl) are added to 20 ml of PDA sterile. The mixtures were cast on Petri dish. Afterwards, the discs of mycelium each mold 5 mm in diameter cut the device from a culture of 7 days are inoculated in the center of the boxes and then incubated at 25 ± 2°C for 3 days for *Rhizopus* and 7 days for others.

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Measuring diameters of hyphal growth relative to the control results in applying the following formula:

$$\textbf{Antifungal index (AI)} = [1 - (D_a / D_b)] \times 100$$

-D_a: diameter growth dish treated (mm)

-D_b: diameter growth control (mm)

Antioxidant activity

The antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and β-carotene bleaching methods. Data collected for each assay was an average of three experiments.

Free radical scavenging assay

The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 517 nm with a deep violet color. The absorption vanishes and the resulting discoloration is stoichiometric with respect to degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant [14]. Fifty microliters of the extracted oil dilutions in ethanol was added to 1 ml of 100 µM solution of DPPH. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm (Jenway 6000 UV/Vis; Staffordshire, UK). Inhibition of DPPH free radical in percent (I %) was calculated as follows:

$$\textbf{I (\%)} = (1 - A_{sample} / A_{blank}) \times 100$$

-A_{blank}: absorbance of the control reaction (containing all reagents except the test compound)

-A_{sample}: absorbance of the test compound

Extract concentration providing 50% inhibition ((IC50) was calculated from the graph plotting inhibition percentage against extract concentration. All tests were carried out in triplicate. Butylated hydroxytoluene (BHT) was used as positive control.

Beta-carotene bleaching assay

The β-carotene method was carried out according to Shahidi *et al* [15]. Two milliliters of β-carotene solution (0.2 mg/ml in chloroform) were pipetted into a round-bottomed flask containing 20 µl linoleic acid and 200 µl Tween 20. The mixture was then evaporated at 40°C for 10 min to remove the solvent, the addition of distilled water (100 ml) followed immediately. After agitating the mixture, 1.5 ml aliquot of the resulting emulsion was transferred into test tubes containing 150 µl of extract and the absorbance was measured at 470 nm against a blank consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored by measuring absorbance at 470 nm after 2 h using spectrophotometry. The same procedure was repeated with the synthetic antioxidant BHT as positive control. The antioxidant capacity (AA%) of the solutions tested was calculated via the following formula:

$$\textbf{AA\%} = \frac{\textbf{β-carotene content after 2 h assay}}{\textbf{initial β-carotene content}} \times 100$$

Results are presented as mean ± SD of three independent tests. All tests were carried out in an identical condition.

RESULTS

Chemical composition of the essential oil

The essential oil was extracted by the hydrodistillation of the dried parts of *L.multifida* from Errachidia region, Morocco and were analyzed by GC-MS. The oil yields were calculated on a dry weight basis as 0.7% (w/w). The *L.multifida* essential oil composition is summarized in Table 1. Briefly, a total of 34 compounds, amounting 96.5% of the oils, were identified; the major components were carvacrol (66.2%), spathulenol (4.9%), p-cymene-8-ol (4.2%), caryophyllene oxide (2.7%) and terpinolene (2.6%).

Antimicrobial activity

The antimicrobial activities of *L.multifida* were evaluated by a paper disc diffusion method against tested bacteria. The result showed that the essential oil is active against the different strains of the microorganism's assayed (Table 2).

The essential oil of *L.multifida* exhibited the strongest activity against *Bacillus cereus*, *Staphylococcus aureus Escherichia coli* with MIC values between 0.039 and 0.078 mg/ml. On the other hand, the MBC values resulted between 0.078 and 0.156 mg/ml.

The antifungal indices presented are a clear demonstration of the excellent antifungal property of the oil against the four agricultural pathogenic fungal strains tested (see Table 3). The growth of *Rhizopus stolonifer* and *Alternaria sp* was completely inhibited by the concentration of 100 µg/ml.

Antioxidant activities

Comparison of the DPPH scavenging activity of the *L.multifida* essential oil (16.83 ± 1.55 µg/ml) and those expressed by BHT (7.73 ± 0.11) showed that the essential oil exhibited weaker antioxidant effects than BHT. The antioxidant effect of the oil was about 3 times lower than that of the synthetic antioxidant BHT.

The potential of *L.multifida* to inhibit lipid peroxidation was evaluated using the β-carotene/linoleic acid bleaching test by measuring the antioxidant capacity (AA%) with values of 78.41 ± 1.22% and 86.2 ± 0.5% obtained for the oil and the positive control BHT, respectively, at the same concentration of 100 µg/ml (Table 4).

Table 1. Chemical composition of essential oil from *Lavandula multifida*

Peak number	Retention time (min)	Compounds (in order of elution)	Composition (%)
1	6.05	α-Pinene	0.8
2	8.49	Camphene	0.2
3	9.7	β-Pinene	0.4
4	9.87	Myrcene	0.5
5	10.56	Octanol	1.4
6	11.39	α-Phellandrene	0.3
7	11.68	Garene 2	0.6
8	11.9	p-Cymene	1.0
9	12.24	1,8-Cineole	1.2
10	12.93	β-Ocimene	1.2
11	13.23	p-Cymenene	0.3
12	13.43	Terpinolene	2.6
13	16.8	Linalool	0.2
14	17.15	α-Thujone	0.8
15	17.48	Camphor	1.8
16	18.99	p-Cymen-8-ol	4.2
17	19.53	α-Terpineol	0.6
18	19.75	Carvacrol	66.2
19	21.54	Eugenol	0.2
20	22.97	Pipertenone oxide	0.4
21	23.8	trans-Caryophyllene	0.9
22	24.12	α-Sesquisabinene	0.5
23	24.21	Spathulenol	4.9
24	25.3	Caryophyllene oxide	2.7
25	26.35	β-Eudesmol	2.6
		Total	**96.5%**

Table 2. Antibacterial activity of *Lavandula multifida* essential oil

Microorganisms	Inhibition zone diameter				Essential oil (mg/ml)		Ampicilin (µg/ml)	
	Essential oil (µl/disc)			Ampicilin (µg/disc)	MIC	MBC	MIC	MBC
	5	10	15					
Gram-negative bacteria								
E.coli ATCC 25922	17.2 ± 0.6	22.7 ± 0.5	26.3 ± 1.2	27.7 ± 1.5	0.078	0.156	1.95	> 3.9
P.aeruginosa ATCC 27853	7.5 ± 0.6	8.1 ± 1.5	11.3 ± 0.4	21 ± 1.2	0.156	1.25	3.9	7.81
Gram-positive bacteria								
S.aureus ATCC 25923	28 ± 1.5	30.7 ± 0.4	37.4 ± 0	32.7 ± 0.6	0.039	0.078	6.25	15.62
B.cerus ATCC 29213	30.9 ± 0.4	40.5 ± 0	44.7 ± 1.5	24 ± 1	0.039	0.078	3.9	7.81

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration. Negative control performed at 0 µl essential oil did not show any inhibitory effect against the tested bacteria.

Table 3. Antifungal activity of *Lavandula multifida* essential oil

Fungal species	Oil growth (mm)	Antifungal index (%)	Control growth (mm)
Alternaria sp	4 ± 0.2	93.72	63.7± 7.2
Penicillium expansum	29.5 ± 2.3	53.84	63.9 ± 7.3
Rhizopus stolonifer	2.9 ± 1.4	92.32	37.8 ± 3.7
Botrytis cinerea	24.5 ± 0.8	44.7	44.3 ± 1.2

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Table 4. Antioxidant capacity of *Lavandula multifida* essential oil

	Lavandula multifida	Butylated hydroxytoluene
DPPH (IC_{50} µg/ml DPPH solution)	16.83 ± 1.55	7.73 ± 1.62
Inhibition in linoleic acid system (%)	78.41 ± 1.22	86.2 ± 0.5

Values are means ± standard deviation of three separate experiments

DISCUSSION

The aim of this study was to evaluate the antioxidant activity by two complementary tests (scavenging of DPPH free radicals and the β-carotene bleaching assay) and the antimicrobial activities of the *Lavandula multifida* essential oils against some pathogenic bacteria and phytopathogenic fungi. The essential oil was characterized by large amounts of monoterpenes (82%) composed of hydrocarbon monoterpenes (8.2%) and oxygenated monoterpenes (73.8%). Sesquiterpenes represented only 8.8% of oil, most of which are oxygenated (7.7%). The first investigation conducted by El Rhaffari *et al* [7] reported that the major components are thymol (32%), carvacrol (27.7%) and p-cymene (15.72%). This difference in the composition is explained by the difference in study techniques and the simultaneous bioconversion of p-cymene and carvacrol in its isomer, thymol [5]; the hydroxylation of the ring of p-cymene lead to occur in the side chain it may lead to p-cymen-8-ol (Fig.1).

The antimicrobial activity of this *Lavandula* oil is mostly attributed to the propriety of the major component (carvacrol), which exhibit significant bactericidal activity [16-17]. It is a monoterpenic phenol which has the closed chemical formula as $C_{10}H_{14}O$, which are synonyms to 2-hydroxy-p-cymene, p-cymen-2-ol and iso-thymol. The other minor compounds (sphathulenol, β-ocimene, γ-terpinene, β-eudesmol, 1,8-cineole and camphene) are also known to have efficient antimicrobial properties [12, 18, 19].

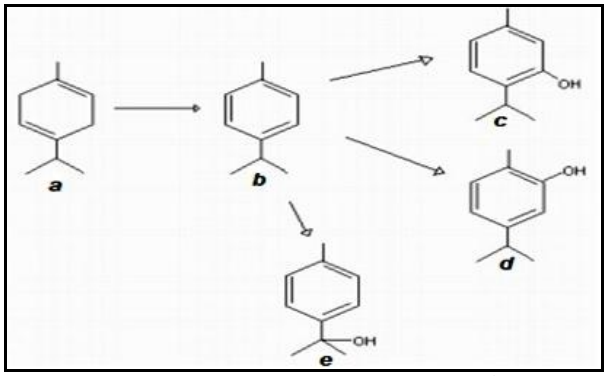

Figure 1. Relationship between the components of *Lavandula multifida* essential oil: (a) y-terpinene, (b) p-cymene, (c) thymol, (d) carvacrol., (e) p-cymen-S-ol.

These results suggest that the *L.multifida oils* would probably be a good therapeutic agent against these bacteria. Some investigations on the composition and biological activities of the *Origanum acutidens* essential oils demonstrated that carvacrol and thymol are the active substances [20]. Other substances such as 1,8-cineole, p-cymene and terpinen-4-ol were also reported to be antifungal [21-22].

The DPPH scavenging ability of this oil can be attributed to the presence of phenolic constituents and to the free radical scavenging activity of some volatile oils [23]. The potential of *L.multifida* to inhibit lipid peroxidation was moderately lower than that of BHT and was attributed to the presence of appreciable amount of antioxidant compounds such as 1,8 cineole, α-pinene and carvacrol and to the presence of phenolic compounds [24, 25].

The results showed that, this oil reveal a relative high antioxidant capacity and inhibit strongly the growth of Gram-positive bacteria at low concentrations. Thus leaves of *Lavandula multifida* could be used as a source of natural antioxidants. Further some future investigations may be helpful in the use of the specific phenolic constituents of the essential oil as food preservatives, as aromatic foods impart flavors and as natural antioxidants to reduce oxidative stress in human beings.

COMPETING INTERESTS

None to declare

REFERENCES

1. Sellam K, Ramchoun M, Alem C, Khallouki F, El Moualij B, El Rhaffari L. Chemical composition, antioxidant and antimicrobial activities of essential oil of *Warionia saharae* from oases of Morocco. In: Salih B (ed) Gas Chromatography – Biochemicals, Narcotics and Essential Oils, Intech Open, pp 213-220, 2012.

2. Naeini AR, Nazeri M, Shokri H. Antifungal activity of *Zataria multiflora*, *Pelargonium graveolens* and *Cuminum cyminum* essential oils towards three species of *Malassezia* isolated from patients with pityriasis versicolor. J Med Mycol 2011; 21:87-91.

3. Cong Y, Abulizi P, Zhi L, Wang X, Mirensha. Chemical composition of the essential oil of *Lavandula angustifolia* from Xinjiang, China. Chem Nat Comp 2008; 44:810.

4. Cavanagh HM, Wilkinson JM. Biological activities of Lavander essential oil. Phytother Res 2002; 16:301-8.

5. Moon T, Wilkinson JM, Cavanagh HM. Antibacterial activity of essential oils, hydrosols and plant extracts from Australian grown *Lavandula* spp. Int J Aromather 2006; 16:9-14.

6. Pala-Paul J, Brophy JJ, Goldsack RJ, Fontaniella B. Analysis of the volatile components of *Lavandula canariensis* (L.) Mill., a Canary Islands endemic species, growing in Australia. Biochem System Ecol 2004; 32:55-62.

7. El Rhaffari L, Ismaili-Alaoui M, Belkamel J, Jeannot V. Chemical composition and antibacterial properties of the essential oil of *Lavandula multifida* L. Int J Essent Oil Therap 2007; 1:122-5.

8. El-Hilaly J, Hmammouchi M, Lyoussi B. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). J Ethnopharmacol 2003; 86:149-58.

9. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing, 6th edition, Approved Standards, M2-A6, Wayne, PA, USA, 2003.

10. Hichri F, Ben Jannet H, Cheriaa J, Jegham S, Mighri Z. Antibacterial activities of a few prepared derivatives of oleanolic acid and of other natural triterpenic compounds. Comptes Rendus Chimie 2003; 6:473-83.

11. May J, Chan CH, King A, Williams L, French GL. Time-kill studies of tea tree oils on clinical solates. J Antimicrobial Chemother 2000; 45:639-43.

12. Burt S. Essential oils: their antibacterial properties and potential applications in foods. International J Food Microbiol 2004; 94:223-53.

13. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10:178-82.

14. Nilsson J, Pillai D, Onning G, Persson C, Nilsson A, Akesson B. Comparison of the 2,2'-azinobis-3-ethylbenzotiazo-line-6-sulfonic acid (ABTS) and ferric reducing anti-oxidant power (FRAP) methods to asses the total antioxidant capacity in extracts of fruit and vegetables. Mol Nutr Food Res 2005; 49:239-46.

15. Shahidi F, Chavan UD, Naczk M, Amarowicz R. Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). J Agric Food Chem 2001; 49:926-33.

16. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mozzanti G, Bisignano G. Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 2005; 49:2474-8.

17. Friedman M, Henika PR, Mandrel RE. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. J Food Prot 2002; 65:1545-60.

18. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totte J, Pieters L, Vlietinck AJ. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 2002; 2:213-20.

19. Nada B, Mirjana S, Valerija D. Phytochemical composition and antimicrobial activity of *Satureja montana* L. and *Satureja cuneifolia* Ten. essential oils. Acta Bot Croat 2005; 64:313-22.

20. Kotan R, Kordali S, Cakir A. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. Z Naturforsch C 2007; 62:507-13.

21. Gallori S, Bilia AR, Mulinacci N, Biccchi C, Rubiolo P, Vincieri FF. Identification of volatile constituents of Tambourissa leptophylla. Planta Med 2001; 67:290-2.

22. Krauze-Baranowska M, Mardarowic M, Wiwart M, Poblocka L, Dynowska M. Antifungal property of the essential oils from some species of the genus Pinus. Z. Naturforsch C 2002; 57:478-82.

23. Galego L, Almeida V, Gonçalves V, Costa M, Monteiro I, Matos F, Miguel G. Antioxidant activity of the essential oils of *Thymbra capitata*, *Origanum vulgare*, *Thymus mastichina* and *Calamintha baetica*. Acta Hortic 2008; 765:325-34.

24. Albano SM, Lima AS, Miguel MG, Pedro LG, Barroso JG, Figueiredo AC. Antioxidant, anti-5-lipoxygenase and antiacetylcholinesterase activities of essential oils and decoction waters of some aromatic plants. Rec Nat Prod 2012; 6:35-48.

25. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 2007; 21:308-23.

 DOI 10.5455/oams.290513.or.044