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Research Article

ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *LAVANDULA ANGUSTIFOLIA* FROM IRAQ

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ABSTRACT
The essential oil of *Lavandula angustifolia* Mill. growing spontaneously in Iraq was investigated by GC and GC/MS for the first time. The oil was extracted from the flowers by hydro-distillation. Thirty-four components amounting to 98.91 % of the oil were identified. The major component being linalool (24.63 %). The other significant constituents were camphor (13.58 %), linalyl acetate (8.89 %), *(Z)*-β-ocimene (7.59 %), 1,8-cineole (7.14 %), borneol (6.41 %), *(E)*-β-ocimene (4.76 %), hotrienol (4.42 %), hexyl butyrate (2.96 %), α-bisabolol (1.13 %) and caryophyllene oxide (1.02 %). The strong antioxidant activity of *L. angustifolia* oil was also examined using the stable 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) free radical scavenging method. Antioxidant activity of the oil was expressed as percentage of DPPH radical inhibition and IC_{50} values (µg/ml). Values of percentage inhibition ranged from 3.28 to 88.91% for 7.81 µg/ml and 1000 µg/ml, respectively with an IC_{50} value of 216 µg/ml for oil. The results suggest the use of lavender oil as effective natural antioxidants.
Keywords: *Lavandula angustifolia,* volatile oil, GC-MS, linalool, DPPH.

INTRODUCTION

Lavandula angustifolia (syn. *L. officinalis* Chaix) is an important perennial shrub of family Labiatae (Lamiaceae). The lavender is widely distributed in the Mediterranean region and cultivated in France, Spain and Italy. Mainly two species, *L. angustifolia* and *L. stoechas* and their subspecies and hybrid forms grow wildly or are cultivated[1]. *L. angustifolia* is reported to have neuroprotective[2], antibacterial[3], antimutagenic[4] and antifungal[5] activities. Lavender oil is known for its excellent fragrance and is comprehensively used in the perfumery, flavour and cosmetic industries as well as medicinal purpose[6]. Lavender oil is also reported to be an effective antifungal agent against *Aspergillus nidulans* and *Trichophyton mentagrophytes*[7]. The essential oil compositions of lavender grown in different countries have been investigated. Oil from India has become more significant competitor with historical sources of lavender oil due to the favorable climatic situations for commercial cultivation in the hilly tracks of Northern India[8,9]. The aim of this study was to evaluate the chemical composition of essential oil of *L. angustifolia* used for the production of lavender oil in Iraq by GC and GC-MS; and its antioxidant potential for the application of the essential oil in food preservation and in medicine.

MATERIAL AND METHODS

Plant Material

Flowers of *L. angustifolia* were collected from the Shaqllawa, Iraq in the month of March-April, 2012. The sample was identified and a voucher specimen of the plant was submitted to the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

Chemicals

BHA (3-tert-butyl-4-hydroxyanisole), BHT (butylated hydroxy toulene), Vitamin C and DPPH (2,2-dyphenyl-1-picrylhydrazyl) were obtained from SIGMA CHEMICALS CO., ST. LOUIS, MO, USA. All other solvents and chemicals were of analytical grade.

Isolation of volatile oil

Samples (200 gm) were hydro-distilled for six hours with Clevenger apparatus. The volatile oil was collected in the graduated tube. The collected volatile oil was dried over anhydrous sodium sulphate and stored at 4 ^{0}C in the dark. The yield of volatile oil obtained was 1.5 *% v/w.*

GC-FID Analysis

GC was carried out on Shimadzu 2010 gas chromatograph (Japan) equipped with a flame ionization detector (FID) and AB-Innowax 7031428 WCOT fused capillary column (60 m x 0.25 mm x 0.25 µm). The injector and detector temperatures were maintained at 250 and 270 ºC, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 ml/min with column pressure of 101.1 kPa. The sample (0.2 µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60-230 ºC at a rate of 3 ºC/min and then held at 230 ºC for 9 min, with a total run time of 50 min. Percentage of the constituents were calculated by electronic integration of FID peak areas.

GC-MS Analysis

The analysis of the volatile constituents were run on a Shimadzu QP-2010 GC-MS system equipped with AB-Innowax 7031428 WCOT column (60 m x 0.25 mm x 0.25 µm) directly coupled to the MS. The carrier gas was helium with a flow rate of 1.21 ml/min with column pressure of 81.7 kPa. Oven temperature was programmed as 50 ºC for 1 min and subsequently held isothermal for 2 min. The injector and detector temperatures were 250 ºC and 280 ºC, respectively. The sample (1 µl) was injected into column with a split ratio of 1:100. Recording was performed at 70 eV, scan time 1.5 s; mass range 40-750 amu. Software adopted to handle mass spectra and chromatograph was Chem station.

Table 1: Essential oil constituents of *Lavandula angustifolia* Mill.

S. No.	Compound	RI	R_t	% Area	EP 5[14], %
1.	*n*-Hexanol	878	2.365	0.45	
2.	3-Nonyne	896	2.644	0.11	
3.	2,7-Dimethyl oxepine	930	3.161	0.52	
4.	Camphene	950	3.450	0.36	
5.	3-Octanone	983	3.954	0.37	0-2
6.	Myrcene	989	4.042	0.74	
7.	*cis*-Linalool oxide	993	4.105	0.95	
8.	Hexyl acetate	1009	4.437	0.96	
9.	1,8-Cineole	1032	4.988	7.14	< 2.5
10.	*γ*-Valerolactone	1040	5.166	0.72	
11.	*(Z) β*-Ocimene	1043	5.253	7.59	2-6
12.	*(E) β*-Ocimene	1074	5.997	4.76	4-10
13.	Linalool	1107	6.828	24.63	20-45
14.	Hotrienol	1110	6.917	4.42	
15.	3-Octyl acetate	1121	7.297	0.28	
16.	Camphor	1149	8.188	13.58	< 1.2
17.	Nerol oxide	1153	8.322	0.74	
18.	Borneol	1170	8.856	6.41	
19.	*trans*-Linalool oxide	1175	9.016	0.57	
20.	Hexyl butyrate	1192	9.542	2.96	
21.	2,6-Dimethyl-3,5,7-octatriene-2-ol	1202	9.881	5.39	
22.	Isobornyl formate	1228	10.835	0.59	
23.	Hexyl-2-methyl butyrate	1234	11.084	0.61	
24.	2-Cyclohexene-1-one-2-methyl-5-(1-methyl ethane)	1244	11.428	0.11	
25.	Linalyl acetate	1256	11.888	8.89	25-46
26.	Lavandulyl acetate	1290	13.129	0.27	> 0.2
27.	Hexyl tiglate	1329	14.638	0.69	
28.	8-Acetoxy linalool	1376	16.495	0.18	
29.	Geranyl acetate	1383	16.748	0.32	
30.	*trans*-Verbenol acetate	1414	17.970	0.58	
31.	Lavandulyl isovalerate	1507	21.589	0.13	
32.	Caryophyllene oxide	1580	24.341	1.02	
33.	*α*-Bisabolol oxide B	1651	26.977	0.13	
34.	*α*-Bisabolol	1682	28.098	1.13	
Total identified, %				98.91	

RI: Retention Index, **R_t:** Retention time on column, *compounds less than 0.1 % was considered minor. [14]European Pharmacopoeia 5.0

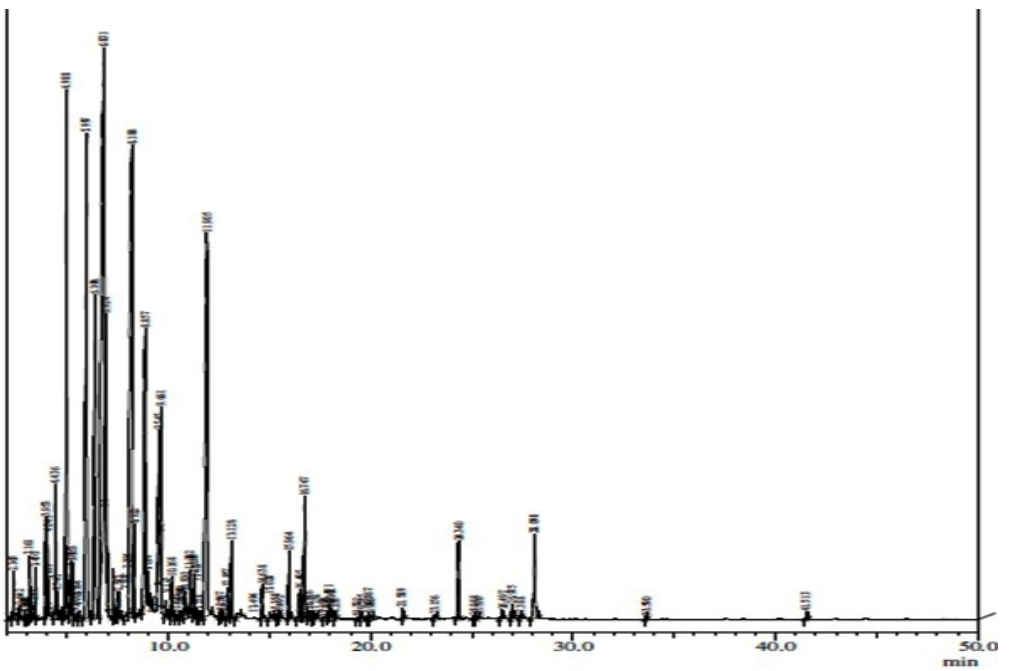

Figure 1. GC-MS chromatogram of essential oil of *Lavandula angustifolia*

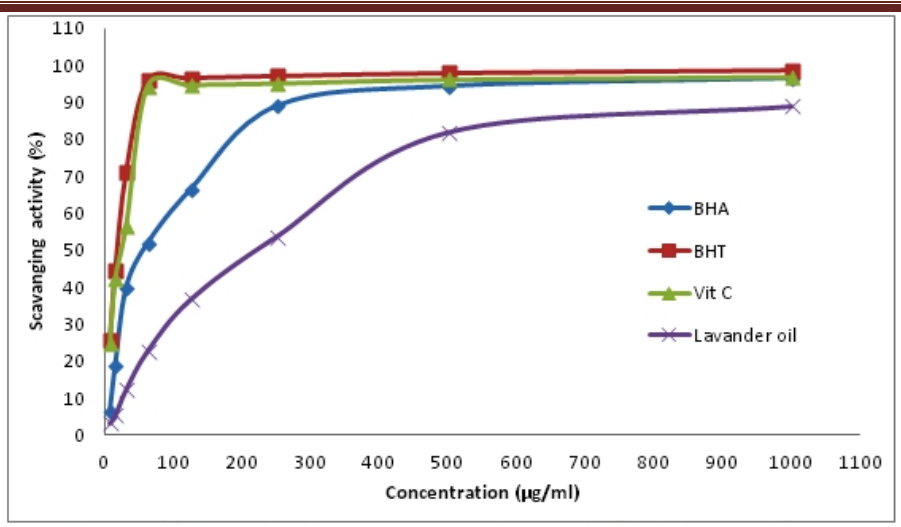

Figure 2. Antioxidant (DPPH scavenging) activity of standard BHA, BHT, Vitamin C and volatile oil of *L. angustifolia*

Evaluation of antioxidant activity

The ability of a substance to scavenge DPPH free radicals was assessed by the standard method[10], adopted with suitable modifications[11]. The stock solution of oil was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/ml were prepared by serial dilution method. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of DPPH (1 mg/ml). After 30 min incubation in darkness at room temperature (23 ^{o}C), the absorbance was recorded at 517 nm. Control sample contained all the reagents except the oil. Percentage inhibition was calculated using equation given below:

$$\% \, Inhibition = \frac{Aco - At}{Aco} \times 100$$

where, A_{CO} is absorbance of the control and A_t is absorbance of the samples.

[IC_{50} values were estimated from the % inhibition versus concentration plot using a non-linear regression algorithm].

Identification of volatile oils

The individual peaks/constituents were identified by GC by comparison of their retention indices (R.I.) either with retention indices of linear compounds available or published literature[9,12] reports. Further identification was made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries. Retention indices of the components were determined relative to the retention time of a series of *n*-alkanes relative to C_9-C_{20} on HPS and HP-20M columns. The relative amount of the individual components was calculated from the peak area without applying an FID response factor correction.

RESULTS AND DISCUSSION

Lavender oil is used traditionally for the treatment of various complaints from ancient timefor its excellent aroma worldwide. It is extensively used in the perfumery, flavor, cosmetic industries and as sedative, carminative, antidepressive, antiinflammatory and antibacterial potential. The essential oil composition of the fresh flowers of *L. angustifolia* was determined by GC and GC-MS analysis. Chromatogram is shown in Fig. 1 and compounds identified in the essential oil are given in Table 1. A total of 34 compounds were identified that constitute 98.91 % of the total volatiles. The major constituents (> 1.0 %) of the oil were linalool (24.63 %), camphor (13.58 %), linalyl acetate (8.89 %), 1,8-cineole (7.14 %), *(Z)*-β-ocimene (7.59 %), borneol (6.41 %), 2,6-dimethyl-3,5,7-octatriene-2-ol (5.39 %), *(E)*-β-ocimene (4.76 %), hotrienol (4.42 %), hexyl butyrate (2.96 %), α-bisabolol (1.13 %) and caryophyllene oxide (1.02 %). Linalool and linalool-rich essential oils are known to exhibit various biological activities such as antimicrobial, antiinflammatory, anticancer and antioxidant properties. Linalool is also a key compound for the industrial production of a variety of fragrance chemicals such as geraniol, nerol, citral and its derivatives, as well as a lead compound in the synthesis of vitamins A and E[13].

On comparison of the present results with those reported from samples of other countries, it is quite evident that the concentrations of 1,8-cineole, camphor, *(Z)*-β-ocimene, *(E)*-β-ocimene, hotrienol and borneol were slightly higher, whereas the concentration of nerol, caryophyllene, elemol, cadinene, terpineol, thymol acetate, lavandulol, limonene and α-pinene were marked less in this sample[9,12]. The concentrations of linalool and linalyl acetate are with the range of European Pharmacopoeia[14]. The observed differences in the constituents of lavender essential oil in the present study may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants.

The antioxidant activity of essential oil of *L. angustifolia* was determined using a methanol solution of DPPH reagent. The antioxidant activity of essential oil of *L. angustifolia* was expressed in terms of percentage of inhibition (%). Parallel to examination of the antioxidant activity of the oil, the values for three standard compounds were obtained and compared with the antioxidant activity of essential oil of lavender. The standard substances were BHA, BHT and Vitamin C. a plot of % inhibition verses concentration given in Fig 3 was used to calculate IC_{50} values. The examination of antioxidant activity of essential oil of *L. angustifolia* showed concentration dependant response and varied from 3.28 to 88.91% for 7.81 to 1000 µg/ml, respectively. The IC_{50} values of standard BHT, Vitamin C and BHA and essential oil of *L. angustifolia* was found to be 16 µg/ml, 24 µg/ml, 52 µg/ml

and 216 µg/ml, respectively. The high content of linalool was postulated to contribute to the antioxidant activity of the oil. The *L. angustifolia* can thus be regarded as promising candidate from natural plant sources of antioxidants with high value.

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