SUPPLEMENTARY MATERIAL

Essential oil constituents, phenolic content and antioxidant activity of *Lavandula stricta* Delile growing wild in southern Iran

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Lavandula stricta belong to the Lamiaceae family and is considered as an endemic medicinal plant in southern Iran. Essential oil composition, total phenolic content and antioxidant activity from two different populations of *L. stricta* were studied for the first time. A GC and GC/MS analysis of essential oil isolated from the aerial part of *L. stricta* identified 31 constituents, the major constituents were α-pinene (58.34- 63.52%), linalool (8.85- 9.36%), 3-methyl butyl 2-methyl butanoate (7.45-7.70%), sabinene (2.84-3.56%), limonene (2.87-3.21%) and myrcene (2.25%). The total phenolic content of methanolic extracts was determined with the Folin-Ciocalteau reagent and the antioxidant activity of methanolic extract and essential oil were determined with the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, respectively. Total phenols varied from 61.05 to 64.45 mg GAE/g dry weight, and IC_{50} values in the radical scavenging assay ranged from 334.11 to 395.23 µg/mL in methanolic extracts and 420-475 µg/mL in essential oil.

Keywords: *Lavandula* species; essential oil composition; phenolic content; antioxidant activity

3. Experimental

3.1. *Plant material*

Aerial parts of *Lavandula stricta* were collected in flowering stage in March 2015, from the wild populations growing in natural habitat at two different location from Genow and Rodan (30 and 100 km from Bandare-Abbas, Capital of Hormozgan Province, in southern region of Iran, near Persian Gulf) (Fig.S1) and were identified and authenticated (voucher no.114) at the herbarium of medicinal and aromatic plants of (IAU), Estahban branch, Fars, Iran. Climatic conditions of *L. stricta* habitats were determined using the nearest meteorology station (Table S1). The harvested plants were dried at room temperature (25°C) for 2 weeks.

3.2. *Essential oil extraction*

Dried leaves were ground into powder (mesh< 35), and 40 g of the powdered tissue was distillated with 1 L of water for 3 h using a Clevenger-type apparatus. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4□C in dark glass vials until analyzed. The yield based on dry weight of the sample was calculated.

3.3. *Identification of the Oil Components*

The essential oil composition was determined by GC and GC-MS analysis. The analysis was performed using a gas chromatograph (Agilent Technologies 7890 GC) equipped with a FID detector, using HP-5MS 5% capillary column (30 m x 0.25 mm, 0.25 μm film thicknesses). The carrier gas was Helium at a flow of 1 ml/min. Initial column temperature was 60°C and was programmed to increase at 3°C/min to 280°C. The injector and detector temperatures were set at 280 °C. The split ratio was 20:1. Oil samples (0.2 μL) were injected manually. The percentage compositions were obtained from electronic integration of peak areas without the use of correction factors.

The GC-MS analysis was done on the Agilent Technologies 5975 Mass system. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 200 °C. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard (Van Den Dool and Kratz, 1963). The compounds were identified by comparison of retention indices (RRI- HP-5) with those reported in the literature and by comparison of their mass spectra with the Willey (WILLEY /Chem Station data system) and mass finder 3 libraries or with the published mass spectra (Adams, 1995).

3.4. *Methanolic extract*

Methanolic extracts of the *L. stricta* plants (7.5 g) were defatted with petroleum ether for 3 h and then, macerated twice for 24 h with 200 mL of methanol/water (90/10) at room temperature. After filtration through Whatman filter paper (Whatman, Little Chalfont, UK), supernatants were combined and the solvent was evaporated to a volume of about 1 mL using a rotary evaporator. The concentrated extracts were freeze-dried and weighed for yield determination, and kept at -20°C until used for antioxidant activity and total phenolic content tests.

3.5. *Phenolic content*

Total phenolic content in *L. stricta* plant extracts was determined by the Folin-Ciocalteau colorimetric method, as described by the method of Singleton and Rossi, (1965). Different concentrations of gallic acid in methanol were tested in parallel to obtain a standard curve. Total phenolic contents were determined as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

3.6. *Free radical scavenging capacity (DPPH)*

Radical scavenging activity of plant extracts and essential oils against the stable free radical DPPH was measured according to the method employed by Brand-Williams et al., (1995) and Wang et al. (1998) respectively. Different concentrations of the plant extract dissolved in methanol were incubated with a methanolic solution of DPPH (100 μM) in 96-well micro plates. The antioxidant activity of the essential oils was determined by dissolving different concentrations in 0.5 mL of 0.2 mM DPPH; all solutions' was allowed to stand at room temperature in the dark. After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. The amount of methanolic extract and essential oils necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically and the percentage inhibition was calculated according to the equation:

Percentage inhibition (%I) = $[(A_{blank} - A_{sample})/ A_{blank}] \times 100$

Where, A_{blank} is the absorbance of the control reaction (DPPH alone), and A_{sample} is the absorbance of DPPH solution in the presence of the plant extract. The IC_{50} values were calculated as the concentration of extracts causing a 50% inhibition of DPPH radical, a lower IC_{50} value corresponds to a higher

antioxidant activity of plant extract sample. Quercetin was used as reference compound. Gallic acid, Quercetin and Vitamin E were used as standard antioxidant.

Table S1.Geographical and environmental conditions of *L. stricta* growing wild in Iran

Region	Altitude (m)	Latitude (UTM)	Longitude (UTM)	Temp.[a] (°C)	Rainfall[b] (mm)	E.C.[c]	pH	R.H.[d]	S.T.[e]
Genow	265	27°29'5''N	56°15'28'' E	28.8	240.3	5.20	7.92	76.7	loam
Rodan	345	29°31'58" N	57°06'43'' E	25.6	265.4	4.58	8.12	65.9	Sandy loam

[a]: Average temperature

[b]:Average annual Rainfall

[c]: Electrical conductivity

[d]: Relative humidity

[e]:Soil texture

Meteorological information was obtained from the nearest meteorology station within the study area and the surrounding zone; each value in the mean of 10 years data.

Physical and chemical Soil characteristics are based on average of three samples taken from each region.

Table S2. Percentage composition of the essential oil of *L. stricta* growing wild in Iran

No	Compound	RI[a]	Genow	Rodan
1	(2E)-Hexenal	851	1.54	1.87
2	α-Thujene	927	0.87	0.37
3	**α-Pinene**	934	58.34	63.52
4	Camphene	949	0.63	0.42
5	Thuja-2,4(10)-diene	954	1.87	1.45
6	Sabinene	974	3.56	2.84
7	β-Pinene	978	0.48	0.23
8	6-methyl-5-Hepten-2-one	987	0.46	0.21
9	**Myrcene**	991	2.25	1.84
10	ρ-Cymene	1025	1.34	0.54
11	**Limonene**	1028	2.87	3.21
12	1,8-Cineole	1032	0.59	0.11
13	(Z)-β-Ocimene	1037	0.78	0.38
14	Benzene acetaldehyde	1044	0.34	0.12
15	(E)-β-Ocimene	1047	0.47	0.11
16	-Terpinene	1058	0.23	0.14
17	*trans*-Linalool oxide	1073	0.18	0.23
18	*cis*-Linalool oxide	1089	0.37	0.25
19	**Linalool**	1099	9.36	8.85
20	**3-Methyl butyl 2-methyl butanoate**	1104	7.45	7.70
21	Isopentylisovalerate	1109	0.64	0.91
22	3-Methyl-3-butenyl-3-methyl butanoate	1117	0.44	0.35
23	α-Campholenal	1127	0.36	0.49
24	*trans*-Verbenol	1145	0.54	0.61
25	*iso*-Menthol	1177	0.23	0.35
26	Verbenone	1207	0.18	0.21
27	Methyl thymol	1228	0.68	0.72
28	Linalyl acetate	1256	1.44	1.04
29	Thymol	1294	0.53	0.36
30	Carvacrol	1300	0.48	0.26
31	(E)-Caryophyllene	1419	0.36	0.26
	Monoterpene hydrocarbons		73.69	75.05
	Oxygenated monoterpenes		25.81	24.64
	Sesquiterpene hydrocarbons		0.36	0.26
	Total		%99.86	%99.95
	Essential Oil Yield (%W/W)		0.36 0.05	0.21 0.04

[a]: RI, retention indices in elution order from HP-5 column. Data expressed as percentage of total. Essential oil yield was obtained by calculating the average of three experiments ±standard deviation.

Table S3. The main identified chemical constituents of essential oil of some *Lavandula* species

Species	Main components	Site	References
L. stricta	α-pinene (58.34-63.52%), linalool (8.85-9.36%), 3-Methyl butyl 2-methyl butanoate (7.45-7.70 %), sabinene (2.84-3.56%), limonene (2.87-3.21%)	Iran	Our study
L. angustifolia	linalyl acetate (26.54%), linalool (20.98%)	Lithuania	Venskutonis' and Dapkevicius,1997
L. angustifolia	linalool (30.6 %), linalyl acetate (14.2 %), geraniol (5.3 %), β-caryophyllene (4.7 %), lavandulyl acetate (4.4 %).	Poland	Smigielski et al., 2009
L. angustifolia	linalool (29-39.2%), α-terpineol (7.1-12.7%), borneol (4.8- 9.3%) and terpinen-4-ol (4.8% -6.9%)	Poland	Prusinowska et al., 2015
L. angustifolia	linalool (23.9–15.8%), linalylanthranilate (12.3–1.6%), 1-terpinen-4-ol (9.7–5.5%), *p*-menth-1-en-8-ol (7.9–4.0%) and linalool oxide (4.7–1.1%).	Poland	Adaszyńska et al., 2013
L. coronopifolia	trans-β-ocimene (26.9%), carvacrol (18.5%), bisabolene (13.1%) and myrcene (7.5%)	Tunisia	Messaoud et al., 2012
L. coronopofolia	linalool (41.2%), 1, 8-cineole (25.4%)	Jordan	Aburjai et al., 2005
L. coronopofolia	carvacrol (48.9%), *E*-caryophyllene (10.8%) and caryophyllene oxide (7.7%)	Morocco	Ait Said et al., 2015
L.dentata	1, 8-cineole (48%)	Algeria	Bousmaha et al., 2006
L. gibsoni	2, 2-diphenylpropane and ethoxylated aromatic monoterpene alcohols	India	Kulkarni and Joshi, 2013
L. intermedia	linalool (34-47%), linalyl acetate (17-34%), camphor (4-9%) and eucalyptol (3-7%)	Spain	Carrasco et al., 2015
L. latifolia	linalool (31.9%, 30.6%), 1, 8-cineole (18.8%, 20.9%) and borneol (10.1%, 8.9%)	Iran	Barazandeh, 2002
L. latifolia	linalool (32.3%), camphor (12.4%), 1, 8-Cineole (11.7%), lavandulol (8.7%), p-cymen-8-ol (7.7%) and bornyl acetate (4.2%)	Tunisia	Alatrache et al., 2007
L.multifida	carvacrol (31.81%), beta-bisabolene (14.89%), and acrylic acid dodecyl ester (11.43%)	Tunisia	Chograni et al., 2010
L. multifida	carvacrol (65.1%) and α-bisabolene (24.7%).	Tunisia	Messaoud et al., 2012
L. officinalis	linalool (34.1%), 1, 8-cineole 18.5%), borneol (14.5%), camphor (10.2%), terpinen-4-ol (4.5%), linalyl acetate (3.7%)	Iran	Afsharypuor and Azarbayejany, 2006
L. stoechas	fenchone (68.2%) and camphor (11.2%)	Tunisia	Bouzouita et al., 2005
L. stoechas	fenchone (34.3%) and comphor (27.4%)	Tunisia	Messaoud et al., 2012

L. vera	linalyl acetate (39.40%), linalool (18%)	Japan	Tsuroa, et al., 2001

Table S4. Phenolic content and free radical scavenging activity of *L. stricta* growing wild in southern Iran

	Total phenolic content [a]	**IC_{50}** [b] (g/mL) **Methanol extract**	**IC_{50}** (g/mL) **Essential oil**
Region			
Genow	64.45 1.25 a	334.11 1.53 b	420 4.67 b
Rodan	61.05 1.18 b	395.23 1.48 a	475 5.27 a
Synthetic antioxidant			
Gallic Acid	ND	24.70 0.48 e	----
Quercetin	ND	35.79 0.88 d	----
Vitamin E	ND	184.15 1.24c	----

Each value in the table was obtained by calculating the average of three experiments ± standard deviation. Means with different letters were significantly different at the level of $p<0.05$.

[a]: Data expressed as mg of gallic acid equivalents per g dry weight (DW).

[b]: $IC_{50:}$ Data expressed as μg per milliliter. Lower IC_{50} values indicated the highest radical scavenging activity.

ND = not determined

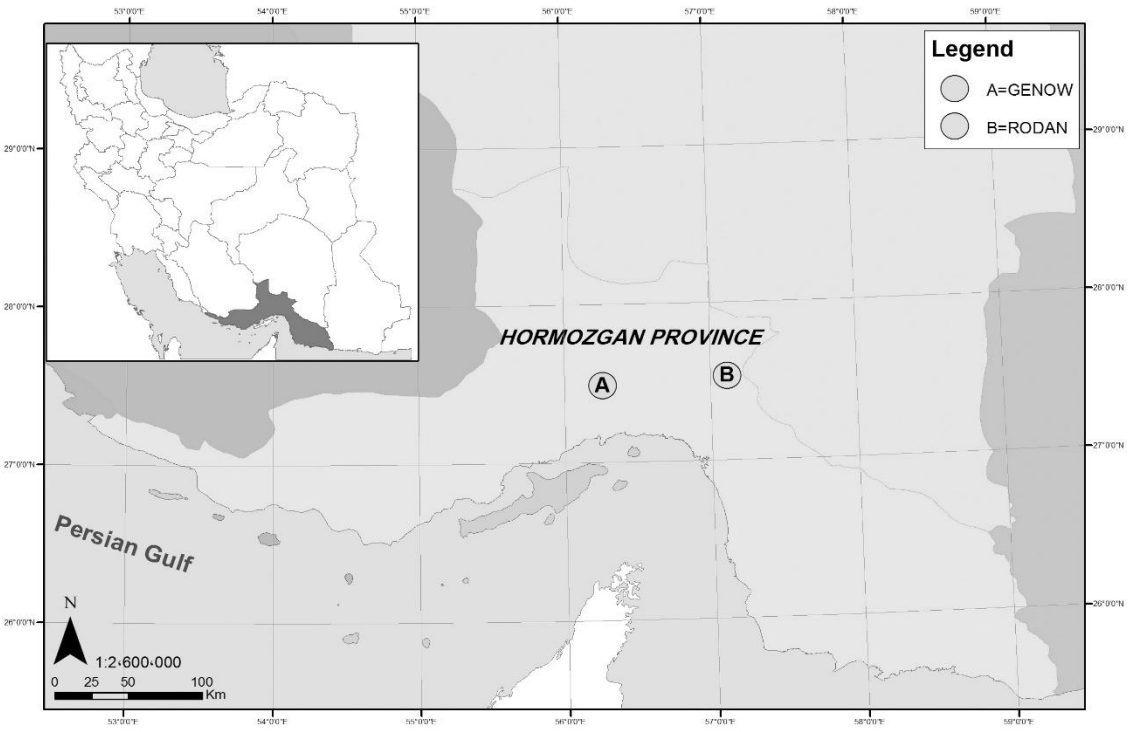

Figure S1. GIS of Sampling locations of *L. stricta* population growing wild in southern Iran.

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