Investigation of some chemical and biochemical properties of locally grown *Lavandula stoechas cariensis*

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Abstract

In this presented work, antioxidant potential of endemic *Lavandula stoechas cariensis* was investigated. *Lavandula stoechas cariensis* plants were locally called as "Karabaş otu" and consumed for its beneficial effects. Fifteen different locations for plant sampling were identified at around Koçarlı (Aydın) region, and plant samples and soil samples were collected in between April and May. Four main antioxidant parameters were used for the evaluation of the antioxidant parameters: proline content; total phenolic compounds; DPPH free radical scavenger activity; and reducing power. Before the antioxidant experiments, water and methanol extracts of the *Lavandula stoechas cariensis* plants were obtained. Essential oil content; and micro and macro nutrient of the *Lavandula stoechas cariensis* samples were also investigated. Soil properties and nutrient content of the soil samples were also evaluated.

Keywords— Antioxidant activity, DPPH Activity, *Lavandula stoechas cariensis*, micro and macro nutrients, reducing power, total phenolic content.

1 Introduction

Investigations about the role of the plant originated antioxidants over the human health have been increased recently. Antioxidants are compounds which protect the organic substances against oxidative damage or preserve them from destructive effects of oxidation. Antioxidant properties of different plant extracts, essential oils and pure chemicals are evaluated by using in vitro tests. These tests divided into two main sub classes: a) evaluation of lipid peroxidation, and b) free radical scavenger competence. To test the lipid peroxidation, various lipid compounds can be used such as fatty acids, linoleic acid, fatty acid methyl esters, low-density lipoproteins. Free radical scavenger activity of an antioxidant compound can be detected two different methods depends on a) hydrogen-atom transfer reactions, and b) single electron transfer reactions. Free radicals responsible for cell damage, and various diseases and disorders such as cancer, cardiovascular diseases, inflammatory disorders, cataract and even aging [1].

Medicinal and aromatic plants have been still used in developing countries in order to treat the certain disorders and have important role at traditional treatment, and 80 % population of the developing countries use traditional drugs as a medicine. Modern drug industry uses plant originated products and 25 % of marketed drugs are obtained from various plant sources [2].

Lavandula is an important genus of the family of *Labiatae* (*Lamiaceae*). *Lavandula* species grow in the Mediterranean basin and are cultured in France, Spain and Italy. Turkey has two species, *Lavandula stoechas* and *Lavandula angustifolia*. *Lavandula* species are redolence and have pleasant aroma, and because of this have

great commercial importance. The plant materials and their essential oils are intensively used in perfume, cosmetic and food industry. Medicinal significance of these plants was tested and drugs prepared from this plant were well characterized. *Lavandula* species have expectorant, antispasmodic, analgesic, sedative, antimicrobial, purgative and wound healing effects. Also this plant have been traditionally used for treatment of central nervous system disorders such as epilepsy and migraine, and used to decrease the blood glucose level. Additionally, anti-cancer efficiently of the *Lavandula* plant extracts has been also reported [3, 4].

In this presented work, antioxidant properties of *Lavandula stoechas cariensis* were investigated and compared by their growth area and soil characteristics. For these purposes, plant materials were collected from different region and different altitudes. Plant materials were extracted by methanol and water, also total essential oil amount was determined by hydrodistillation. Soil samples were also collected, in order to investigate the soil characteristics.

2 Materials and Methods

2.1 Materials

Lavandula stoechas cariensis (which are locally called as "karabaş otu") samples were collected from the Koçarlı (Aydın) region between the months of March and April. Coordinates and altitudes of the plant materials were determined by using a GPS device and are summarized in Table 1.

2.2 Methods

2.2.1 Plant analysis

Total essential oils were determined by using Clevenger type distillation device and expressed as volume for per g of plant materials. Macro (N, P, K, Ca, Mg, Na) and micro (Fe, Mn, Zn, Cu, B) nutrients of *Lavandula stoechas cariensis* samples were determined by using the method of Kaçar and İnal [5]. For this, 1.0 g of plan material was mixed with 12.0 mL of Nitric-perchloric acid solution (4:1, v/v). After wet decomposition, samples were washed 5 times with distilled water, and then filtered and the final volume of the solution adjusted to 100 mL. P was analyzed spectrophotometrically and total P content was expressed as % [5]. K and Ca amount of the sample determined by using a flame photometer (Jenway PFP7), while Mg, Fe, Mn, Zn and Cu were detected by atomic absorption spectrophotometer (Varian SpectrAA 220FS) [5]. B content of the samples was determined by dry combustion method. For this, samples were ignited at 550 °C and then complexed with azomethine-H, resulting colored product detected spectrophotometrically [6].

Table 1. General information about sampling area.

Samples	Coordinates	Altitude (m)	Land conditions
1	N 37°42.456' E 027°46.406'	457	Westward slope
2	N 37°41.732' E 027°46.045'	546	South-eastern slope
3	N 37°41.137' E 027°45.393'	666	Southbound slope
4	N 37°39.722' E 027°44.187'	637	Southbound slope
5	N 37°38.012' E 027°44.696'	418	Westward slope
6	N 37°36.124' E 027°43.900'	476	Southbound slope
7	N 37°32.472' E 027°38.562'	597	Southbound slope
8	N 37°42.536' E 027°44.850'	456	South-eastern slope
9	N 37°42.905' E 027°45.214'	432	Eastern slope
10	N 37°42.464' E 027°50.306'	300	Eastern slope
11	N 37°42.485' E 027°50.195'	146	Flat land
12	N 37°43.878' E 027°45.436'	132	Westward slope
13	N 37°44.458' E 027°45.509'	65	Eastern slope
14	N 37°42.865' E 027°37.073'	247	Northbound slope
15	N 37°42.120' E 027°38.831'	543	Northbound slope

Antioxidant activity of *the Lavandula stoechas cariensis* samples were investigated by using two different extracts. For this purpose, collected plant materials were dried, grinded and extracted by using methanol and water separately. Five grams of samples were mixed with solvent (methanol or water) and mixed for 6 h at room temperature (with the mixing rate of 100 rpm). Then, the extracts were filtered and the solvent phase was removed by using lyophilization (-50 °C, 0.070

mBar; for water extract) or evaporation (40 °C; for methanol extract). In order to evaluate the antioxidant efficiency of the *Lavandula stoechas cariensis* extracts, 4 main antioxidant activity methods were used. For these purposes, proline analysis was carried out spectrophotometrically by the method of Bates et al. [7]. Total phenolic contents of the extracts were determined by using the reactive of Folin-Ciolcalteu [8]. DPPH free radical scavenger effects were investigated by using the method of Blois [9]. And finally, reducing power of the extracts was studied by the method of Oyaizu [10].

2.2.2 Soil analysis

Soil samples were collected from the same point of the plants collected [11]. Structure analysis of soil was carried out by using a hydrometer [12]. For this; sand, silt and clay fractions of oil samples were analyzed and evaluated by using a structure analysis triangle [13]. pH of the soil sample was determined by using a pH meter. For this, soil samples (10 g) were mixed with 25 mL of water for 30 min and then pH of the sample was recorded [14, 15]. Lime content of soil was calculated by using a Scheibler calcimeter [16, 17]. Salt content of soil was measured by determining the electrical conductivity of the samples by using Conductivity Bridge device [18, 19]. Soil P content determined by the method of Olsen [20]. For this, soil samples were mixed with 0.5 M of sodium bicarbonate solution (pH 8.5) and then filtered. Five mL of the supernatant was mixed with sulphuric acid and ammonium molybdate solution and then resultant blue colored product was measured by using a spectrophotometer. In order to determine the K, Ca, and M contents, soil samples were extracted by ammonium acetate solution (pH 7.0; 1.0 N). Ca and Mg were measured by using atomic absorption spectrophotometer, while K was determined by using flame photometer [21, 22]. Fe, Mn, Cu and Zn amount were determined by using atomic absorption spectrophotometer after extraction with diethylenetriamine pentaacetic acid [23]. B content of the soil samples was determined by the same method used for the plant materials [6].

3 Results and Discussion

After pioneering works around the mountainous region of Koçarlı (Aydın, TURKEY), plant materials were collected from 15 different areas. Sampling areas are demonstrated in Figure 1.

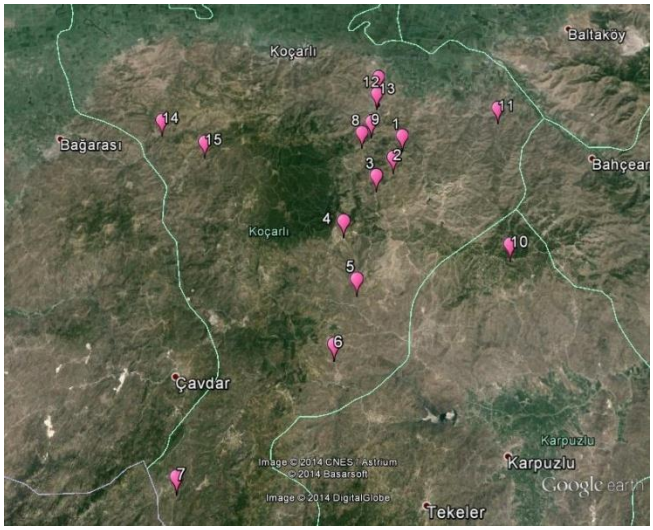

Figure 1. Geographic location of sampling areas for *Lavandula stoechas cariensis* plants.

3.1 Plant analysis

Dry mater ratio and essential oil content of *Lavandula stoechas cariensis* samples were summarized in Table 2.

Table 2. Dry mater ratio and essential oil content of *Lavandula stoechas cariensis* samples.

Samples	Dry mater ratio (%)	Essential oil content (v/w)
1	6.78	1,88
2	7.13	1,89
3	7.43	1,74
4	7.98	1,64
5	6.93	1,48
6	8.04	1,62
7	8.45	2,57
8	8.13	2,96
9	7.75	3,42
10	7.52	2,05
11	6.65	1,69
12	7.47	1,45
13	6.28	1,66
14	7.84	1,42
15	8.88	1,73

As clearly seen in this Table, plant dry mater ratio was changed between 6.28 % and 8.88 %, and the highest essential oil content was found to be as 3.42 (v/w) by sample 9. Samples 7 and 8 were also demonstrated higher essential oil content.

P content of *Lavandula stoechas cariensis* samples were found to be between 20 % and 37 % (Table 3). In general, P contents were found to be "adequate" whereas P contents of samples 1, 2, 3 and 7 were detected as "high". K content of the plant samples were found to be as "adequate", while Na and Mg content was found to be as "inadequate" for all tested materials (Table 3).

Fe, Zn, Cu, Mn and B content of *Lavandula stoechas cariensis* samples were demonstrated in Table 3. As seen here, Fe content varied between 466 ppm and 891 ppm, Zn content was found between 63 and 93 ppm. All tested plant samples exhibited "adequate" Cu content except sample 13 (inadequate). Mn contents of *Lavandula stoechas cariensis* samples were found to be as "adequate", whereas B contents varied between 13.14 ppm and 45.03 ppm.

Table 3. P, K, Ca, Mg, Fe, Zn, Cu, Mn and B contents of *Lavandula stoechas cariensis* samples

	P	K	Ca	Mg	Fe	Zn	Cu	Mn	B
Samples	%				ppm				
1	0,35	2,88	0,46	0,26	491	78	21	366	19,47
2	0,35	3,07	0,52	0,25	707	78	14	344	18,86
3	0,37	3,07	0,55	0,24	569	71	18	198	18,38
4	0,30	2,79	0,51	0,24	514	67	16	225	18,01
5	0,20	2,62	0,44	0,21	466	74	18	265	13,99
6	0,30	2,79	0,43	0,20	573	63	18	219	30,42
7	0,31	2,36	0,56	0,26	668	86	17	196	16,92
8	0,22	2,79	0,63	0,27	690	74	25	299	19,11
9	0,23	2,98	0,55	0,25	793	80	27	426	29,09
10	0,26	2,11	0,87	0,26	602	70	32	320	45,03
11	0,25	2,53	0,84	0,25	719	81	25	262	22,76
12	0,29	2,62	0,53	0,24	765	75	18	212	13,14
13	0,28	2,03	0,77	0,28	891	89	2	312	43,45
14	0,24	2,62	0,82	0,24	769	82	18	236	39,92
15	0,30	2,62	0,65	0,24	794	93	4	321	24,46

Extraction yields of the *Lavandula stoechas cariensis* samples were summarized in Table 4. As clearly seen here, yields of the water extracts was found to be lower than 10 %, while methanol extracts showed higher yields than that of water extracts.

Table 4. Extract yields of *Lavandula stoechas cariensis* samples.

	Water Extract			**Methanol Extract**	
	Yield (%)	g/g		Yield (%)	g/g
1	8,15	0,0815	**1**	10,83	0,1083
2	10,79	0,1079	**2**	13,15	0,1315
3	6,51	0,0651	**3**	11,46	0,1146
4	9,80	0,0980	**4**	12,78	0,1278
5	9,76	0,0976	**5**	12,66	0,1266
6	9,45	0,0945	**6**	15,42	0,1542
7	11,87	0,1187	**7**	13,69	0,1369
8	7,36	0,0736	**8**	12,14	0,1214
9	10,81	0,1081	**9**	10,50	0,1050
10	6,50	0,0650	**10**	12,89	0,1289
11	9,45	0,0945	**11**	13,43	0,1343
12	8,47	0,0847	**12**	13,88	0,1388
13	9,41	0,0941	**13**	14,49	0,1449
14	5,92	0,0592	**14**	12,86	0,1286
15	7,81	0,0781	**15**	13,75	0,1375

Proline content of the *Lavandula stoechas cariensis* samples were listed in Table 5. As seen in this table, samples 1, 6, 10 and 15 had high proline content while samples 5, 8, 11 and 13 had lower content.

Table 5. Total proline content of *Lavandula stoechas cariensis* samples

Samples	**Proline content (μg/g)**
1	1651,52 ± 4,29
2	1202,42 ± 82,28
3	1209,09 ± 4,29
4	1351,52 ± 17,14
5	1078,79 ± 42,85
6	1415,15 ± 21,43
7	1375,76 ± 25,71
8	784,85 ± 30,00
9	1236,36 ± 8,57
10	1530,30 ± 132,85
11	1003,03 ± 55,71
12	1148,48 ± 72,85
13	1021,21 ± 38,57
14	1272,73 ± 25,71
15	1427,27 ± 149,99

Total phenolic compounds amount of *Lavandula stoechas cariensis* samples were summarized in Table 6. As seen here, sample 9 exhibited the highest phenolic content for all tested two extracts (water and methanol).

Table 6. Phenolic compound content, DPPH activity and Reducing Power of water and methanol extracts of *Lavandula stoechas cariensis* samples.

Samples	Phenolic Compound (mg/g)	IC_{50} (µg/mL)	Ascorbic acid (%)
Water Extract			
1	27,24 ± 3,09	81,32 ± 1,45	8,719 ± 0,676
2	19,81 ± 2,06	115,25 ± 3,64	8,805 ± 0,392
3	30,59 ± 1,24	69,40 ± 3,09	12,122 ± 0,406
4	22,00 ± 0,62	93,95 ± 1,99	8,939 ± 1,663
5	24,04 ± 1,03	105,80 ± 4,61	10,373 ± 0,608
6	34,67 ± 4,53	74,06 ± 2,87	14,627 ± 0,297
7	45,60 ± 2,68	45,37 ± 1,94	17,667 ± 1,839
8	48,51 ± 0,21	39,58 ± 0,56	18,987 ± 0,324
9	90,18 ± 3,91	28,30 ± 0,21	28,920 ± 0,068
10	35,69 ± 0,62	59,01 ± 3,11	16,740 ± 1,717
11	25,35 ± 0,82	80,33 ± 3,53	13,289 ± 0,297
12	26,52 ± 2,06	87,41 ± 2,63	12,801 ± 1,690
13	28,41 ± 1,44	72,14 ± 1,59	13,031 ±1,879
14	32,78 ± 0,21	94,31 ± 2,77	12,887 ± 0,879
15	34,67 ± 7,83	68,20 ± 1,17	13,853 ±2,799
Methanol Extract			
1	30,01 ± 6,18	79,40 ± 2,62	8,891 ± 0,865
2	25,50 ± 0,62	174,99 ± 4,53	10,679 ± 0,744
3	27,24 ± 5,56	86,53 ± 3,52	11,501 ± 1,879
4	17,34 ± 1,44	157,78 ± 3,10	9,207 ± 0,500
5	21,71 ± 4,33	62,15 ± 0,56	9,426 ± 0,027
6	38,75 ± 2,06	107,63 ± 4,72	12,734 ± 0,406
7	27,39 ± 2,06	92,31 ± 5,09	11,711 ± 0,825
8	61,92 ± 7,21	21,18 ± 0,57	21,702 ± 0, 595
9	119,90 ± 11,33	8,56 ± 0,28	36,090 ± 2,231
10	40,21 ± 2,88	42,51 ± 2,73	16,874 ± 0,338
11	28,26 ± 8,24	111,29 ± 6,25	13,547 ± 0,879
12	24,18 ± 4,53	112,78 ± 5,39	11,558 ± 0,203
13	18,07 ± 2,06	151,73 ± 3,96	9,455 ± 0,122
14	20,98 ± 2,47	110,22 ± 2,63	11,119 ± 0,284
15	18,79 ± 1,44	109,32 ± 3,03	11,864 ± 0,149

Table 6. also demonstrates the DPPH free radical scavenger ability of the extracts of *Lavandula stoechas cariensis* samples. DPPH activity was expressed as IC50 value, which means that the extract amount to reach the 50 % inhibition and this means that lower IC50 values are higher free radical scavenger activity. As clearly seen in this table, water extracts exhibited higher DPPH activity than that of methanol extracts. Also, two extracts of sample 9 exhibited the highest free radical scavenger activity than that of other samples. This sample also had the highest phenolic content and there are a correlation between phenolics and antioxidant activity.

Reducing power of the *Lavandula stoechas cariensis* samples were evaluated as ascorbic acid % and summarized in Table 6. As clearly seen here, sample 9 had the highest reducing power that that of all other samples. This sample had also highest phenolic content and DPPH activity.

3.2 Soil analysis

Structure analysis of the soil was briefly demonstrated in Table 7. As seen here, soil samples generally had loamy structure. pH profiles of the soil samples were summarized in Table 10. As clearly seen here, pHs of the samples were varied between 5.25 and 6.62. Lime and total salt content of the soil samples were demonstrated in Table 7. As seen here, the soil samples contained low amounts of lime, while all tested soil samples were found to be as salt-free.

P, K, Ca and Mg contents of soil samples were summarized in Table 8. P content of tested materials was found to be as "adequate", while K amount of soil samples were varied between 19 ppm and 128 ppm. Ca content of soil samples were also varied between 312 ppm and 906 ppm, while Mg content was found to be in the concentration range of 47-136 ppm.

Fe, Zn, Mn, Cu and B content of the soil samples were also demonstrated in Table 8. As seen here, all tested soil samples exhibited high Fe content, while Zn and Mn content of the soil samples was found to be as "adequate". All soil samples showed "adequate" Cu content except the sample 15 (inadequate Cu content). B content of the all tested materials was found to be as very low, except that sample 10 exhibited low B content.

Table 7. Structure properties, pH, lime and salt content of soil samples.

Samples	Sand %	Silt %	Clay %	pH	Total salt (%)	$CaCO_3$ (%)
1	68,52	24,36	7,12	5,86	0,0038	0,32
2	70,52	21,36	8,12	5,93	0,0047	0,32
3	73,52	19,36	7,12	6,01	0,0030	0,32
4	80,52	12,36	7,12	6,12	0,0028	0,32
5	75,52	17,36	7,12	6,22	0,0035	0,32
6	71,52	20,36	8,12	5,6	0,0022	0,32
7	67,52	25,36	7,12	6,08	0,0027	0,32
8	79,52	13,36	7,12	6,33	0,0033	0,32
9	70,52	21,36	8,12	5,99	0,0036	0,32
10	71,52	20,36	8,12	5,25	0,0555	0,32
11	74,52	18,36	7,12	6,12	0,0219	0,47
12	73,52	19,36	7,12	6,62	0,0040	0,32
13	64,52	23,36	12,12	5,69	0,0471	0,32
14	73,52	19,36	7,12	6,29	0,0038	0,47
15	67,52	25,36	7,12	5,98	0,0033	0,47

Table 8. P, K, Ca, Mg Fe, Zn, Mn, Cu and B contents of soil samples.

Samples	P	K	Ca	Mg	Fe	Zn	Cu	Mn	B
	ppm								
1	12,1	57	493	91	86,8	2,5	0,50	10,4	0,29
2	14,6	101	634	119	101,5	2,3	0,41	29,0	0,22
3	12,9	114	594	108	80,0	2,6	0,50	13,5	0,19
4	9,5	35	644	125	59,2	2,5	0,32	9,3	0,09
5	8,6	41	413	67	80,8	2,5	0,30	7,7	0,17
6	8,6	69	453	75	80,3	2,5	0,30	6,3	0,19
7	9,5	25	312	47	60,6	1,9	0,50	7,0	0,12
8	15,5	63	403	84	60,7	2,3	0,65	6,4	0,22
9	10,3	128	463	69	92,7	2,2	0,69	12,7	0,31
10	9,5	52	704	86	101,7	2,4	0,86	16,8	0,61
11	18,1	19	906	136	38,1	3,0	1,00	7,5	0,29
12	12,1	63	554	62	76,6	2,3	1,13	10,0	0,18
13	8,6	35	523	104	121,2	2,7	1,29	7,3	0,19
14	10,3	69	906	122	71,6	2,2	1,35	11,7	0,17
15	9,5	63	906	104	92,3	2,7	0,10	39,4	0,17

4 Conclusion

In this presented study, antioxidant properties, and macro and micro nutrition elements of *Lavandula stoechas cariensis* which is an endemic plant grown in Aydın-Koçarlı vicinity were investigated. Soil properties of plants were also evaluated. The altitude at which this plant generally grown was around 350-400 m, in addition this plant also intensively grown at around Cincin village which has altitude of 65 m.

The highest essential oil yields were found to be with samples 8 and 9 which were collected from Kuşlarbeleni region and interborough of Kuşlarbeleni-Cincin, respectively. P content of plant was generally found to be as "adequate"; while K contents of all tested plant samples were of "adequate". On the other hand, Ca and Mg content of this plant were found to be as "inadequate".

Higher proline contents were detected by the samples of 1, 10, 15 and 6, which are Zeytinköy vicinity, interborough of Gözkaya-Taşoluk, interborough of Güdüşlü-Çallı and Yağcıdere vicinity, respectively. Sample 9 exhibited the highest phenolic content followed by the sample 8. Samples 8 and 9 were also identified as the most essential oil rich plants.

DPPH activity of *Lavandula stoechas cariensis* showed the parallel results with phenolic compounds and essential oil content. The most active sample for DPPH was 9, the second was 8. Reducing power activity followed the same order for samples 9 and 8.

pH profile of the soil samples was found as low and moderate acidic, lime and salt contents were low and salt-free, respectively. Fe content of sampling soil was high; Zn, Mn and Cu amounts were "adequate", while B content was found as "low".

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