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Evaluation of Bioactivity of Ethanol and Water Extracts and Determination of Nutrient Concentrations of Laurel (Laurus nobilis) Plants Grown in İzmir Province

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Research Article

History Received: 29/01/2024 Accepted: 19/04/2024 ABSTRACT This study was conducted to determination of ethanol and water extracts obtained from the leaves of Laurus nobilis (Laurel) plant of the general content by GC-MS analysis, antimicrobial activities of these extracts on some microorganisms, total antioxidant levels (TAS), total oxidant levels (TOS), oxidative stress index (OSI) values, anticarcinogenic effects on various cell lines and macro and micronutrient concentrations of Laurel (Laurus nobilis) plants cultivated for decorative purposes in parks and gardens of Izmir province of Turkey. TAS, TOS, and OSI values were determined with the aid of Rel Assay Diagnostics kits. In antimicrobial activity analysis, the minimum inhibition concentration (MIC) of the plant extracts against microorganisms was determined by microdilution broth method. Staphylococcus aureus (ATCC 29213), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Bacillus cereus (ATCC11778), Candida albicans (ATCC 10231) and Candida tropicalis (DSM11953) microorganisms were used in this analysis. Furthermore, cytotoxic activities of plant extracts were determined by XTT method in normal rat fibroblast cell line, HUVEC, and 2 different human cancer cell lines. At the end of the study, it was found that ethanol and water extracts of the Laurel plants had no antimicrobial activity, low oxidative stress index and good antioxidant activity, and moderate cytotoxic activity on experimented cell lines. Nitrogen (N), Phosphorus (P), Potassium (P), Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) concentrations of plant extracts were respectively identified as 1.68% N, 0.72% P, 1.12% K, 2.40% Ca, 0.45% Mg, 147.4 mg/kg Fe, 13.6 mg/kg Zn, 326.3 mg/kg Mn and 27.8 mg/kg Cu.

Keywords: Antimicrobial activity, Antioxidant activity, Nutrient, Laurus nobilis (Laurel).

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Introduction

The use of plants for treatment purposes has a long history as old as the history of humanity [1-2]. Since synthetic drugs are found to be insufficient for various diseases and have various side effects, the use of herbal natural products has become quite widespread. Therefore, many plant species have been investigated in many ways not only from microbiological and pharmacological aspects but also in terms of plant defense mechanisms [2]. Researchers especially focus on obtaining antioxidant and antimicrobial substances from the plant extracts [3].

Laurus nobilis L. (L. nobilis, Laurel) is a characteristic species of maguis plant cover of Mediterranean climate. Laurel is defined as ever-green shrub or tree with a height of 3-10 m [4]. Dried laurel leaves are commonly used as spice [5] and Turkey is the leading exporter of laurel leaves [6]. Researchers revealed antibacterial, diaphoretic, analgesic, antiseptic, and insecticidal effects of laurel leaves, and plant leaves were also found to be effective and alleviative for various diseases including stomach disorders, diabetes, migraine, asthenia, dyspepsia, abnormal menstruation, rheumatism, and insomnia [1, 6]. Anthocyanins in fruits of laurel plants are used in food, pharmaceutics, and cosmetic industries as pigment material [7-11].

All biological activities of the laurel plant, which is ethnobotanically widespread among people and consumed in many countries as a spice, have not been studied experimentally and the available literature information is insufficient. In addition, depending on the geography where it is grown, different activities can be observed in plants. So, this study was conducted to analyze the antimicrobial activities of ethanol and water extracts of laurel (L. nobilis) leaves, widespread in Turkey, against some microorganisms, determine the total antioxidant levels (TAS), total oxidant levels (TOS), oxidative stress index (OSI) values, anticarcinogenic effects on various cell lines, and concentrations of macro and micronutrients in laurel leaves.

Material and Methods

Plant Material

Laurel (L. nobilis) plants were selected as the plant material. Plants were collected from the parks and gardens of İzmir province in July 2019 in Turkey. Plants were taxonomically identified by Dr. Mustafa Sevindik. Leaves were separated from the shoots and dried in the shade under room conditions.

Extraction

Dried leaves were ground into powder form in a hometype grinder. For extractions, 100 g ground leaf sample and 1000 mL ethanol and water solvents were used. For water extraction, weighed ground samples were supplemented with 80°C distilled water and brewed for 30 min [12]. Following the brewing, it was filtered through filter paper (Whatman blue band) and lyophilized to remove water. For ethanol extraction, weighed samples were supplemented into 70% ethanol and extraction was performed for 6 hours in a Soxhlet device. Resultant extracts were filtered through filter papers (Whatman blue band) and evaporated in an alcohol rotary evaporator (Buchi) at 40°C.

Macro and Micronutrient Analyses

Dry laurel leaves were ground in an agate mortar and subjected to wet-digestion in H_2O_2 -HNO₃ acid mixture. Phosphorus (P) concentration was determined colorimetrically in a spectrophotometer at 882 nm in accordance with method of Murphy and Riley [13]; calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) concentrations was determined in an Atomic Absorption Spectrophotometer (Shimadzu AA-7000). Nitrogen (N) concentration of laurel leaves was determined by Kjeldahl distillation method [14]. Each analysis was conducted 3 times.

GC-MS Analyses

In GC-MS analyses of resultant plant extracts, Shimadzu GCMS QP 2010 ULTRA device equipped with RTX-5MS capillary column (30 m; 0.25 mm; 0.25 μ m) apparatus was used. Helium (0.7 mL/min) was used as the carrier gas, the column oven temperature was set at 40°C and the injection temperature was set at 250°C. Pressure was set at 100 kPa, injection mode was selected as split and split rate was selected as 5. Injection volume was 1.0 microliter, and oven temperature program was arranged as: 3 min at 40°C, 53 min from 40°C to 240°C with 4°C/min gradients. Interface temperature was preferred as 250°C and ion source temperature as 200°C. Finally, dilution was performed with 1/10 hexane.

Antimicrobial Activity

The antimicrobial activity of laurel plants was determined through finding out the minimum inhibition concentration (MIC) of the extracts against microorganisms with the aid of the "Microdilution Broth Method" [15]. The microorganism strains used in this study were *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC11778), *Candida albicans* (ATCC 10231) and *Candida tropicalis* (DSM11953).

Plant extracts were dissolved in 40% dimethyl sulfoxide (DMSO) to get concentrated solutions. Mueller Hinton Broth (Accumix[®] AM1072) broth medium was used for bacteria strains and Saboraud Dextrose Broth (Himedia ME033) broth medium was used for *Candida albicans* and *Candida tropicalis*. The wells on the first row of microtiter plates were supplemented with 90 μ L broth medium and the other wells were supplemented with 50 μ L broth medium. The wells on

the 11th row were used as sterile control and each of them was supplemented with 100 µL broth medium [16-17]. The wells on the 12th row were used as propagation control. The wells on the first row were then supplemented with 10 µL extract and serial dilutions were performed. Microorganisms propagated in blood agar broth medium were taken with a loop and a suspension was prepared with these microorganisms at McFarland 0.5 turbidity. Each well was supplemented with 50 µL bacterial suspension as to have 5 x10⁵ CFU/mL for bacteria and 0.5-2.5 x10³ CFU/mL for Candida albicans and Candida tropicalis. Plates containing bacteria were incubated at 37°C, while those containing Candida albicans and Candida tropicalis were incubated at 35°C for 16-24 hours. Herein, the first wells with the reduced image of bacteria colony were accepted as MIC values. Tests were repeated 3 times and similar results were obtained on each time.

Antioxidant Activity

Total antioxidant levels (TAS), total oxidant levels (TOS) and oxidative stress index (OSI) values were determined with the aid of commercially available Rel Assay Diagnostic kits [18-19]. Trolox standard was used for TAS analyses, hydrogen peroxide standard was used for TOS analyses. Oxidative stress index (OSI) was calculated with the aid of the following equation (Arbitrary Unit = AU) [19].

$$OSI (AU) = \frac{TOS, \ \mu mol \ H_2O_2 \ equiv./L}{TAS, \ mmol \ Trolox \ equiv./L \ X \ 10}$$
(1)

Cell Cultures

Breast cancer cell line (MCF-7) (Human breast adenocarcinoma cell), healthy human endothelial cell line (HUVECs; human umbilical vein endothelial cell), human glioblastoma cell line (U87) and rat fibroblast cell line (L929) were used in this study.

Cell Growth and Propagation

All cell lines were cultured in an incubator including 37°C 5% CO₂ in 25 cm² flasks (Corning-Sigma-Aldrich St. Louis, MO, USA) with Dulbecco's modified Eagle's medium (DMEM) containing high glucose, 2 mM L-glutamine and sodium pyruvate and 10% Fetal Bovine serum (FBS) medium. Cell growth and morphology were monitored and sowing was performed when 90% density was achieved (Nuve MN 120). Each well (96 of them, 100 µl/plate empty space as to have $5x10^3$ cells) was supplemented with 200 µL mixture. DMEM, fetal bovine serum (FBS), and sterile phosphate buffer (PBS) were supplied from commercial Gibco (Invitrogen). Before sowing them into plates, cells were passaged, stained with Triptan Blue and cell counts were performed on the Thoma Glass slide (Iso Lab) just to sow certain number of cells to each well. For this process, counting regions over the Thoma glass slide was used. In this stage, dead cells were stained with Methylene Blue and living cells were not stained. Only the unstained cells were counted to get living cell counts. A dilution factor was also considered in calculation of number of cells per mL to get quantity of cells to be sown in each cell.

Cell proliferation test

Cytotoxic effects of plant extracts on cancerous and healthy cell lines were measured with the aid of XTT cell proliferation kit (Biotium). About 5 mL XTT solution was supplemented with 25 μ L PMS and vortexed. The plant extracts prepared at 1 mg/mL stock concentration were arranged between 1 μ g/mL – 1000 μ g/mL doses and applied to cell cultures for 24 hours. At the end of this process, 50 μ L XTT solution was supplemented into each well to get number of living cells, 96-well plates were then placed into incubator and incubated at 37°C for 5 hours. At the end of the incubation duration, plates were placed into a microplate reader and absorbance readings were performed at 450-500 nm. At the end of XTT tests, IC_{50} values were calculated with the aid of GraphPad software. Detailed anti-cancer impact potential works were not performed on plant extracts with IC₅₀ values over 100 μ M. The Cancer Institute of NCBI (National Center for Biotechnology Information) assumed that the isolates with an IC_{50} value of greater than 100 μM did not have a remarkable anti-cancerogenic effect.

Statistical Analyses

SPSS 22.0 (IBM Corporation, Armonk, New York, United States) software was used in statistical analyses. Data were assessed at 95% confidence level and the ones with a p-value of less than 0.05 were considered as significant.

Results and Discussion

GC-MS Analyses Results

The GC-MS analysis results indicating the chemical composition of water and ethanol extracts of laurel leaves collected from İzmir province are provided in Table 1 and 2. GC-MS chromatograms and chromatogram peaks revealed that there were 50 different components in water extracts and 43 different components in ethanol extracts. The 2,3 Butanediol was the highest component of water and ethanol extracts (Fig. 1-2, Table 1-2).



Figure 1: GC-MS chromatogram of ethanol extract of L. nobilis



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Peak	R. Time	Area	Area%	Name	
1	3.624	178222	1.00	(S)-(+)-1,2-Propanediol	
2	3.698	939944	5.25	1,2-PROPANEDIOL	
3	4.041	234799	1.31	Propane, 2-fluoro-2-methyl- (CAS)	
4	4.141	972207	5.43	Propanoic acid, 1-methylethyl ester (CAS)	
5	4.478	759617	4.24	2,3 Butanediol (CAS)	
6	4.620	549220	3.07	Propanoic acid, 2-oxo-, methyl ester (CAS)	
7	4.691	3632265	20.28	2,3 Butanediol (CAS)	
8	4.825	622335	3.47	Hydroperoxide, 1,1-dimethylethyl (CAS)	
9	5.038	407783	2.28	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester	
10	5.950	114915	0.64	2-Pentanone, 4-hydroxy-4-methyl- (CAS)	
11	6.002	246118	1.37	2-Pentanone, 4-hydroxy-4-methyl- (CAS)	
12	6.936	280395	1.57	2-Propanone, 1-(acetyloxy)- (CAS)	
13	7.506	167990	0.94	Styrene	
14	8.082	114813	0.64	2-Cyclopenten-1-one, 2-methyl- (CAS)	
15	8.371	166509	0.93	2(3H)-Furanone, dihydro- (CAS)	
16	11.420	617991	3.45	2-Hydroxy-gamma-butyrolactone	
17	11.555	241142	1.35	1,2-Cyclohexanedione	
18	13.117	269924	1.51	2-Cyclopenten-1-one, 2,3-dimethyl-	
19	14.162	475753	2.66	Cyclohexanone, 3-methyl-, (2,4-dinitrophenyl)hydrazone (CAS)	
20	17.006	366085	2.04	Silane, diethoxydimethyl-	
21	19.067	227307	1.27	3,7-dimethyl-1,5-octadien-3,7-diol	
22	19.710	123698	0.69	exo-2-Hydroxycineole	
23	19.806	126887	0.71	Propanoic acid, di(tert-butyl)silyl ester	
24	20.266	605836	3.38	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-	
25	22.131	318864	1.78	2,6-DIMETHYL-1,7-OCTADIENE-3,6-DIOL #	
26	25.709	522274	2.92	2b,4-dihydroxy-1,8-cineole	
27	26.100	205166	1.15	Skatole	
28	27.284	114754	0.64	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane-6,7-endo,endo-diol	
29	30.192	200771	1.12	Phenol, 3,5-bis(1,1-dimethylethyl)-	
30	30.672	252910	1.41	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	
31	33.575	295694	1.65	exo-7-(2-Propenyl)bicyclo[4.2.0]oct-1(2)-ene	
32	33.746	118015	0.66	Triisobutyl (3-phenyl propoxy) silane	
33	34.670	464829	2.60		
34	35.712	154377	0.86		
35	37.123	845090	4.72	Bicyclo[3.2.0]hept-2-ene, 2-methyl-	
36	37.987	214742	1.20	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	
37	39.535	254650	1.42	Santalol <beta-></beta->	
38	41.706	391154	2.18	Hexadecanoic acid, methyl ester	
39	42.544	129062	0.72	Bicyclo[3.2.0]hept-2-ene, 2-methyl-	
40	42.828	256160	1.43		
41	42.966	227098	1.27		
42	46.598	317364	1.77	Methyl stearate	
43	47.728	185409	1.04	1H-Pyrrole, 1-butyl-	
		17910138	100.00		

Table 1. Chemical composition of *L. nobilis* ethanol extract

Peak	R. Time	Area	Area%	Name	
1	3.617	328072	1.46	Propanoic acid, 2-hydroxy-, methyl ester, (,+,)-	
2	3.703	637042	2.83	1,2-PROPANEDIOL	
3	3.850	106994	0.48	2-Propyn-1-ol (CAS)	
4	4.047	331013	1.47	N-methyl-N-(methyl-d3)aminoheptane	
5	4.160	1268209	5.64	1-Propanol, 2,2-dimethyl-, acetate	
6	4.433	176771	0.79	2,3 Butanediol (CAS)	
7	4.494	467707	2.08	2,3 Butanediol, [R-(R@,R@]-	
8	4.663	2755610	12.25	2-Butanone, 3-hydroxy- (CAS)	
9	4.713	3063565	13.61	2,3 Butanediol (CAS)	
10	4.850	399272	1.77	Propane, 2-methoxy- (CAS)	
11	5.035	294948	1.31	Propanoic acid, 2-hydroxy-, methyl ester	
12	5.711	158896	0.71	2-Cyclopenten-1-one	
13	5.984	612068	2.72	2-Pentanone, 4-hydroxy-4-methyl- (CAS)	
14	6.931	340143	1.51	2-Propanone, 1-(acetyloxy)-	
15	8.373	126530	0.56	2(3H)-Furanone, dihydro- (CAS)	
16	8.525	140948	0.63	1-Octene, 3-ethyl- (CAS)	
17	8.850	80759	0.36	2-Hydroxy-2-cyclopenten-1-one	
18	9.636	74909	0.33	2,5-DIMETHYL-3(2H)FURANONE	
19	10.252	95358	0.42	2-Cyclopenten-1-one, 3-methyl-	
20	11.420	886872	3.94	2-Hydroxy-gamma-butyrolactone	
21	11.555	195772	0.87	1,2-Cyclohexanedione	
22	12.753	159276	0.71	1,2-Cyclopentanedione, 3-methyl- (CAS)	
23	15.305	166064	0.74	anhydro - sugar	
24	17.015	328462	1.46	Silane, diethoxydimethyl- (CAS)	
25	17.255	69145	0.31	1-Tridecene	
26	18.192	105795	0.47	Silanediol, dimethyl-, diacetate (CAS)	
27	19.811	101333	0.45	Propanoic acid, di(tert-butyl)silyl ester	
28	20.279	372956	1.66	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-	
29	20.644	144869	0.64	2-Hexenal, 2-ethyl-	
30	25.716	411269	1.83	2b,4-dihidroxy-1,8-cineole	
31	27.289	131764	0.59	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane-6,7-endo,endo-diol	
32	29.724	131911	0.59		
33	30.195	286917	1.28	Phenol, 3,5-bis(1,1-dimethylethyl)-	
34	33.587	242622	1.08	(3aS*, 5aR*, 9aS*)-3Methyldecahydro-4H-cyclopenta[c]inden-4-one	
35	33.751	377720	1.68	Triisobutyl(3-phenylpropoxy)silane	
36	34.688	145653	0.65		
37	35.716	772294	3.43		
38	37.127	244520	1.09	Bicyclo[3.2.0]hept-2-ene, 2-methyl-	
39	37.994	306136	1.36	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	
40	38.338	73733	0.33	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	
41	39.903	99154	0.44	Bicyclo[3.2.0]hept-2-ene, 2-methyl-	
42	41.166	88777	0.39		
43	41.710	1460232	6.49		
44	42.547	161820	0.72		
45	42.837	1779015	7.91		
46	45.585	164212	0.73	1-Octadecanol (CAS)	
47	46.124	116565	0.52		
48	46.602	1165995	5.18	Methyl stearate	
49	47.203	258338	1.15		
50	47.728	93953	0.42	1H-Pyrrole, 1-butyl-	

Table 2. Chemical composition of *L. nobilis* water extract

	E.coli	S.aureus	P.aeruginosa	B.cereus	C.albicans	C.tropicalis
	ATCC 25922	ATCC 29213	ATCC 27853	ATCC 11778	ATCC 10231	DSM 11953
<i>L. nobilis</i> water extract	2.5	5	>5	>5	>5	>5
<i>L. nobilis</i> ethanol extract	5	>5	>5	>5	>5	>5

Antimicrobial Activity Results

Table 3. Antimicrobial activities of L. nobilis extracts

The antimicrobial activity of water and ethanol extracts of laurel leaves are provided in Table 3. When the MIC values were $\leq 0.1 \text{ mg/mL}$, the substance is identified as important; if they have MIC values of 0.1 < MIC $\leq 0.625 \text{ mg/mL}$, they are classified as moderately effective and the ones with a MIC value of > 0.625 mg/mL are classified as poorly effective [20-21]. Present findings revealed that laurel leaf extracts did

not have antimicrobial effects on 6 different microorganism strains.

Nutrient Concentrations Results

Macro and micronutrient concentrations of water and ethanol extracts of laurel plants collected from İzmir province are provided in Table 4.

Table 4. Some macro and micronutrient concentrations of L. nobilis leaf

Macro alamanta	N	Р	K	Ca	Mg	
Macro elements	(%)					
<i>L. nobilis</i> Leaf	1.68	0.72	1.12	2.40	0.45	
Micro elements	Fe	Zn	Mn		Cu	
(mg/kg)						
<i>L. nobilis</i> Leaf	147.4	13.6	32	6.3	27.8	

For macronutrients, nitrogen concentration was identified as 1.68% N, phosphorus concentration as 0.72% P, potassium concentration as 1.12% K, calcium concentration as 2.40% Ca, and magnesium concentration as 0.45% Mg. For micronutrients, iron concentration was identified as 147.4 mg/kg Fe, zinc concentration as 13.6 mg/kg Zn, manganese concentration as 326.3 mg/kg Mn, and copper concentration as 27.8 mg/kg Cu (Table 4). Saraç with co-workers [22] reported nutrient concentrations of knotweed plants collected from Sivas region as 3.5% N, 0.259% P, 3.9% K, 0.51% Ca, 0.44% Mg, 144.7 mg/kg Fe, 40.3 mg/kg Zn, 30.1 mg/kg Mn and 7.5 mg/kg Cu. In another study, macro and micronutrient concentrations of redweed plants were reported as 3.25% N, 0.110% P, 2.13% K, 0.44% Ca, 0.10% Mg,

205.9 mg/kg Fe, 21.1 mg/kg Zn, 22.7 mg/kg Mn and 6.1 mg/kg Cu [23].

Antioxidant Activity Results

Antioxidant potentials of medicinal plants generally come from their anti-scavenging activity on reactive oxygen species. The greater the antioxidant quantity is, the greater the therapeutic impact is [23]. In the present study, water and ethanol extracts of *L. nobilis* plants had a good antioxidant capacity and extracts had oxidant substances. However, the oxidative stress index (OSI) is principally important in the assessment of antioxidant-oxidant loads of the plant extracts. *L. nobilis* extracts had low OSI values (Table 5).

Table 5. TAS, TOS, and OSI values of L. nobilis leaf extract

	TAS (mmol/L)	TOS (μmol/L)	OSI
L. nobilis water extract	4.839±0.102	3.712±0.076	0.075±0.063
L. nobilis ethanol extract	4.746±0.187	3.843±0.249	0.080±0.214

Values are presented as mean±SD; Experiments were made in 3 parallels.

In vitro Cytotoxic Activity Results

Different concentrations (1, 10, 100, and 1000 μ g/mL) of two different extracts of laurel plants were applied to 4 different cell lines and left for incubation for 24 hours. Cytotoxic effects of extracts on different cell lines (IC₅₀ values) are provided in

Table 6. IC_{50} values were generally quite greater than 100 µg/mL dose. Therefore, it is possible to state that present isolates did not have remarkable cytotoxic effects on experimented cell lines. While both extracts had a moderate cytotoxic effect on MCF-7 and U87 cell lines, they had a poor cytotoxic effect on HUVEC and L929 cell lines (Table 6).

Table 6. IC ₅₀ levels in different cell lines of a	L. nobilis extracts
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	IC-50 (μg/mL)					
	HUVEC MCF-7 U87 L92					
	Human umbilical vein endothelial cell line	Human breast adenocarcinoma cell line	Human gliablastoma cell line	Mouse fibroblast cell line		
<i>L. nobilis</i> water extract	459.63	178.84	285.905	415.70		
<i>L. nobilis</i> ethanol extract	548.12	237.48	621.631	472.41		

As it was in the world, medicinal and aromatic plants, their extracts and drugs are widely used in treatment of various diseases in Turkey. There is still limited information available about the biological effects and impact mechanism of extracts of medicinal plants. In this study, nutrient concentrations of L. nobilis plants were investigated and their nutritive values, antimicrobial, antioxidant, and anticancerogenic characteristics were assessed in an integrated fashion. With IC₅₀ values of 237.48 µg/mL (ethanol extract) and 178.84 µg/mL (water extract) on MCF-7 cell line, present extracts reduce the cell vigor in breast cancer cell line (MCF-7), but slightly affected cell vigor in human healthy endothelial cell line (HUVEC). With an IC₅₀ value of 285.905 μ g/mL, water extracts of L. nobilis plants had a moderate cytotoxic effect on U87 cell line. For rat fibroblast cell line, plant extracts did not have any harmful cytotoxic effects. Since a natural plant extract did not generate any damages on healthy cells, but had cytotoxic effects on cancerous cells, it was thought that these extracts could potentially be used in the development of drug preparations.

On the other hand, both water and ethanol extracts of L. nobilis plants did not have antimicrobial effect on the experimented microorganism (MIC > 0.625 mg/mL). However, there are several other microorganisms to be investigated for antimicrobial effects of these extracts. Antimicrobial effects on the other microorganisms could be investigated to better elucidate the antimicrobial activity of these extracts. Different from the present findings, antimicrobial effects of essential oils of L. nobilis plants collected from different countries, especially on S. aureus, C. albicans, E. coli-like microorganisms were reported [24-26]. In another study [27-28], antimicrobial effects of the extracts of L. nobilis plants collected from different provinces of Turkey on S. aureus and C. tropicalis-like various microorganisms were reported. In previous studies [24-26, 29], essential oils of L. nobilis plants were subjected to GC-MS analysis and 34-68 different components were identified. In the present study, 50 components were identified in water extracts and 43 components were identified in ethanol extracts of L. nobilis plants collected from Izmir province. In both extracts, 2,3 Butanediol was the major component. It is possible to identify different chemical compositions of the same plant just because of polarity of extraction solvent and geographical status of the plant collection locations [30] (Jamilah et al., 2012).

There are some earlier studies investigating antioxidant activity of different extracts of laurel plants [29, 31]. However, Rel Assay Diagnostic kits with the ability to yield outcomes at 99% confidence were used for the first time in the assessment of TAS, TOS, and OSI values of laurel extracts [32]. Antioxidant substances eliminate harmful reactions of free radicals and thus prevent degenerative diseases [23]. Present findings revealed that laurel extracts exhibit a high antioxidant capacity and low oxidative stress index thus they can be identified as a promising source of antioxidants. Therefore, they could be further investigated for their potential in obtaining and producing effective substances for the prevention of free radical-induced damages. Antioxidant activity of various other medicinal plants was investigated in previous studies. In a previous study, TAS of Mentha longifolia subsp. longifolia species, a medicinal and aromatic plant, was reported as 3.628, TOS value as 4.046 and OSI value as 0.112 [33]. In another study [34], TAS value of Salvia multicaulis plants was reported as 6.434, TOS value as 22.441, and OSI value as 0.349. When the present findings on antioxidant activity of laurel plants were compared with the above-mentioned plants, it was seen that TAS values were between these two plants, TOS and OSI values were quite lower than Mentha sp. and Salvia sp. plants. The water extract of laurel plants had a slightly greater antioxidant capacity than the ethanol extract.

Conclusions

In this study, antioxidant, oxidant, antimicrobial, and anticancerogenic potential of L. nobilis plants collected from İzmir province were investigated, nutrient concentrations were determined spectrophotometrically, and the chemical composition of water and ethanol extracts were investigated through GC-MS analysis. The findings indicate that the L. nobilis plant has a high antioxidant potential. Although the plant extracts did not have antimicrobial effect on experimented microorganisms, they had a moderate cytotoxic effect on the tested cell lines. This study conducted to investigate the bioactive pharmacological characteristics of laurel plants, is believed to provide significant contributions to existing literature. Present findings could be used in model molecules for further studies and the design of further pharmacognosy projects.

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Conflicts of interest

The authors declare no conflict of interest.

References

- Baytop T. Therapy by Plants in Turkey. İstanbul University Faculty of Pharmacy Press. No: 40, İstanbul, Turkey, (1984).
- [2] Kırbağ S., Kurşat M., Zengin F.K., Elazığ'da Tıbbi Amaçlar İçin Kullanılan Bazı Bitki Ekstraktlarının Antimikrobiyal Aktiviteleri, Fırat Üni. Doğu Araş. Derg., 3(3) (2005) 168-171.
- [3] Faydaoğlu E., Sürücüoğlu, M., Tıbbi ve Aromatik Bitkilerin Antimikrobiyal, Antioksidan Aktiviteleri ve Kullanım Olanakları, Erzincan Uni. J. Sci. and Tech., 6(2) (2013) 233-265.
- [4] Bilgin F., Şafak İ., Kiracıoğlu, Ö., Ege Bölgesinde Defne Üreticilerinin Profili, 1st International Non-Wood Forest Products Symposium, Trabzon, Turkey, Karadeniz Technical University (KTU): Faculty of Forestry, (2006) 60-65.

- [5] Ölmez F. N., Farklı Kaynatma Sürelerinde Defneden (*Laurus nobilis* L.) Elde Edilen Renkler ve Bazı Haslık Değerleri, *YYU J. Agri. Sci.*, 14(1) (2004) 35-40.
- [6] Karık Ü., Çiçek F., Oğur E., Tutar M., Ayas, F., Essential Oil Compounds of Turkey Laurel (*Laurus nobilis* L.) Populations, *Anadolu J. AARI*, 25 (2015) 1-16.
- [7] Özer, S., Ülkemizdeki Bazı Önemli Orman Tali Ürünlerinin Teşhis ve Tanıtım Kılavuzu. Orman Genel Müdürlüğü Yayını, Yayın 659, Ankara, (1987).
- [8] Hammer K.A., Carson C.F, Riley T.V., Antimicrobial Activity of Essential Oils and Other Plant Extracts, J. Appl. Microbio., 86 (1999) 985-990.
- [9] Cooper-Driver G.A., Contributions of Jeffrey Harborne and Co-workers to the Study of Anthocyanins, *Phytochem.*, 56(3) (2001) 229-236.
- [10] Longo L., Vasapollo G., Anthocyanins from Bay (*Laurus nobilis* L.) Berries, J. Agric. Food Chem., 53(20) (2005) 8063-8067.
- [11] Ayanoğlu F., Mert A., Kaya A., Köse, E., Hatay Yöresinde Doğal Olarak Yetişen Defne (*Laurus nobilis* L.) Bitkisinin Kalite Özelliklerinin Belirlenmesi ve Seleksiyonu. Tübitak Project Report No: 1080878, 268p, Hatay, Turkey, (2010).
- [12] Mata A.T., Proença C., Ferreira A.R., Serralheiro M.L.M., Nogueira J.M.F., Araújo, M.E.M., Antioxidant and Antiacetylcholinesterase Activities of Five Plants Used as Portuguese Food Spices, *Food Chem.*, 103(3) (2007) 778-786.
- [13] Murphy J., Riley J.P., A Modified Single Solution for the Determination of Phosphate in Natural Waters, *Analtica Chemica Acta.*, 27 (1962) 31-36.
- [14] Bremner J.M., Method of soil analysis, Part 2, Chemical and Microbiological Methods. Madison, USA: American Society of Agronomy Inc., (1965) 1149-1178.
- [15] Eloff J.N., A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria, *Planta Med.*, 64 (1998) 711–713.
- [16] CLSI, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard. 2nd ed., NCCLS document M27- A2. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, USA, (2002) 19087-1898.
- [17] CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard. 9th ed., CLSI document M07-A9. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania USA, (2012) 19087.
- [18] Erel O., A Novel Automated Direct Measurement Method for Total Antioxidant Capacity Using A New Generation, More Stable ABTS Radical Cation, *Clinical Biochem.*, 37(4) (2004) 277-285.
- [19] Erel O., A New Automated Colorimetric Method for Measuring Total Oxidant Status, *Clinical Biochem.*, 38(12) (2005) 1103-1111.
- [20] Kuete V., Potential of Cameroonian Plants and Derived Products Against Microbial Infections: A Review, *Planta Med.*, 76 (2010) 1479–1491.
- [21] Awouafack M.D., McGaw L.J., Gottfried S., Mbouangouere R., Tane P., Spiteller M., Eloff J.N., Antimicrobial Activity and Cytotoxicity of the Ethanol Extract, Fractions and Eight Compounds Isolated from *Eriosema robustum* (Fabaceae), *BMC Complementary and Alternative Med.*, 13(1) (2013) 1-9.
- [22] Saraç H., Daştan T., Demirbaş A., Daştan S.D., Karaköy T., Durukan, H., Madımak (*Polygonum cognatum* Meissn.) Bitki Özütlerinin Besin Elementleri ve In vitro Antikanserojen Aktiviteleri Yönünden

Değerlendirilmesi, SDU Ziraat Fakül. Derg., (2018a) 340-347.

- [23] Saraç H., Daştan T., Durukan H., Daştan S.D., Demirbaş A., Karaköy T., Kırmızı Gelincik (Fam: Papaveraceae, Glaucium grandiflorum Boiss.&Huet var. grandiflorum) Bitkisinin Farklı Özütlerinin Besin Elementi İçeriğinin ve In vitro Antiproliferatif Etkilerinin Değerlendirilmesi, SDU Ziraat Fakül. Derg., (2018b) 417-428.
- [24] Merghni A., Marzouki H., Hentati H., Aouni M., Mastouri M., Antibacterial and Antibiofilm Activities of *Laurus* nobilis L. Essential Oil Against Staphylococcus aureus Strains Associated With Oral Infections, Current Research in Translational Med., 64 (2016) 29-34.
- [25] Ivanović J., Mišić D., Ristić M., Pešić O., Žižović I., Supercritical CO₂ Extract and Essential Oil of Bay (*Laurus nobilis* L.) – Chemical Composition and Antibacterial Activity, J. the Serbian Chem. Soci., 75(3) (2010) 395-404.
- [26] Marzouki H., Khaldi A., Chamli R., Bouzid S., Piras A., Falconieri D., Marongiu B., Biological Activity Evaluation of the Oils from *Laurus nobilis* of Tunisia and Algeria Extracted by Supercritical Carbon Dioxide, *Natural Product Res., 23*(3) (2009) 230-237.
- [27] Digrak M., Alma M.H., Ilçim A., Antibacterial and Antifungal Activities of Turkish Medicinal Plants, *Pharmaceutical Bio.*, 39(5) (2001) 346-350.

- [28] Dadalioğlu I., Evrendilek G.A., 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, J. Agric. Food Chem., 52(26) (2004) 8255-8260.
- [29] Li R., Jiang S., Jiang Z.T., β-cyclodextrin Supramolecular Microcapsules and Antioxidant Activity of *Laurus nobilis* Essential Oil, *J. Essen. Oil Bearing Plants, 20*(6) (2017) 1511-1524.
- [30] Jamilah J., Sharifa A., Sharifah N.R.S.A., GC-MS Analysis of Various Extracts from Leaf of *Plantago major* Used as Traditional Medicine, *World Appl. Sci. J.*, *17* (2012) 67-70.
- [31] Simić M., Kundaković T., Kovačević N., Preliminary Assay on the Antioxidative Activity of *Laurus nobilis* Extracts, *Fitoterapia*, 74 (2003) 613-616.
- [32] Sevindik M., Investigation of Antioxidant/Oxidant Status and Antimicrobial Activities of *Lentinus tigrinus*, *Advan. in Pharma. Sci.*, (2018) vol. 2018, Article ID 1718025.
- [33] Sevindik M., Akgul H., Pehlivan M., Selamoglu Z., Determination of Therapeutic Potential of *Mentha longifolia* ssp. *longifolia*, *Fresen. Environ. Bull.*, 26 (2017) 4757-4763.
- [34] Pehlivan M., Sevindik M., Antioxidant and Antimicrobial Activities of Salvia multicaulis, Turkish J. Agri.-Food Sci. and Tech., 6(5) (2018) 628-631.