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Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil

Birgul Ozcan*[1], Mari Esen[1], M. Kemal Sangun[2], Arzu Coleri[3] and Mahmut Caliskan[1]

[1]Letters Biology Department, [2]Letters Chemistry Department, Faculty of Sciences,
Mustafa Kemal University, Hatay - 31040, Turkey
[3]Biology Department, Faculty of Science, Ankara University, Ankara - 06100, Turkey

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Abstract: *This study was carried out to determine the in vitro antimicrobial and antioxidant activities of the essential oil, seed oil, and methanolic extract of seed oil obtained from Laurus nobilis L. (Lauraceae). The methanolic extract of seed oil exhibited more effective antibacterial activity comparing to essential oil and seed oil. GC-MS analyses of the essential oil resulted in the identification of 25 compounds. 1.8-Cineol (44.72%), a-Terpinyl acetate (12.95%), Sabinene (12.82%) were the main components. The fatty acid composition was characterized with the high content of linoleic acid (40.79%) and lauric acid (38.08%). The 50% (IC50) inhibition activity of the essential oil on the free radical DPPH was determined as 94.655 mgml^{-1}, whereas IC50 value of methanolic extract of seed oil was found unstable. In the case of the linoleic acid system, oxidation of linoleic acid was inhibited by essential oil and methanolic extract of seed oil, which showed 64.28 and 88.76% inhibition, respectively. The inhibition value of the methanolic extract of seed oil was quite close to the synthetic antioxidant BHT, 92.46% inhibition.*

Key words*: Seed oil, Antimicrobial activity, Antioxidant activity, Laurus nobilis*
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Introduction

Since the ancient times, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Rios and Recio, 2005). These infections may be locally within the dermis and some can subsequently become generalized as a blood infection. Because of the side effect and the resistance that pathogenic microorganisms build against the antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Essawi and Sour, 2000). Plant volatile oils are generally isolated from nonwoody plant material by distillation methods, usually steam or hydrodistillation, and are variable mixtures of principally terpenoids, specifically monoterpenes, diterpenes, and sesquiterpenes. Terpenes are amongst the chemicals responsible for the medicinal, culinary and fragrant uses of aromatic and medicinal plants (Dorman and Deans, 2000). Known for their antiseptic, *i.e.* bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies (Bakkali *et al*., 2008).

The generation of reactive oxygen species (ROS) beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases such as atherosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS (Burits and Bucar, 2000). Currently used synthetic antioxidants have been suspected to cause or promote negative health effects, hence stronger restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants (Koleva *et al*., 2002). Many essential oils, more recently, have been qualified as natural antioxidants and proposed as potential substitutes of synthetic antioxidants in specific sectors of food preservation where their use is not in contrast with their aroma (Ruberto and Barata, 2000).

The *Lauraceae* comprise 32 genera and about 2000-2500 species. *Laurus nobilis* L., (bay) a member of the family named Apollo's Laurel in mythology, is a plant native to the Southern Mediterranean region including Hatay (Antioch) Turkey. The leaves of *L. nobilis* L. are traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence (Kivcak and Mert, 2002). The chemical composition and antimicrobial activities of the essential oil of *Laurus nobilis* L. has been analyzed before (Ozcan and Erkmen, 2001; Simic *et al*., 2003; Sangun *et al*., 2007). The essential oil of leaves have antimicrobial properties (Fiorini *et al*., 1997) and it is used as an antirheumatic, antiseptic, diaphoretic, digestive and diuretic. It is also used as a fragrance component in the cosmetics and food industry (Simic *et al*., 2003).

The current study was carried out to determine the *in vitro* antimicrobial and antioxidant activities of the essential oil, seed oil, and methanolic extract of seed oil obtained from *Laurus nobilis*. This study is the first report giving the composition, antimicrobial and

* **Corresponding author:** birgulozcan@gmail.com

antioxidant properties of the seed oil and methanolic extract of seed oil obtained from *Laurus nobilis*.

Materials and Methods

Plant material and isolation of the oils: The seeds and leaves of *Laurus nobilis* was collected in July from Harbiye (Daphne), Antakya-Hatay region in Turkey. Air dried leaves were hydrodistilled for 3 h using a Clevenger-type apparatus. Boron trifluoride-methanol reagent (Merck) was used for the esterification of fatty acids before the fatty acid analysis of seed oil by GC and GC/MS (Nikolaos *et al*., 1983). Seed oil was extracted by using (1/3) (v/v) oil/MeOH in ultrasonic bath for 15 min. Oil extract was filtered, and the filtrate was then evaporated under reduced pressure and dried using a rotary evaporator at 50°C. Dried extract and essential oil were stored in dark at +4°C until use. All solvents obtained from Merck, Darmstad, Germany.

The gas chromatography (GC) analyses were carried out using Hewlett-Packard 6890 GC with FID. A HP-5 MS capillary column (30 m x 0.25 mm *i.d.* 0.25 mm film thickness) was used. Helium was used as a carrier gas (1.4 ml min^{-1}). The column was temperature programmed as follows: 5 min at 45°C; then at 3°C min^{-1} to 220°C and held for 10 min. The injector and detector temperatures were to 220 and 250°C respectively. Samples (0.5 ml of the oil solution in hexane, 1:100) were injected by the splitless technique into Helium carrier gas. Peak areas and retention times were measured by Electronic Integration.

Gas chromatography mass spectrometry (GC/MS) analyses of the essential oils were carried out on Hewlett Packard 597°A mass selective detector (MSD), directly coupled to a HP 6890 GC. The column, temperature programme and injection were performed as described for GC. Library search was carried out using "Wiley Library, WILEY275, NBS75K, NIST98, FLAVOR". EI mass spectra were measured at 70eV ionization voltage over the mass range 10-400u.

Antioxidant activity:

DPPH assay: The DPPH test was carried out as described (Cuendet *et al*., 1997; Burits and Bucar, 2000). 50 ml of various dilutions of the extract was mixed with 5 ml of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm in Biospec-mini Shimadzu spectrophotometer (Shimadzu Biotech, Japan). Butylhydroxytoluene (BHT) and ascorbic acid were used as positive controls. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

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

A_{blank} is the absorbance of control reaction (without test compound) and A_{sample} is the absorbance of test compound. The concentration of extract which provide the 50% was calculated from the graph plotting inhibition percentage against extract concentration.

β-Carotene/linoleic acid assay: This assay was used to visual instrumentally bleaching of the carotene/linoleic acid solution (Dapkevicius *et al*., 1998). The model test mixture was prepared by dissolving 0.5 mg β-carotene in 1 ml chloroform, 25 µl of linoleic acid and 200 mg Tween 40 were added to the β-carotene solution. Chloroform was removed using a rotary evaporator at 50°C. 100 ml of distilled water saturated with oxygen during 30 min, flow rate 100 ml min^{-1}, was added and the mixture was vigorously shaken. 250 µl of this model test mixture was distributed in each of the test tube. 35 µl of an ethanolic solution of the extract was added to each test tube. The test tubes were then placed in an incubator at 55°C for 48 hr. After incubation period the absorbance was measured in Biospec-mini Shimadzu spectrophotometer (Shimadzu Biotech, Japan) at 490 nm. An equal amount of ethanol was used for the blank samples and BHT used as synthetic antioxidant as explained before (Dapkevicius *et al*., 1998).

Antimicrobial activity:

Microorganisms: The various extracts of *Laurus nobilis* were individually tested on different microorganisms, including *Staphylococcus aureus* ATCC 29213, *Staphylococcus .aureus* ATCC 25923, *Staphylococcus aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *Enterococcus gallinarium* CDC-NJ-4, *Enterococcus faecium* NJ-1, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* ATCC 19615, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* O157 H:7, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Salmonella typhi* NCTC 8394, *Haemophilus influenzae* ATCC 49247, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. Microorganisms were cultured overnight at 37°C.

Agar-well diffusion method: The seed oil and the methanolic extract of seed oil of *Laurus nobilis* were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 200 mg ml^{-1}. The wells (6 mm) were cut from the agar surface and each well inoculated with 50 µl of extract (10 mg $well^{-1}$) at a concentration of 200 mg ml^{-1} (Dulger and Gonuz, 2004). The inoculums were suspended in sterile saline and diluted according to 0.5 Mc Farland standards. They were "flood-inoculated" on to surface of MHA. After incubation period, 24 hr at 37°C, diameters of the zones were measured in millimetres.

Disc diffusion method: The agar disc diffusion method was employed for the determination of antimicrobial activity of essential oil of *L. nobilis* (NCCLS, 1997). The inoculums were suspended in sterile saline and diluted according to 0.5 Mc Farland standard and then spread on solid media plates. Empty paper discs (6 mm in diameter) were soaked with 15 µl of the essential oil and placed on the inoculated plates. These plates, after remaining 2 hr at 4°C, were incubated at 37°C for 24 hr. The diameters of the inhibition zones were measured in millimetres.

Determination of minimum inhibitory concentration (MIC): Broth microdilution assay was used for determination of the MIC (NCCLS, 1999). The tests were performed in Mueller Hinton broth

supplemented with Tween 80 detergent. Overnight grown culture was used in broth microdilution assay. Geometric dilution was used ranging from 0.25 mg ml^{-1} to 36 mg ml^{-1}. The appropriate dilutions were inoculated on to plate and incubated at 37°C for 24 hr. The presence of colony on the surface of agar indicated the bacterial growth.

Results and Discussion

The current study was carried out to determine the *in vitro* antimicrobial and antioxidant activities of the essential oil, seed oil, and methanolic extract of seed oil. While the antimicrobial activities of essential oil were determined by disc diffusion method, the activities of the other extracts were determined by agar well method, on the gram positive and gram negative bacteria and one fungus. The possible antioxidant activities of the essential oil and methanolic extract of seed oil of *Laurus nobilis* were performed with two complementary test systems, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. The chemical composition of the essential oil and seed oil was determined by GC/MS.

Chemical composition of the essential oil and seed oil: As shown in Table 1, GC-MS analysis of the essential oil from leaves of *L. nobilis* resulted in the identification of 25 compounds. 1.8-cineol (44.72%), α-terpinyl acetate (12.95%), sabinene (12.82%) were the main components. The composition of essential oil of *L. nobilis* has also been reported previously (Fiorini *et al*., 1997; Simic *et al*., 2004; Dadalioglu and Evrendilek, 2004; Sangun *et al*., 2007). In conclusion our study gave the results that close to those in the literature, with 1.8-cineole, sabinene and a-terpinyl acetate as the major components. The fatty acid composition of seed oil of *L. nobilis* has been represented in Table 2. The linoleic acid and lauric acid

Table - 1: Chemical composition of the essential oil from leaves of *Laurus nobilis* harvested from Daphne-Antioch.

Components	RRI	%	Components	RRI	%	Components	RRI	%
α-Thujene	1110	0.50	Trans-Sabinene hydrate	1376	1.37	β-Elemene	2368	0.95
α-Pinene	1119	3.44	Cis-Sabinene hydrate	1394	0.59	β-Caryophyllene	2444	t
Camphene	1157	0.47	α-Terpinolene	1425	t	Methyl eugenol	2451	3.35
Sabinene	1185	12.82	Linalool	1475	0.78	α-Humulene	2543	t
Myrcene	1219	0.77	Terpinen-4-ol	1663	3.38	Caryophyllene oxide	2666	2.24
α-Phellandrene	1236	t	α-Terpinenol	1712	7.85	Calamenene	2688	0.51
1.8-Cineole	1293	44.72	α-Terpinyl acetate	2243	12.95	β-Eudesmol	2822	1.85
Trans-b-osimen	1312	t	Eugenol	2275	t	α-Eudesmol	2824	1.16
γ-Terpinene	1350	0.3						

t = Trace amount, RRI = Relative retention index

Table - 2: Chemical composition of the fatty acid of seed oil from *Laurus nobilis* harvested from Daphne-Antioch.

Fatty acid components	%
Caprinic acid	0.99
Lauric acid	38.08
Myristic acid	2.66
Palmitic acid	14.33
Linoleic acid	40.79
Stearic acid	1.53
Eicosanoic acid (Arachidic)	1.62

Table - 3: Antioxidative capacities of the essential oil and methanolic extract of seed oil of *L. nobilis* measured in DPPH and β-carotene/linoleic acid assays

Extract and controls	DPPH[a] (mg ml^{-1})	β-Carotene / linoleic acid [b]
Essential oil	94.655	64.28
Methanolic extract of seed oil	Changeable	88.76
BHT	1.52	92.46
Ascorbic acid	0.45	NT[c]

[a]IC50 values of DPPH assay, [b]% inhibition rate of linoleic acid oxidation, [c]Not tested

Table - 4: Antimicrobial activity of the essential oil, seed oil and the methanolic extract of seed oil of *L. nobilis* using agar-well, disc diffusion (DD), and minimum inhibitory concentration (MIC) methods.

Microorganisms	Essential oil		MeOH	Seed
	DD[a]	MIC[b]	extract[c]	oil[c]
S. aureus ATCC 29213	10	3	11.33	8.66
S.aureus ATCC 25923	11	4.5	12.33	10
S.aureus ATCC 43300 (MRSA)	11	10	12.66	8.33
B. subtilis ATCC 6633	11	3	12	10.33
E. gallinarium CDC-NJ-4	8	6.5	7.66	NA
E. faecium NJ-1	9	4.5	7.66	NA
E. faecalis ATCC 29212	9	4	7	NA
L. monocytogenes ATCC 7644	9	1	12	7
S. pyogenes ATCC 19615	8	4.5	7.33	NA
E. coli ATCC25922	10	4	NA	NA
E. coli ATCC 35218	10	4	NA	NA
E. coli O 157 H:7	11	2.5	NA	NA
H. influenzae ATCC 49247	11	6	15.33	9.66
P. aeruginosa ATCC 27853	-	6	NA	NA
S. typhi NCTC 8394	11	1.5	NA	NA
C. albicans ATCC 10231	11	0.1	12	12

[a] = Diameter of inhibition zone (mm) including disc diameter of 6 mm, [b] = Minimum inhibitory concentration values given as mgml^{-1} for the essential oil, [c] = Agar well diffusion method, including well diameter of 6 mm, NA = Not active

Table - 5: Antibiotic susceptibility tests

Microorganisms	Antimicrobial agents*										
	Nst. (100 U)	TE (30 µg)	AMP (10 µg)	P (10 U)	E (15 µg)	NOR (10 µg)	C (30 µg)	RD (5 µg)	SXT (25 µg)	N (30 µg)	S (25 µg)
S. aureus ATCC 29213	NT	20	33	33	24	19	22	28	22	15	13
S.aureus ATCC 25923	NT	20	24	22	23	20	13	22	23	16	14
S.aureus ATCC 43300 MRSA	NT	21	9	11	-	17	24	24	23	9	15
B. subtilis ATCC 6633	NT	20	18	20	21	29	21	15	31	20	21
E. gallinarium CDC-NJ-4	NT	8	25	19	-	21	11	15	20	-	-
E. faecium NJ-1	NT	-	16	-	11	15	13	21	-	-	-
E. faecalis ATCC 29212	NT	11	27	22	17	19	18	17	20	15	15
S. pyogenes ATCC 19615	NT	12	23	20	15	18	16	19	18	15	16
L. monocytogenes ATCC 7644	NT	22	26	24	25	17	25	25	18	17	17
E. coli O157 H:7	NT	19	17	-	14	28	19	11	30	15	15
E. coli ATCC 25922	NT	20	15	-	10	30	21	12	27	11	13
E. coli ATCC 35218	NT	15	-	-	11	25	9	12	16	12	-
S. typhi NCTC 8394	NT	20	25	13	-	33	30	-	31	15	15
H. influenzae ATCC 49247	NT	20	18	15	25	29	23	40	22	10	16
P. aeruginosa ATCC 27853	NT	10	-	-	-	25	-	-	-	8	18
C. albicans ATCC 10231	16	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

* = Values in parenthesis indicates dose of antibiotics, NST = Nystatin, TE = Tetracycline, AMP = Ampicillin, P = Penicillin, E = Erythromycin, NOR = Norfloxacine, C = Chloramphenicol, RD = Rifampicin, SXT = Trimethoprim sulfamethoxazole, NEO = Neomycin, S = Streptomycin, NT = Not tested. Diameter of inhibition zone (mm) including disc diameter of 6 mm

were found as the major components with a value of 40.79 and 38.08% respectively.

Antioxidant activity: The potential antioxidant activity of the essential oil and methanolic extract of seed oil from *L. nobilis* were determined by employing two complementary tests, namely DPPH free radical scavenging and β-carotene/linoleic acid test systems. Total antioxidant activity of the plant extracts are recommended to carry out by employing two or more methods (Politeo *et al*., 2007). Based on this recommendation, in the current work two complementary test systems were used to evaluate the antioxidant properties of the essential oil and methanolic extract of seed oil of *L. nobilis* L. In both test systems the essential oil and the methanolic extract of seed oil of *L. nobilis* L. exhibited antioxidant properties; however, in the DPPH system it was not possible to get a stable value for the methanolic extract of seed oil. Free radical scavenging properties and the inhibition effects on the linoleic acid oxidation of methanolic extract and essential oil of *L. nobilis* is given in Table 3. The IC50 value of essential oil is 94.65 mg ml^{-1} which is higher than the synthetic antioxidant agents, BHT (1.52 mg ml^{-1}) and ascorbic acid (0.45 mg ml^{-1}), whereas IC_{50} value of methanolic extract of seed oil was found changeable from one work to others. In the β-carotene/linoleic acid assay, the essential oil and methanolic extract seed oil seemed to inhibit the oxidation of linoleic acid with the value of 64.28 and 88.76% inhibition respectively (Table 3). It is worth to mention that the value of the methanolic extract of seed oil (88.76%) is quite close to synthetic antioxidant, BHT (92.46%).

The results of this study confirm the previous results which have been showing the antioxidant properties of essential oil from *L. nobilis* L (Simic *et al*., 2003; Politeo *et al*., 2007). This is the first study which shows that the antioxidant property of the methanolic extract of seed oil is higher than the essential oil. Several studies have been conducted to clarify the possible substances involved in antioxidant properties of the essential oil. Among the identified compounds in the essential oil from laurel, methyl eugenol may be considered the main contributors to the antioxidant activity. The antioxidant activity of eugenol has been reported several times (Politeo *et al*., 2007). And there are reports that, monoterpene hydrocarbons (sabinene, α-terpinene) and oxygeneted monoterpenes (1.8-cineole, α-terpinenol, terpinen-4-ol) have shown antioxidant activity and it's likely that the activity of essential oil of *L. nobilis* L. is due to this compounds (Ruberto and Barata, 2000).

Antimicrobial activity: While the antimicrobial activity of the essential oil, seed oil and methanolic extract of seed oil was represented in Table 4, the susceptibility of the tested microorganisms against various antibiotics was represented in Table 5. In the current study the antimicrobial activities of essential oil was determined by disc diffusion and minimum inhibitory concentration methods. The activities of the other extracts were determined by agar well method. It was observed that the essential oil has more generalized activity against bacteria comparing to seed oil. For instance, it was seen that both seed oil and methanolic extract of seed oil did not show activity against Gram-negative bacteria except for *H. influenzae*. But they exhibited remarkable antimicrobial activity against Gram-positive bacteria. The methanolic extract of seed oil exhibited more effective antimicrobial activity comparing to the seed oil (Table 4). The antimicrobial (Dadalioglu and Evrendilek, 2004) and antifungal activity (Simic *et al*., 2004) of the essential oil of *L. nobilis* have been shown previously. They reported low antifungal activity. In the

current study we found that the essential oil, seed oil and methanolic extract of seed oil have almost the equal properties of antibacterial and antifungal activity.

The activity of the essential oils would be expected to relate to the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components (Dorman and Deans, 2000). The phenolic components of essential oils monoterpene hydrocarbons, oxygenated components showed the strongest antimicrobial activity, followed by aldehydes, ketones, and alcohols. There are some reports about antimicrobial activity of terpinen-4-ol, eugenol, and -linalool components (Ozcan and Erkmen, 2001; Simic *et al*., 2004).

In conclusion the essential oil, seed oil and methanolic extract of seed oil from *L. nobilis* exhibited antimicrobial and antioxidant activity. The methanolic extract of seed oil seemed to have more effective, antibacterial and antioxidant properties than the essential oil of leaves and the seed oil.

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References

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar: Biological effects of essential oils - A review. *Food Chem. Toxic*., **46**, 446-475 (2008).

Burits, M. and F. Bucar: Antioxidant activity of *Nigella sativa* essential oil. *Phytother. Res*., **14**, 323-328 (2000).

Cuendet, M., K. Hostettmann, O. Potterat and W. Dyatmiko: Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv. Chim. Acta*, **4**, 1144-1152 (1997).

Dadalioglu, I. and G.A. Evrendilek: Chemical composition and antibacterial effect of essential oils of Turkish Oregano (*Origanum minutifolium*), Bay Laurel (*Laurus nobilis*), Spanish Levander (*Lavandula stoechas* L.), and Fennel (*Foeniculum vulgare*), on common Foodborne pathogens. *J. Agric. Food Chem*., **52**, 8255-8260 (2004).

Dapkevicius, A., R. Venskutonis, T.A. van Beek and J.P.H. Linssen: Antioxidant activity of extracts obtained different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric*., **77**, 140-146 (1998).

Dorman, H.J.D. and S.G. Deans: Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. App. Microbiol*., **88**, 308-316 (2000).

Dulger, B. and A. Gonuz: Antimicrobial activity of some endemic *Verbascum*, *Salvia*, and *Stachys* species. *Pharm. Biol*., **42**, 301-304 (2004).

Essawi, T. and M. Sour: Screening of some Palestinian medicinal plants for antibacterial activity. *J. Ethnopharmacol*., **70**, 343-349 (2000).

Fiorini, C., I. Fouraste, B. David and M. Bessiere: Composition of the flower, leaf and stem essential oils from *Laurus nobilis* L. *Flavour Frag. J*., **12**, 91-93 (1997).

Kivcak, B. and T. Mert: Preliminary evaluation of cytotoxic properties of *Laurus nobilis* leaf extracts. *Fitoterapia*, **73**, 242-243 (2002).

Koleva, I., T. Van Beek, J.P.H. Linssen, A. Groot and L.N. Evstatieva: Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem. Analysis*, **13**, 8-17 (2002).

Nikolaos, B., N.B. Kyriakidis and G. Dionysopoulos: Preparation of fatty acid methyl esters from olive oil and other vegetable oils using aqueous hydrochloric acid-methanol. *Analyst*, **108**, 738-741 (1983).

NCCLS (National Committee for Clinical Laboratory Standards): Performance standards for antimicrobial disc susceptibility test. 6th Edn. Approved Standart. M2-A6 (1997).

NCCLS (National Committee for Clinical Laboratory Standards): Performance standards for antimicrobial susceptibility testing. 9th International Supplement, M100-S9 (1999).

Ozcan, M. and O. Erkmen: Antimicrobial activity of the essential oils of Turkish plant spices. *Eur. Food Res. Technol*., **212**, 658-660 (2001).

Politeo, O., M. Juki and M. Milos: Chemical composition and antioxidant activity of free volatile aglycones from Laurel (*Laurus nobilis* L.) compared to its essential oil. *Croatica Chemica Acta*, **80**, 121-126 (2007).

Rios, J.L. and M.C. Recio: Medicinal plants and antimicrobial activity. *J. Ethnopharmacol*., **100**, 80-84 (2005).

Ruberto, G. and M.T. Barata: Antioxidant activity of selected essential oil components in two lipid model system. *Food Chem*., **69**, 167-174 (2000).

Sangun, M.K., E. Aydin, M. Timur, H. Karadeniz, M. Caliskan and A. Ozkan: Comparison of chemical composition of the essential oil of *Laurus nobilis* L. leaves and fruits from different regions of Hatay, Turkey. *J. Environ. Biol*., **28**, 731-733 (2007).

Simic, A., M.D. Sokovic, M. Ristic, G.S. Jovanovic, J. Vukojevic and P.D. Marin: The chemical composition of some *Lauraceae* essential oils and their antifungal activities. *Phytother. Res*., **18**, 713-717 (2004).

Simic, M., T. Kundakovic and N. Kovacevic: Preliminary assay on the antioxidative activity of *Laurus nobilis* extracts. *Fitoterapia*, **74**, 613-616 (2003).