Medicinal and Aromatic Plant Science and Biotechnology ©2009 Global Science Books

In Vitro Cytotoxic, Antioxidant and Antimicrobial Activities of Essential Oil of Leaves of *Laurus nobilis* L. Grown in Egypt and its Chemical Composition

Salma Ahmed El-Sawi[1*] • Mohamed Elsayed Ibrahim[2] • Amal M. Ali[3]

[1] Pharmacognosy Department, National Research Center, Dokki, 12622, Cairo, Egypt
[2] Cultivation and Production of Medicinal and Aromatic Plants Dept, National Research Center, Dokki, 12622, Cairo, Egypt
[3] Microbial Chemistry Department, National Research Center, Dokki, 12622, Cairo, Egypt

Corresponding author: * Selsawi7@yahoo.com

ABSTRACT

In this study, the essential oil (EO) of leaves of *Laurus nobilis* L. (family Lauraceae) was evaluated as a new source of EO under Egyptian conditions. The EO was extracted by hydrodistillation yielding 0.5-0.8% (v/w). The EO was analyzed by GC/MS. Fifty three compounds were identified accounting for 96% of the total EO constituents. The fresh EO contains approximately 50.38% 1,8-cineole. Other major oxygenated monoterpenes were α-terpenyl acetate (19.97 %) and 4-terpineol (6.48%). α-Terpenine, γ-eudesmole, α-terpineole, and 3-carene were identified in concentrations above 1%. Minor qualitative and quantitative differences were reported in the constituents of the leaf EO after cold storage (4°C) for one year. The *in vitro* cytotoxicity of the EO on five human cancer cell lines (Hela, U-251, HepG2, MCF7 and H460) was examined. The EO was found to be very active against all five cell lines tested. The EO was also found to possess antioxidant activity as demonstrated by the 1-diphenyl-2 picrylhydrazyl (DPPH) radical method. The antimicrobial activities of the EO were tested using the inverted Petri-plate method. The EO showed prominent antimicrobial activities at a very low concentration (100 µl).

Keywords: antibacterial, antifungal, bay laurel, 1,8-cineol, electron spin resonance, human cancer cell lines

INTRODUCTION

As a part of an intensive screening program to introduce new species of medicinal and aromatic plants to Egyptian cultivation and industry, we decided to evaluate the plant *Laurus nobilis* L. (bay laurel) as a new source of essential oil (EO) under Egyptian conditions. A comparison between the composition of the fresh and stored EOs from leaves was carried out. Also, we aimed to evaluate the antibacterial, antioxidant and cytotoxic activities of both EOs.

Bay laurel is an evergreen shrub belonging to the family Lauraceae. It is native to the southern Mediterranean region and is widely cultivated mainly in Europe and the USA as an ornamental plant (Garg *et al*. 1992).

Bay leaf has traditionally been used as a herbal medicine to treat rheumatism, earaches, indigestion, sprains, to promote perspiration (Heinerman 1983), or more recently in treating diabetes and preventing migraine (Duke 1997). They have anticonvulsive and antiepileptic activities (Sayyah *et al.* 2002). The alcoholic extract of leaves has a radical scavenging activity; leaves and fruit are excite-aromatic and they were used as nerve agent against hysteria and emmenagogue; infusion of the fruits possesses diuretic and carminative properties; fatty oil from fruit is used externally for treatment of furuncles, sprains, bruises and rheumatism and as an insect repellent (Simic *et al.* 2003).

There are many studies on the chemical composition of the EO obtained from the leaves of Mediterranean and European *L. nobilis*: Turkey (Ozcan and Chalchat 2005; Sangun *et al.* 2007), Cyprus (Yalçin *et al.* 2007), Lebanon (Loizzo *et al.* 2008), Iran (Verdian-rizi and Hadjiakhoondi 2008) and Tunisia (Marzouki *et al.* 2009).

To the best of our knowledge, only two investigations of the EO of *L. nobilis* from Egypt (Baghdadi *et al.* 1992; Soliman *et al.* 1994) were carried out.

MATERIALS AND METHODS

Plant materials

The leaves of 10-year-old *L. nobilis* shrubs grown in the National Research Centre garden at Cairo-Egypt were collected in May (vegetative stage).

Plant extraction

The EO of the fresh leaves was extracted by hydro-distillation for 3 hr. The EO was dehydrated over anhydrous sodium sulfate and subjected to GC/MS analysis.

Chemical analysis

1. Gas Chromatography/Mass Spectrometry

A GC-MS system (FID Hewlett-Packard 5989) equipped with library software (Wiely138 and NBS75 database) was used. A capillary DB5 (methyl-silicone containing 5% phenyl groups) column (30 m × 0.25 mm i.d.) was used. Temperature program: 2 min at 60°C, 60-100°C (2°C/min) and 100-250°C (5°C/min). Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Injection volume: 1.0 µl at a 1:50 split. A mass spectrometer (EI-MS 70 eV) was used with a scan mass range of 40-350 u.

2. Identification of components

The chemical constituents of *L. nobilis* EO were identified based on the data base of mass spectra from the MS library (software Wiely138 and NBS75 database). The obtained data were confirmed by injecting the authentic samples of the different components in GC-MS under the same conditions and in comparison also with the data obtained from the literature (Adams 1995).

Received: 8 April, 2009. Accepted: 1 July, 2009.

Original Research Paper

Biological activities

1. Microbiological techniques

Microbial strains: The antimicrobial activity of the EOs was tested against four bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus*), four fungal strains (*Aspergillus niger*, *Aspergillus flavus*, *Maccrophomina phasioli* and *Botryties allii*) and six yeast strains (*Candida pseudotropicalis*, *Candida albicans*, *Saccharomyces cerevisiae*, *Saccharomyces carlspergensis*, *Saccharomyces chevallii* and *Rhodotorula minuta*). Test organisms used were obtained from the Faculty of Agriculture, Cairo University.

2. Agar diffusion method

This method was carried out according to Collins and Lyne (1985). Nutrient agar (NA) was used for the cultivation of bacteria and yeast, and Czapek-Dox's medium (Dox 1910) for cultivation of fungal species. In this method, pre-sterilized Whatman No.1 filter paper discs (0.5 mm in diameter) (Whatman International Ltd., Maidstone, England) were impregnated with 100 µl of the EO and applied on the surface of agar plates freshly seeded with standard inocula of young cultures, 24-hrs-old bacteria and yeast, and 7-days-old fungi. The plates of test organisms were then incubated at 27°C for 24 hrs for bacteria and yeast and for 48 hrs for fungi. At the end of the incubation period, the inhibition zones were measured (results are the average of triplicate measurements).

3. Determination of minimum inhibitory concentration (MIC)

MIC values were determined by testing representative organisms in liquid NA (for bacteria and yeast) and Czapek-Dox solutions, using the method reported by Gardner and Provine (1984). Stock solutions of known concentrations of the tested EOs were prepared and serial dilutions (ranging from 333.33 to 0.016 mg/ml) were made. The assay tubes were inoculated with standard inocula prepared from young cultures. The minimum dilution in which no visible growth was observed is the lowest inhibitory concentration of the extract. MIC were determined as µg/ml media after 24 hrs incubation at 27 ± 1°C for bacteria and yeast and after 48-72 hrs for fungi.

4. Measurement of potential cytotoxicity by SRB assay

Potential cytotoxicity of leaf EO was tested using the method of Skehan *et al.* (1990). Cells were plated in a 96-multiwell plate (Cellstar greiner bio-one, Germany) (10^4 cells/well) for 24 hrs before treatment with the EO to allow attachment of cells to the wall of the plate. Different concentrations of the EOs (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.

Monolayer cells were incubated with the EOs for 48 hrs at 37°C and in a 5% CO_2 atmosphere. After 48 hrs, cells were fixed, washed and stained with sulforhodamine B stain (Sigma-Aldrich, St. Louis, USA). Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer (Sigma-Aldrich, USA).

Color intensity was measured in an ELISA reader (Tecan Group Ltd., Sunrise, Germany). The relation between surviving fraction and EO concentration was plotted to obtain the survival curve of the tumor cell line after treatment.

5. Electron spin resonance (ESR) measurements: (DPPH) radical scavenging activity

The potential antioxidant activity of the EO was assessed on the basis of scavenging activity of the stable DPPH free radical. Reaction mixtures contained test samples (5-20 mg/ml) dissolved in ethanol, and an equal volume of DPPH ethanolic solution (5×10^{-4} M) (Calliste *et al.* 2001).

$$\text{The inhibition ratio} = \frac{ref - extract}{ref} \times 100$$

Statistical analysis

All values of the antimicrobial activity were expressed as the mean of inhibition zones (mm) with three replicates for each treatment. Values of the antioxidant activity were the means of three measurements of the double integration area produced by ESR measurements. Data were subjected to a paired-sample *t*-test using SPSS (ver. 9.0). $P<0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Chemical composition

The EO components of *L. nobilis* cultivated in Egypt were identified by GC/MS. The EO yield on a fresh weight basis ranged between 0.5-0.8% (v/w). Fifty three components were identified representing approximately 96.48% of the EO constituents (**Table 1**). The leaf EO was characterized by high amounts of 1,8-cineole (50.38%), α-terpinenyl acetate (19.97%), 4-terpineol (6.84%) and sabinene (4.82%). Lower amounts of γ-eudsmol (2.41%), α-pinene (1.97%), γ-terpinene (1.91%), α-terpineol (1.99%) and spathulenol (1.35 %) were detected. Significant amounts of some other terpenoid constituents such as α-terpinene, terpinolene, isobornyl acetate, β-elemene and caryophyllene oxide were identified. Other components were detected in lower amounts (**Table 1**).

The overall view of the obtained data showed that EO of the Egyptian *L. nobilis* was rich in oxygenated monoterpenes, which recorded 80.19%. 1,8-cineol and α-terpinenyl acetate represented the main oxygentated monoterpenes. The total hydrocarbon terpenes (monoterpenes and sesquiterpenes) recorded 11.31% including sabinene as the major constituent of the monoterpene hydrocarbons, in addition to α-pinene and γ-terpinene, which represented 1.97 and 1.91%, respectively. Sesquiterpene hydrocarbons were found in low amounts (1.03%), while the oxygenated sesquiterpenes recorded 4.72% of the EO. γ-eudesmol was the major oxygenated sesquiterpene followed by spathulenol.

The yield and composition of laurel leaf EO from different countries are listed in **Table 2**. Except for the Turkish EO, the yield of the EO ranged from 0.2 to 0.78% which means that the Egyptian EO had a high yield. Laurel EOs from different countries were characterized by high contents of monoterpenes (70-90%), while the concentration of sesquitrepenes was much lower (1-15%). These ranges were in agreement with those of the Egyptian EO. The major constituent in all EOs is 1,8-cineole, and its concentration is within 22 to 68%, so the Egyptian leaf EO had a high content which is important for the characteristic flavor of this spice. α-Terpeninyl acetate, another flavor-important compound in laurel was detected at a higher concentration (19.97%) than that reported in other EOs in the concentration ranges of 8.8 to 11%. The concentration of 4-terpineol (6.84%) and sabinene (4.82%) in the Egyptian EO is in accordance with EO from the Mediterranean region. The presence of α-pinene, phellandrene, neryl acetate, β-elemene, limonene, β-selinene and γ-cadenine previously detected (Guenther 1949; Gildmeister and Hoffman 1959; Masada 1976), were also approved in the sample under investigation.

On the other hand, linalool and eugenol, which were reported by Quijano *et al*. (2007) in the concentration ranges of 6 to 18%, and 1 to 6%, respectively were not present in the Egyptian EO. Borneol, linalyl acetate, β-terpineol, geraniol, nerol, eugenol acetate, *p*-cymene, myrcene, and methyl eugenol previously detected (Guenther 1949; Gildmeister and Hoffman 1959; Masada 1976) were not found in the sample under investigation. Volatile organic acids *viz*. acetic, propionic, butyric, valeric, caproic, pelargonic and enanthic acids were also reported (Guenther 1949; Gildmeister and Hoffman 1959; Masada 1976) as constituents in the EO of laurel. They were not detected in the sample.

There are major differences between our study and those reported by Baghdadi *et al.* (1992) and Soliman *et al.* (1994) carried out on leaf EO of *L. nobilis* grown in Egypt.

Table 1 GC-MS profile of fresh and stored leaf essential oil of *laurus nobilis* L. grown in Egypt.

Compound		Fresh oil	Stored oil
Monoterpene hydrocarbons			
1	α-thujene	0.11	0.17
2	α-pinene	1.97	4.08
3	Sabinene	4.82	3.60
4	β-pinene	0.03	0.07
5	α-phellandrene	0.14	0.16
6	3-carene	0.15	0.16
7	α-terpinene	0.40	0.43
8	*para*-cymene	0.00	0.05
9	Limonene	0.11	0.18
10	β-phellandrene	0.04	0.00
11	γ-terpinene	1.91	5.14
12	1,3,8-*para*-menthatriene	0.00	0.05
13	Terpinolene	0.45	4.54
14	*trans*-sabinene hydrate	0.15	0.00
	Total	10.28	18.63
Oxygen-containing monoterpenes			
15	1,8-Cineole	50.38	54.30
16	*cis*-Linalool oxide	0.11	0.18
17	linalool	0.00	0.05
18	*trans*-Pinocarveol	0.23	0.21
19	Pinocarvone	0.23	2.96
20	β-*Z*-terpineol	0.23	2.96
21	4-Terpineol	6.48	4.00
22	α-Terpineol	1.99	2.96
23	Myrtenal	0.00	0.08
24	*cis*-Carveol	0.01	0.00
25	Isobornyl acetate	0.43	0.44
26	Thymol	0.02	0.16
27	Sabinyl acetate	0.02	0.62
28	Carvacrol	0.02	0.13
29	α-Terpinenyl acetate	19.97	8.29
30	Neryl acetate	0.07	0.22
31	Geranyl acetate	0.00	0.10
	Total	80.19	77.66
Sesquiterpene-hydrocarbons			
32	β-Elemene	0.36	0.14
33	Caryophyllene	0.09	0.05
34	α-Humulene	0.06	0.00
35	Alloaromadendrene	0.02	0.04
36	Acoradiene	0.02	0.07
37	γ-Gurjunene	0.12	0.23
38	β-Chamigrene	0.08	0.63
39	Germacrene D	0.04	0.07
40	*Ar*-Curcumene	0.00	0.86
41	β-Selinene	0.12	0.23
42	Germacrene B	0.02	0.17
43	β-Bisabolene	0.02	0.17
44	γ-Cadinene	0.08	0.16
45	β-Sesquiphellandrene	0.00	0.21
	Total	1.03	3.03
Oxygen-containing sesquiterpenes			
46	Elemol	0.24	0.00
47	Spathulenol	1.35	0.04
48	Caryophyllene oxide	0.40	0.08
49	Globulol	0.17	0.00
50	β-Elemenone	0.02	0.00
51	Cubenol	0.07	0.04
52	$C_{15}H_{24}$	0.00	0.04
53	γ-Eudesmol	2.41	0.66
54	α-Cadinol	0.00	0.18
55	β-Bisabolol	0.00	0.35
56	Germacrone	0.06	0.33
57	Geranyl tiglate	0.00	0.08
	Total	4.72	1.80
Various compounds			
58	3-Hexenol	0.01	0.03
59	2-Heptanone	0.01	0.03
60	Tricyclene	0.00	0.02
61	Methyl salicylate	0.00	0.05
62	1-Dodecene	0.00	0.07
63	1-Tridecene	0.03	0.12
64	Methyl eugenol	0.00	0.08
65	Hexadecane	0.06	0.05
66	$C_{15}H_{22}O$	0.00	0.06
67	Phytol	0.02	0.18
68	Docosane	0.05	0.00
69	Tetracosan	0.05	0.00
	Total	0.23	0.69

In the first study 22 compounds were identified representing 96.7% of the total EO contents. The main constitutuent was 1,8-cineole (54.9%). Camphene (0.2%), myrcene (0.2%), 4-terpinene (0.6%), 4-cymene (0.7%), linalool (1.1%), linalyl acetate (0.2%), bornyl acetate (3.9%), citronellyl acetate (0.5%), borneol (10.8%), nerol (0.9%), geraniol (0.6%), caryophyllene epoxide (0.8%) and eugenol (0.8%) were detected in the laurel EO in this study, while they were not detected in our study. Moreover, there were big differences between Baghdadi *et al.*'s study and our study in the concentrations of the other constituents as α-pinene (3.9 and 1.97%, respectively), α-thujene (0.3 and 0.11%, respectively), β-pinene (3.6 and 0.03%, respectively), phellandrene (7.7 and 0.14%, respectively), limonene (2.5 and 0.11%, respectively), terpinolene (0.2 and 0.45%, respectively), caryophyllene (3.9 and 0.09%, respectively) and α-terpineol (2.1 and 1.99%, respectively). The other 44 compounds found in **Table 1** were not detected in Baghadi and *et al.*'s study.

In the second study (Soliman *et al*. 1994), 28 compounds were identified representing 98% of the EO composition. The major compound was 1,8-cinoele (37.58%) followed by *p*-cymene (19.83%). Beside *p*-cymene, there were 12 other compounds detected in the EO in this study and were not detected in ours namely, camphene (0.4%), myrcene (4.68%), *cis*-*p*-menth-2-en-1-ol (0.69%), *cis*-sabinene hydrate (0.57%), linalool (2.85%), citronellal (0.16%), *trans*-α-dihydoterpineol (1.48%), estragole (1.00%), *trans*-anethole (0.46%), bornyl acetate (1.98%), eugenol (1.48%) and methyl eugenol (2.33%). The concentrations of some compounds were similar to each other in Soliman *et al.*'s study and our study: α-pinene (2.02 and 1.97%, respectively), δ-carene (0.20 and 0.15%, respectively), carvacrol (0.02% for both), spathulenol (0.32 and 0.35%, respectively) and caryophyllene oxide (0.64 and 0.40%, respectively). On the other hand, there are major differences between the concentrations of the other compounds such as sabinene (0.4 and 4.8%, respectively), β-pinene (3.73 and 0.03%, respectively), γ-terpinene, (0.16 and 1.91%, respectively), terpinolene (0.12 and 0.45%, respectively), 4-terpineol (3.72 and 6.48%, respectively), α-terpineol (3.50 and 1.99%, respectively), sabinyl acetate (0.70 and 0.02%, respectively), α-terpinyl acetate (7.14 and 19.97%, respectively) and β-caryophyllene (0.18 and 0.09%, respectively). Thirty eight compounds were identified in our study that were not detected in Soliman *et al.*'s study (**Table 2**). The big differences between this study and the other Egyptian studies may be due to the difference in plant age or the use of more modern techniques.

Effect of cold storage

Terpenic constituents showed variable responses to storage conditions; some increased while others decreased during a course of 12 months cold storage. Monoterpene hydrocarbons increased from 10.68% in the fresh EO to 18.63% after storage. Slight variations were recorded between fresh and stored EOs in the case of oxygen-containing monoterpenes, as they decreased from 80.19% in fresh EOs to 77.66% after 12 months.

Regarding the sesquiterpene hydrocarbons, cold storage

Table 2 Parameters of *Laurus nobilis* leaf oil grown in different countries.

Country / Comparison	Croatia (Politeo *et al.* 2007)	Cyprus (Yalçin *et al.* 2007)	Italy (Flamini *et al.* 2007)	Italy (Macchioni *et al.* 2006)	Iran (Verdian-rizi and Hadjiakhoondi 2008)	Turkey (Ozcan and Chalchat 2005)	Colombia (Quijano *et al.* 2007)
Yield %	0.62	-	0.78	0.60	0.78	1.4-2.4	0.20
Main component	1,8-cineole (45.5%)	1,8-cineole (58.59%)	1,8-cineole (35.7%)	1,8-cineole (39.2%)	1,8-cineole (35.7%)	1,8-cineole (51.73-68.48%)	1,8-cineole (22.0%)
Other major constituents	methyl eugenol (10.0%), α-terpinyl acetate (9.1%), linalool (8.5%), sabinene (5.7%)	α-terpinyl acetate (8.82%), terpinene-4-ol (4.25%), α-pinene (3.39), β-pinene (3.25%), sabinene (3.32%)	*trans*-sabinene hydrate (9.7%), α-terpinyl acetate (9.3%), methyl eugenol (6.8%)	α-terpinyl acetate (11.3%), sabinene (10.6%), linalool (7.4%)	*trans*-sabinene hydrate (9.7%), α-terpinyl acetate (9.3%), methyl eugenol (6.8%)	α-terpinyl acetate (4.04-9.87%), sabinene (4.44-7.75%), α-pinene (2.93-4.89%), β-pinene (2.58-3.91%), terpinene-4-ol (1.33-3.24%), and α-terpineol (0.95-3.05%)	Linalool (16.4 %), α-terpinyl acetate (11.1%), β-caryophyllene (9.0%)
Monoterpenes	79.9	-	77.80	91.8	77.80	-	71.5
Sesquiterpenes	6.1	-	5.98	1.4	5.98	-	15.8
Number of identified compounds	22	81	37	-	37	-	112
% of the identified compounds	98.5	98.74	95.8	99.1	95.8	-	95

Table 3 Antimicrobial activities of fresh and stored leaf volatile oils of *Laurus nobilis* L grown in Egypt.

Test organism	Fresh essential oil (100 µl/disc)	Stored essential oil (100 µl/disc)	Control (100 µg/ml)
	inhibition zone (mm ± SE)		
Bacteria (Gram-negative)			
Escherichia coli	NA	NA	16± 0.6
Bacteria (Gram-positive)			
Staphylococcus aureus	35 ± 0.4*	35 ± 0.6*	22 ± 0.8
Bacillus subtilis	30 ± 0.6*	32 ± 0.7*	24 ± 0.5
Bacillus cereus	45 ± 0.4*,[a]	47 ± 0.2*,[a]	10 ± 0.0
Fungi			
Aspergillus niger	NA	NA	9 ± 0.3
Aspergillus flavus	NA	NA	20 ± 0.5
Maccrophomina phasioli	NA	NA	21 ± 0.7
Botryties allii	15 ± 0.3	NA	NT
Yeast			
Candida pseudotropicalis	35 ± 0.2**	30 ± 0.2	NT
Candida albicans	36 ± 0.2	36 ± 0.4	NT
Saccharomyces cerevisiae	32 ± 0.4	30 ± 0.1	NT
Saccharomyces carlspergensis	20 ± 0.2[a]	20 ± 0.6[b]	11 ± 0.2[a,b]
Saccharomyces chevallii	35 ± 0.8*	35 ± 0.3*	12 ± 0.1
Rhodotorula minuta	40 ± 0.6**	30 ± 0.3	NT

NA: not active. NT: not tested. Control: Amoxycillin for bacteria and Canestin for fungi and yeast.
Each value represents the mean of inhibition zones (mm) of three replicate ± SEM (standard error of means).
* significantly different from the reference drug at $p<0.05$ according to paired-sample *t*-test.
** significantly different from the stored EO at $p<0.05$ according to paired-sample *t*-test.
[a,b] the correlation and *t* can not computed because the standard error of the difference is zero.

of *Laurus* EO enhanced the accumulation of these components. Total sesquiterpene hydrocarbons increased from 1.03% at the start of the experiment to 3.03% after 12 months. This may be due to the transformation of other terpenes to sesquiterpene hydrocarbons during storage.

Tracing the fate of major constituents during storage course revealed variable sensitivities from each component to another. Stored EO samples showed increase in the number of compounds and slight differences in all major constituents, except α-terpinenyl acetate which decreased from 19.97% at the beginning of storage to 8.29% after storage.

Our results are consistent with those reported in the literature. Several studies such as those of Njoroge *et al*. (1996) on *Citrus junos*, El Nikeety *et al*. (2000) on parsley herb EO, Cesare *et al.* (2001) on the volaile fraction of basil leaves and Canbas *et al*. (2001) on hop pellets have revealed increasing of some terpenic constituents and decreasing of others during storage i.e. storage influenced terpenoids qualitatively and quantitatively, which depended on storage conditions (temperature, and storage period).

Antimicrobial activity

The antimicrobial activity of the EO was evaluated *in vitro* by using the disc diffusion and serial dilution methods against 14 microorganisms. The disc diameters of zones of inhibition (DD) and minimum inhibitory concentrations (MIC) of the fresh and stored EOs for the microorganisms tested are shown in **Tables 3** and **4**, respectively. The data obtained from the disc diffusion method indicated that *B. cereus* was the most sensitive Gram-positive bacteria tested, with the strongest inhibition zone (45 mm), followed by *S. aureus* and *B. subtilis* with strong inhibition zones (30-35 mm), while the Gram-negative bacterium *E. coli* was insensitive to the EO. Most of the fungi tested were insensitive to the EO, except for *B. allii*, which exhibited a moderate inhibition zone (15 mm). The EO exhibited high activity against all tested yeasts. The strongest activity was against *R. minuta* (inhibition zone = 40 mm) followed by *C. albicans* (inhibition zone = 36 mm). The EO had the same activity against *C. pseudotropicalis* and *S. chevallii* (35 mm) followed by *S. cerevisiae* (32 mm). The least activity was against *S. carlspergensis* (inhibition zone = 20 mm).

The results of the MIC determination (**Table 4**) indi-

Table 4 Minimal inhibitory concentrations of leaf volatile oil *Laurus nobilis* L. grown in Egypt.

Test organism	Leaf essential oil (mg/ml)
Bacteria (Gram-negative)	
Escherichia coli	NA
Bacteria (Gram-positive)	
Staphylococcus aureus	4.11
Bacillus subtilis	0.45
Bacillus cereus	0.15
Fungi	
Aspergillus niger	NA
Aspergillus flavus	NA
Maccrophomina phasioli	NA
Botryties allii	NT
Yeast	
Candida pseudotropicalis	0.45
Candida albicans	12.34
Candida carlspergensis	4.11
Saccharomyces cerevisiae	0.45
Saccharomyces chevallii	4.11
Rhodotorula minuta	0.45

cated that the EO inhibited all the sensitive microorganisms tested. *B. cereus* had the lowest MIC (0.15 mg/ml). *C. albicans* had the highest MIC value (12.34 mg/ml). *B. subtilis*, *C. pseudotropicalis*, *S. cerevisiae* and *R. minuta* had an equal MIC value (0.45 mg/ml), while *S. aureus*, *C. carlspergensis* and *S. chevallii* had a higher MIC value (4.11 mg/ml).

Except for *B. allii*, the stored EO exhibited the same trend as the fresh EO with slight changes in the inhibition zone diameters.

The antimicrobial activity of *Laurus* EO may be due to its monoterpene contents. Many monoterpenes have been found to be active against a variety of microorganisms. α- and β-pinene have been reported to have significant antimicrobial activity (Dorman and Deans 2000; Yff *et al.* 2002; Benkendorff *et al.* 2005). A mixture of terpinen-4-ol, α-terpineol, 1,8-cineole, and linalool (monoterpenes) has been shown to possess antibacterial activity against microorganisms isolated from the oral cavity, skin, and respiratory tract (Hammer *et al.* 2004). The development of *C. albicans* and *C. tropicalis* was also limited by a composition of monoterpenes, which included terpinen-4-ol, α-pinene, β-pinene, 1,8-cineole, linalool, and α-terpineol (Kêdzia *et al.* 2000).

It has been shown that microorganisms express a differentiated sensitivity to plant-derived agents. Tests have been performed on Gram-positive and -negative bacteria and also on fungi (Trombetta *et al.* 2005). In general, Gram-positive bacteria are more sensitive to terpenes than Gram-negative bacteria (Kêdzia *et al.* 2000). This is mainly determined by differences in the permeability, composition, and charge of the outer structures of the microorganisms. The mechanism of antimicrobial action of terpenes is closely associated with their lipophilic character. Monoterpenes preferentially influence membrane structures which increase membrane fluidity and permeability, changing the topology of membrane proteins and inducing disturbances in the respiration chain (Trombetta *et al.* 2005).

Athough α-terpinene was reported to have antifungal activity similar to that of commonly used antifungal drugs (Parveen *et al.* 2004), weak antifungal activity was realized because high doses of terpenes or terpenoids have to be used.

Since most of these compounds comprise a small percentage of the EO, therefore the main antimicrobial activity may be due to the total monoterpenes in the EO.

Antioxidant activity

Antioxidant activities of the fresh and stored EOs from *L. nobilis* were tested by the DPPH radical scavenging assay (**Fig. 1**). The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenypicrylhydrazine (non radical) with the loss of this violet color (Molyneux 2004). Free radical scavenging properties of the fresh and stored EOs are presented in **Table 5**. A lower IC_{50} value indicates higher antioxidant activity. The EO of *L. nobilis* exhibited remarkable antioxidant activities. The fresh EO showed higher scavenging ability on DPPH

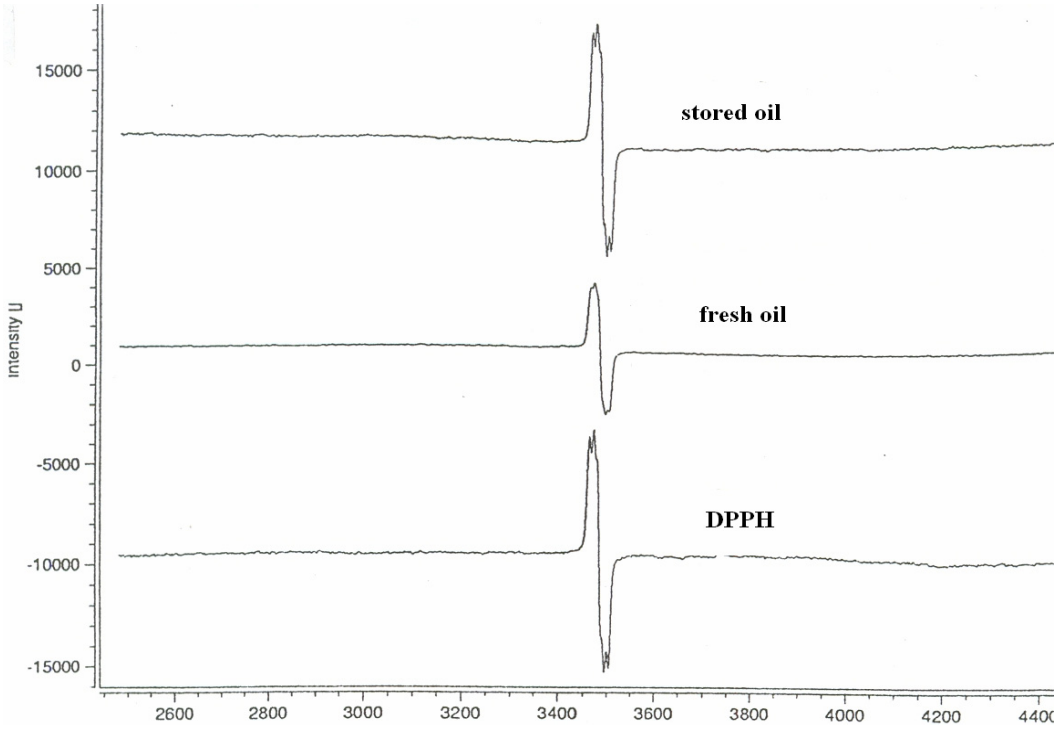

Fig. 1 DPPH radical scavenging assay of fresh and stored oils of leaves of *Laurus nobilis* grown in Egypt using electron spin resonance spectroscopy.

Table 5 Radical scavenging activities of fresh and stored leaf essential oils of *Laurus nobilis* L. grown in Egypt.

Substance	Concentration (mg/ml)	Double integration area	% peak inhibition	IC_{50} (mg/ml)
DPPH	0.5	4421614 ± 8.08	0%	-
Fresh leaf oil	0.5	2339034 ± 11.54*,***,***	47.1%	0.52
Stored oil	0.5	4110449 ± 5.20*,**	7.04%	NT
Ascorbic acid	0.5	0	100%	4.2×10^{-3}

DPPH: 2,2-diphenyl-1-picrylhydrazyl.
* significantly different from DPPH at $p < 0.05$ according to paired sample *t*-test
**significantly different from ascorbic acid at $p < 0.05$ according to paired sample *t*-test.
*** significantly different from stored oil at $p < 0.05$ according to paired sample *t*-test

radicals than the stored EO. DPPH scavenging abilities of the fresh EO (IC_{50} = 0.52 mg/ml) were lower than that of ascorbic acid ($IC_{50} = 4.2 \times 10^{-3}$ mg/ml).

The antioxidant activity of the EO may be due to its contents of 1,8-cineole and linalool. These monoterpenes have been reported to have antioxidant properties (Celik and Ozkaya 2002; Wang *et al.* 2008).

Cytotoxic activity

The growth inhibitory effects of the fresh and stored EOs on five human cancer cell lines were examined. The fresh EO exhibited marked growth inhibitory effects on all cell lines tested in a dose-dependent manner (**Fig. 2**), while there was a dramatic decrease in cytotoxic activity in the case of stored EO (**Fig. 3**). The IC_{50} values are shown in **Table 6**. The growth inhibition effects of the EO on human liver cancer cell lines were stronger than those on other cell lines, with IC_{50} value of 0.6 µg/ml. Among the remaining four human cancer cell lines breast cell line was of similar sensitivity to the EO to lung cell lines (IC_{50} value of 0.8 µg/ml), followed by brain cancer cell line with an IC_{50} value of 0.9 µg/ml. Cervix cell line exhibited the lowest sensitivity to the EO with an IC_{50} value of 1.8 µg/ml.

The cytotoxic activity of *L. nobilis* leaf EO may be attributed to the mono- and sesquiterpene contents of the EO. Many mono- and sesquiterpenes in the EO have been reported to have cytotoxic acivity. α-humulene showed activity against MCF-7, PC3, A-549, DLD-1, M4BEU and CT-26 cell lines (Legault *et al.* 2003). Kubo *et al.* (1996) and Sibanda *et al.* (2004) reported that caryophyllene oxide exhibited a modest cytotoxic activity on PC-3, MDA-MB-231, Hs 578T, MCF7, SK-MEL-28, and 5637 human tumour cells. Sesquiterpenes have been reported to be responsible for the cytotoxic activity of *Myrica gale* L. EO against lung carcinoma cell line A-549 and human colon adenocarcinoma cell line, DLD-1 (Sylvestre *et al.* 2005). β-elemene has been reported to have strong cytotoxicity angaist non-small-cell lung cancer cells (Wang *et al.* 2005). Monoterpenes are also helpful in the prevention and therapy of cancers as mammary, liver, and/or lung carcinogenesis (Reddy *et al.* 1997; Carvalho and Fonseca 2006). Among monoterpenes, d-limonene and α-pinene have been shown to possess chemopreventive and therapeutic properties against many human cancers. D-limonene exhibited anticancer activity against both spontaneous and chemically induced mammary tumors in rats, as well as liver, lung and forestomach tumors (Crowell and Gould 1994). It had also chemopreventive effect against breast and colorectal human cancers (Sun 2007). Tatman and Mo (2002) verified that α-pinene present cytotoxic activity against murine B16 melanoma and human HL-60 leukemia cells, while Setzer *et al.* (2000) reported that α–pinene was active against Hep-G2 and Sk-MEL-28 human tumor cell lines. However, the low concentration of the aforementioned compounds cannot fully explain the high cytotoxic activity of *L. nobilis* leaf EO, which means that the cytotoxicity of the EO could be due to the synergistic effects of these active chemicals with the main constituents of the EO as well as the antioxidant activity of the EO.

In the human body, the oxidant–antioxidant balance is critical because it maintains cell membrane integrity and

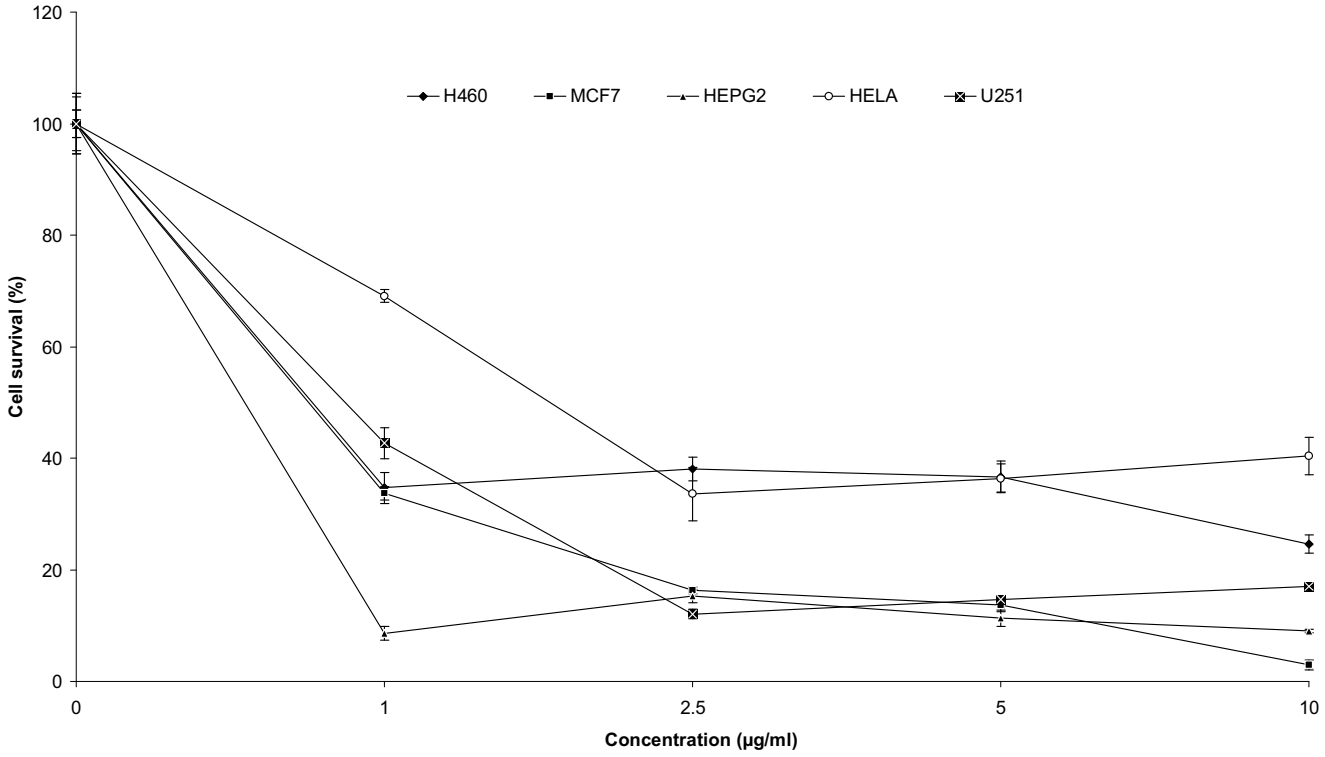

Fig. 2 Dose-dependent cytotoxic activity of fresh leaf oil of *Laurus nobilis* grown in Egypt against human tumor cell lines.

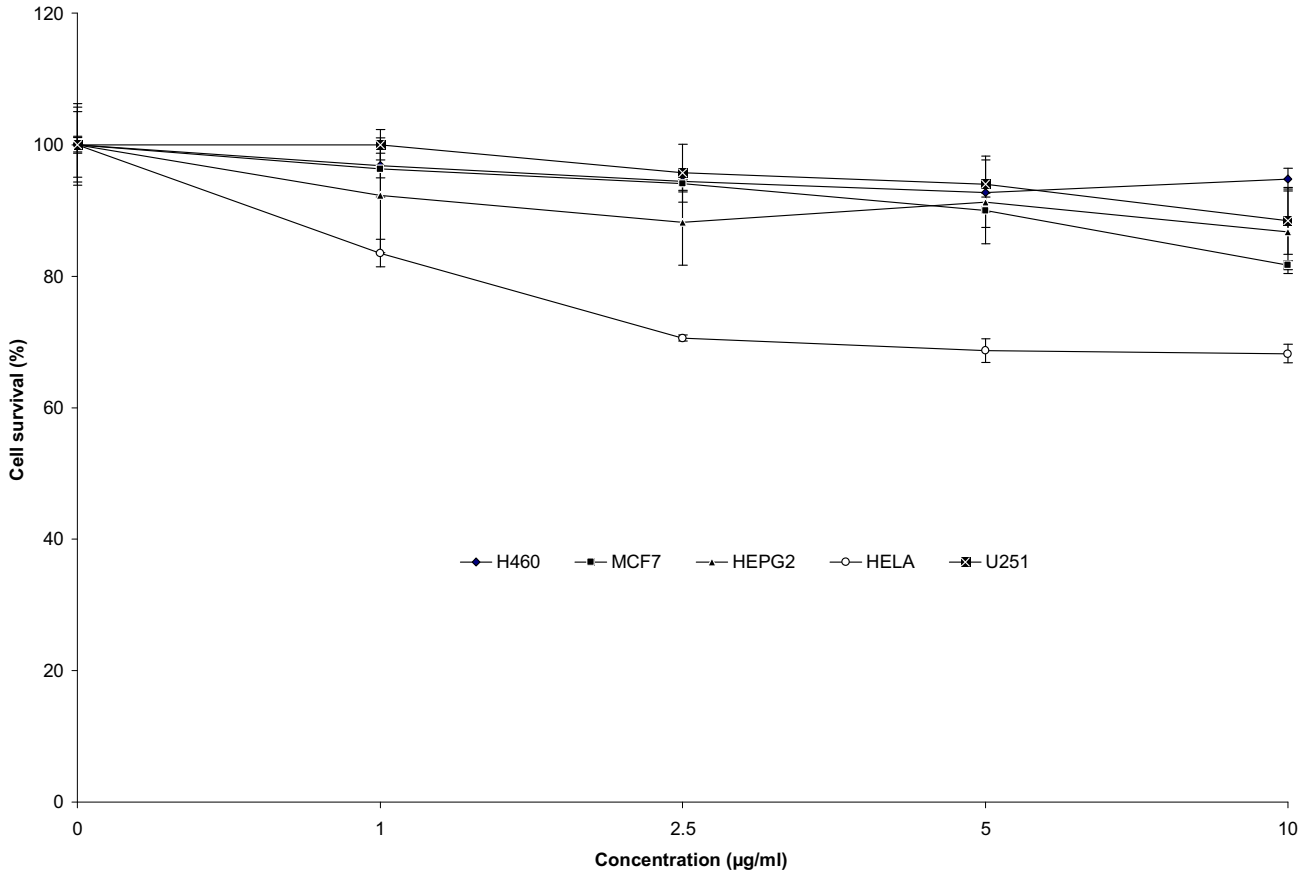

Fig. 3 Dose dependent cytotoxic activity of stored leaf oil of *Laurus nobilis* grown in Egypt against human tumor cell lines.

Table 6 IC_{50} of fresh and stored essential oils of of *Laurus nobilis* L against human tumor cell lines.

Extract	[a]IC$_{50}$				
	[b]U251	[c]H460	[d]Hepg2	[e]MCF7	[f]Hela
Leaf essential oil	0.9	0.8	0.6	0.8	1.8
Stored oil	> 10	> 10	> 10	> 10	> 10
Cisplatin	9.8	4.77	9.83	NT	2.37
Doxorubicin	NT	NT	0.6	0.7	0.85
5-flurouracil	NT	NT	0.67	NT	1.01

[a]extract concentration (µg/ml) required to reduce cell survival by 50%, [b]brain tumor cell line, [c]lung carcimona cell line, [d]liver carcinoma cell line, [e]breast carcinoma cell line, [f]cervix carcinoma cell line.

functionality, cell proteins, and nucleic acids (Knight 2000). In healthy humans, free radicals, such as reactive oxygen species levels are controlled; however, the concept of oxidative stress hypothesizes that exposure to adverse physiochemical, environmental, and pathological agents disrupts the body's natural balance, and if excess free radicals are not eliminated by antioxidants, they may damage crucial extracellular or cellular components. Examples of potential damage caused by oxidant–antioxidant imbalance include impaired cell functions, cell death, impaired immunity, and DNA damage, which can cause mutations and ultimately contribute towards the development of chronic diseases, such as cancer (Sies 1986; Serafini *et al.* 2002; Devasagayam *et al.* 2004).

Many antioxidants have been demonstrated to be cytotoxic, such as *Panax ginseng* (Keum *et al.* 2000), xanthones (Lee *et al.* 2005) and methanol extract of *Betula platyphylla* (Ju *et al.* 2004). Some reports support the relationship of cytotoxicity with antioxidant activity (Owen *et al.* 2000; Dwivedi *et al.* 2003).

CONCLUSION

Most components of leaf EO of *L. nobilis* grown in Egypt were identified and its antimicrobial, antioxidant and cytotoxic activities were investigated. The chemical composition of the Egyptian EO resemble that of *Laurus* plants grown in other countries which means that this plant can be grown in Egypt as a new source of EO. Minor qualitative and quantitative changes in EO composition were observed after cold storage. The study revealed significant antimicrobial activity of the investigated EO. The EOS as antimicrobial agents present two main characters: the first is their natural origin which means more safety to people and the environment, the second is that they have been considered at low risk for resistance development by pathogenic organisms as it was clear from comparing the antimicrobial activities of fresh and stored EOs. This result indicated that *Laurus* EO could serve not only as a flavor agent but also as a safe antiseptic supplement in preventing deterioration of foodstuff and beverage products and pharmaceuticals. The EO presented strong cytotoxic activity against five human tumor cell lines tested which could be related to its proved antioxidant activity.

Since there were minor qualitative and quantitative changes in the EO composition after cold storage, a further study is needed to find the reason for losing antioxidant and cytotoxic activities in the stored *Laurus* EO.

REFERENCES

Adams RP (1995) *Identification of Essential Oil Components by Gas Chromatography and Mass Spectroscopy*, Allured, Carol Stream, IL, 463 pp

Baghdadi HH, Ahmed SS, Fournier G, Refaat AM (1992) On the essential oil of *Laurus nobilis* grown in Egypt. *Egyptian Journal of Horticulture* **19 (1)**, 93-97

Benkendorff K, Davis AR, Rogers CN, Bremner JB (2005) Free fatty acids and sterols in the benthic spawn of aquatic mollusks, and their associated antimicrobial properties. *Journal of Experimental Marine Biology and Ecology* **316**, 29-44

Calliste CA, Trouillas P, Allais D, Simon A, Dutoux J (2001) Free radical scavenging activities measured by electron spin resonance spectroscopy and B16 cell antiproliferative behaviors of seven plants. *Journal of Agriculture and Food Chemistry* **49**, 3321-3327

Canbas A, Erten H, Ozasahin F (2001) The effect of storage temperature of the chemical composition of hop pellets. *Process Biochemistry* **36**, 1053-

1058

Carvalho CC, Fonseca MM (2006) Carvone: Why and how should one bother to produce this terpene. *Food Chemistry* **95**, 413-422

Celik S, Ozkaya A (2002) Effects of intraperitoneally administered lipoic acid, vitamin E, and linalool on the level of total lipid and fatty acids in guinea pig brain with oxidative stress induced by H_2O_2. *Journal of Biochemistry and Molecular Biology* **35**, 547-552

Cesare LF, Nani RC, Fusare EL, Viscardi D, Vital R (2001) The influence of blanching of drying and storage on the volatile fraction of the basil leaves. *Industri Alimentari* **40 (407)**, 1007-1013

Collins CT, Lyne PM (1985) *Microbiological Methods* (5th Edn), Butterworth and Co. Publishers Ltd, London and Toronto, pp 167-181

Crowell PL, Gould MN (1994) Chemoprevention and therapy of cancer by d-limonene. *Critical Reviews in Oncogenesis* **5**, 1-22

Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD (2004) Free radicals and antioxidants in human health: current status and future prospects. *Journal of Association of Physicians of India* **52**, 794-804

Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology* **88**, 308-316

Dox AW (1910) The intracellular enzymes of *Penicillium* and *Aspergillus* with special references to those of *P. camenberti*. *The United States Department of Agriculture Bureau of Animal Industry Bulletin* **120**, 170 pp

Duke JA (1997) *The Green Pharmacy: New Discoveries in Herbal Remedies for Common Diseases and Conditions from the World's Foremost Authority on Healing Herb*s, Rodale Press, New York, NY, 507 pp

Dwivedi C, Muller LA, Goetz-Parten DE, Kasperson K, Mistry VV (2003) Chemopreventive effects of dietary mustard oil on colon tumor development. *Cancer Letter* **196**, 29-34

El Nikeety MMA, El-Akel ATM, El-Hady MM, Badei AZM (2000) Change in physical properties and chemical constituents of parsley herb volatile oil during storage. *Egyptian journal of Food Science* **(26/28)**, 35-49

Flamini G, Tebano M, Cioni PL, Ceccarini L, Ricci AS, Longo I (2007) Comparison between the conventional method of extraction of essential oil of *Laurus nobilis* L. and a novel method which uses microwaves applied in situ, without resorting to an oven. *Journal of Chromatography A* **1143**, 36-40

Gardner P, Provine AT (1984) *Manual of Acute Bacterial Infections* (2nd Edn), Little, Brown and Co. Inc., Boston, 328 pp

Garg SN, Siddiqui MS, Agarwal SK (1992).) New fatty-acid esters and hydroxy ketones from fruits of *Laurus nobilis*. *Journal of Natural Products* **55**, 1315-1319

Gildmeister E, Hoffman F (1959) *Die Atherischen Ole*, Akademic-Verlag, Berlin, 142 pp

Guenther E (1949) *The Essential Oils*, D. Van Nostrand Company, Inc., New York, 204 pp

Hammer KA, Carson CF, Riley TV (2003) Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *Journal of Applied Microbiology* **95**, 853-860

Heinerman J (1983) *The Complete Book of Spices, their Medical, Nutritional and Cooking Uses*, Keats Publishing Inc., New Canaan, CT, 183 pp

Ju EM, Lee SE, Hwang HJ, Kim JH (2004) Antioxidant and anticancer activity of extract from *Betula platyphylla* var. *japonica*. *Life Sciences* **74**, 1013-1026

Kêdzia B, Alkiewicz J, Han S (2000) The significance of tea tree oil in phytotherapy [Znaczenie olejku z drzewa herbacianego w fitoterapii]. *Fitoterapii* **2**, Borgis online library

Keum YS, Park KK, Lee JM, Chun KS, Park JH, Lee SK (2000) Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. *Cancer Letters* **150**, 41-48

Knight JA (2000) Review: Free radicals, antioxidants, and the immune system. *Annals of Clinical and Laboratory Science* **30 (2)**, 145-158

Kubo I, Chaudhuri SK, Kubo Y, Sanchez Y, Ogura T, Saito T (1996) Cytotoxic and antioxidative sesquiterpenoids from *Heterotheca inuloides*. *Planta Medica* **62**, 427-430

Lee BW, Lee JH, Lee ST, Lee HS, Lee WS, Jeong TS (2005) Antioxidant and cytotoxic activities of xanthones from *Cudrania tricuspidata*. *Bioorganic and Medicinal Chemistry Letters* **15**, 5548-5552

Legault J, Dahl W, Debition E, Pichette A, Maldemont JC (2003) Antitumor activity of balsam fir oil: production of reactive oxygen species induced by α-humulene as possible mechanism of action. *Planta Medica* **69**, 402-407

Loizzo MR, Saab AM, Tundis R, Statti GA, Menichini F, Lampronti I, Gambari R, Cinatl J, Doerr HW (2008) Phytochemical analysis and *in vitro* antiviral activities of he essential oils of seven Lebanon species. *Chemistry and Biodiversity* **5**, 461-470

Macchioni F, **Perrucci S**, **Cioni P, Morelli I** (2006) Composition and acaricidal activity of *Laurus novocanariensis* and *Laurus nobilis* essential oils against *Psoroptes cuniculi*. *Journal of Essential Oil Research* **18 (1)**, 43-49

Marzouki H, Piras A, Salah KB, Medini H, Pivetta T, Bouzid S, Marongiu B, Falconieri D (2009) Essential oil composition and variability of *Laurus nobilis* L. growing in Tunisia, comparison and chemometric investigation of different plant organs. *Natural Product Research* **23**, 343-354

Masada Y (1976) *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*, John Wiley and Sons, Inc., New York, 208 pp

Molyneux P (2004) The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology* **26**, 211-219

Njoroge SM, Ukeda H, Sawamura M (1996) Changes in the volatile composition of Yuzu (*Ctrus junos* Tanaka) cold-pressed oil during storage. *Journal of Agriculture and Food Chemistry* **44**, 550-556

Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B (2000) Olive-oil consumption and health: the possible role of antioxidants. *The Lancet Oncology* **1**, 107-112

Ozcan M, Chalchat JC (2005) Effect of different locations on the chemical composition of essential oils of laurel (*Laurus nobilis* L.) leaves growing wild in Turkey. *Journal of Medicinal Food* **8 (3)**, 408-411

Parveen M, Kamrul H, Junko T, Yoshinori M, Emiko K, Osamu K, Hitoshi I (2004) Response of *Saccharomyces cerevisiae* to a monoterpene: evaluation of antifungal potential by DNA microarray analysis. *Journal of Antimicrobial Chemotherapy* **54**, 46-55

Politeo O, Juki M, Milo M (2007) Chemical composition and antioxidant activity of free volatile aglycones from laurel (*Laurus nobilis* L.) compared to its essential oil. *Croatica Chemica Acta* **80 (1)**, 121-126

Quijano CE, Jorge Y, Pino A (2007) Characterization of the leaf essential oil from laurel (*Laurus nobilis* L.) grown in Colombia. *Revista CENIC Ciencias Químicas* **38 (3)**, 371-374

Reddy BS, Wang CX, Samaha H, Lubet R, Steele VE, Kelloff GJ, Rao CV (1997) Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. *Cancer Research* **57**, 420-425

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

Sayyah M, Valizadeh J, Kamalinejad M (2002) Anticonvulsant activity of the leaf essential oil of *Laurus nobilis* against pentylenetetrazole- and maximal electroshock-induced seizures. *Phytomedicine* **9 (3)**, 212-216

Serafini M, Bellocco R, Wolk A, Ekstrom AM (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology* **123 (4)**, 985-991

Setzer WN, Shen X, Bates RB, Burns JR, McClure KJ, Zhang P, Moriarity DM, Lawton RO (2000) A phytochemical investigation of *Alchornea latifolia* from Monteverde, Costa Rica. *Fitoterapia* **71**,195-198

Sibanda S, Chigwada G, Poole M, Gwebu ET, Noletto JA, Schmidt JM (2004) Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. *Journal of Ethnopharmacology* **92**, 107-111

Sies H (1986) Biochemistry of oxidative stress. *Angewandte Chemie International Edition* **25**, 1058-1071

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

Skehan P, Storeng R, **Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd R** (1990) New colorimetric cytotoxicity assay for anticancer drug screening. *Journal of National Cancer Institute* **82**, 1107-1112

Soliman FM, El-Kashoury EA, El-Fishawy AM, El-Kawy MAA (1994) Analysis of essential oil of *Laurus nobilis* L. *Bulletin of Faculty of Pharmacy, Cairo University* **32 (3)**, 387-389

Sun J (2007) D-limonene: safty and clinical application. *Alternative Medicine Review* **12**, 259-264

Sylvestre M, Legault J, Dufour D, Pichette A (2005) Chemical composition and anticancer activity of leaf essential oil of *Myrica gale* L. *Phytomedicine* **12**, 299-304

Tatman D, Mo H (2002) Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60 leukemia cells. *Cancer Letters* **175**, 129-139

Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G (2005) Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy* **49**, 2474-2478

Verdian-rizi M, Hadjiakhoondi A (2008) Essential oil composition of *Laurus nobilis* L. of different growth stages growing in Iran. *Zeitschrift für Naturforschung C* **63**, 785-788

Wang G, Li X, Huang F, Zhao J, Ding H, Cunningham C, Coad JE, Flynn DC, Reed E, Li QQ (2005) Antitumor effect of β-elemene in non-small-cell lung cancer cells is mediated via induction of cell cycle arrest and apoptotic cell death. *Cellular and Molecular Life Sciences* **62**, 881-893

Wang W, Wu N, Zu YG, Fu YJ (2008) Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. *Food Chemistry* **108**, 1019-1022

Yalçin H, Anik M, Sanda MA, Cakir A (2007) Gas chromatography/mass spectrometry analysis of *Laurus nobilis* essential oil composition of northern Cyprus. *Journal of Medicinal Food* **10**, 715-719

Yff BTS, Lindsey KL, Taylor MB, Erasmus DG, Jäger AK (2002) The pharmacological screening of *Pentanisia prunelloides* and the isolation of the antibacterial compound palmitic acid. *Journal of Ethnopharmacology* **79**, 101-107