Cytotoxic Activity of Essential Oils from Labiatae and Lauraceae Families Against *In Vitro* Human Tumor Models

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Abstract. *Background: The aim of this work was to study the cytotoxicity of essential oils and their identified constituents from Sideritis perfoliata, Satureia thymbra, Salvia officinalis, Laurus nobilis and Pistacia palestina. Materials and Methods: Essential oils were obtained by hydrodistillation and were analysed by gas chromatography (GC) and GC/mass spectrometry (MS). The cytotoxic activity was evaluated in amelanotic melanoma C32, renal cell adenocarcinoma ACHN, hormone-dependent prostate carcinoma LNCaP, and MCF-7 breast cancer cell lines by the sulforhodamine B (SRB) assay. Results: L. nobilis fruit oil exerted the highest activity with IC_{50} values on C32 and ACHN of 75.45 and 78.24 μg/ml, respectively. The activity of S. perfoliata oil on both cell lines (IC_{50} of 100.90 mg/ml for C32 and 98.58 μg/ml for ACHN, respectively) was also interesting. Among the tested constituents the highest activity was found when α-humulene was applied to LNCaP cells (IC_{50} of 11.24 μg/ml). Conclusion: This study suggests for the first time the ability of S. perfoliata, S. thymbra, S. officinalis, L. nobilis and P. palestina essential oils and some identified terpenes to inhibit human tumor cell growth.*

Nature is a rich source of biological and chemical diversity. The unique and complex structures of natural products cannot be obtained easily by chemical synthesis. Interest in medicinal plant research has increased in recent years, especially for the treatment of pathologies of relevant social impact such as cancer (1-3). Cytotoxicity has been reported for many essential oils (4-10) but no previous study has been undertaken on the cytotoxic activity of essential oils from *Sideritis perfoliata*, *Satureia thymbra*, *Salvia officinalis*, *Laurus nobilis* or *Pistacia palestina*.

The genus *Sideritis* (Labiatae) is of great botanical and pharmacological interest, in fact many species are reported to have analgesic, anti-inflammatory, antibacterial, antirheumatic, anti-ulcer, digestive and vaso-protective properties and have been used in Mediterranean folk medicine (11). No reports have been found concerning the phytochemical composition or biological or cytotoxic activity of *S. perfoliata* (12). *S. thymbra* (Labiatae) is the most common *Satureja* specimen and is known as a herbal home remedy, due to its antiseptic, gastro-sedative and diuretic properties. Biological activity studies have established that the essential oil possesses significant antibacterial and antifungal activities (13). *S. officinalis* and *P. palestina* also belong to the Labiatae family. Sage essential oils have been widely studied and possess carminative, antispasmodic, antiseptic and astringent properties. They can be used for inflammations and infections of the mucous membranes of throat and mouth such as stomatitis, gingivitis and pharyngitis, as well as dyspeptic symptoms and excessive perspiration (14). No previous investigations have been reported for *P. palestina* chemical composition and biological activity. From the Lauracae family, *L. nobilis* L. has been used as a folk remedy in different countries to treat numerous diseases. In Iranian traditional medicine, the leaves have been used topically for relieving rheumatic pains, while laurel essential oil has been reported as being used in the preparation of hair lotion for its antidandruff activity and for the external treatment of psoriasis (15). Many phytochemical analyses have been reported (16) and the essential oil has broad antibacterial, antifungal, analgesic, and anti-inflammatory activity (17-19).

A study of five plants collected in Lebanon and submitted to hydrodistillation are presented here. The essential oils obtained were investigated by gas chromatography (GC) and GC/mass spectrometry (MS) analysis and some of the

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Key Words: Satureia thymbra, *Pistacia palestina*, *Salvia officinalis*, *Sideritis perfoliata*, *Laurus nobilis*, essential oils, cytotoxicity.

Table I. *Plant data.*

Plant species	Family	Place of Collection	Voucher No.	% w/w
Satureja thymbra L.	Labiatae	Ayoun-kourkoush	AS2	1.5
Sideritis perfoliata L.	Labiatae	Tarshich	AS4	1.8
Pistacia palestina Boiss.	Labiatae	Ain-Saadé	AS10	2
Salvia officinalis L.	Labiatae	Ain-Saadé	AS13	2.25
Laurus nobilis L. (fruits)	Lauraceae	Nahr-Ibrahim	AS14	2.9
Laurus nobilis L. (leaves)	Lauraceae	Nahr-Ibrahim	AS14	3

% w/w, hydrodistillation yield.

terpenes identified together with essential oils were screened for their cytotoxic activity against a panel of human tumor cell lines.

Materials and Methods

Essential oils. Leaves of *Sideritis perfoliata* L., *Satureja thymbra* L., *S. officinalis* L. and *Laurus nobilis* L. and fruits from *L. nobilis* and *P. palestina* Boiss. were collected from June to November 2003 in Lebanon. Voucher specimens were authenticated botanically by Prof. S. Safi, Biology Department, Faculty of Sciences II, Lebanese University and deposited in the Herbarium of the Faculty of Sciences II, Lebanese University. Two hundred grams of fresh material was submitted to hydrodistillation for 3 h using a Clevenger-type apparatus (20). The white-yellow essential oils were dried over anhydrous sodium sulphate to remove traces of moisture and stored at 4˚C. Place of collection and percent yield are reported in Table I.

GC and GC/MS analysis. GC analysis was performed on a Shimadzu GC17A gas chromatograph equipped with a flame ionization detector (FID) and controlled by Borwin Software (Milan, Italy). The samples were analysed on a fused silica 30 m SE-30 capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 μm. Nitrogen was used as the gas vector. Injector and detector were maintained at 250˚C and 280˚C, respectively. The oven temperature programming was 50˚C during injection, then increased from 50 to 280˚C at a rate of 13˚C/min. GC/MS analysis of the oils was carried out using a Hewlett-Packard 6890 gas chromatograph equipped with a methylsilicone SE-30 capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) and interfaced with a Hewlett Packard 5973 Mass Spectrometer (Milan, Italy). Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The carrier gas was helium and the analytical conditions were: oven temperature 3 min isothermal at 50˚C; 50-280˚C at a rate of 16˚C/min; isothermal for 10 min. The injector and detector were maintained at 250˚C and 280˚C, respectively. The identification of the compounds was based on comparing the mass spectral data with Wiley 138 and Wiley 275 libraries and by referring to compounds known in the literature (21). The relative quantity of compounds was estimated by integrating the total ion content of individual peaks.

Cancer cell lines. The human amelanotic melanoma cell line C32, (American Type Culture Collection, ATCC, Rockville, MD, USA) (ATCC No: CRL 1585), renal cell adenocarcinoma ACHN (ATCC No: CRL-1611) and hormone-dependent prostate carcinoma LNCaP (ATCC No: CRL-1740) were cultured in RPMI-1640 medium while the human breast cancer cell line MCF-7 (ATCC No: HTB-22D) was cultured in DMEM medium. Both media were supplemented with 10% foetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin (Sigma-Aldrich, Milan, Italy). The cell lines were maintained at 37˚C in a 5% CO_2 atmosphere and 95% humidity. The cultures were passed once a week by trypsinization using a 1:30 dilution of standard trypsin-EDTA solution (Gibco, Milan, Italy).

Assessment of cytotoxicity. The cytotoxic assay was performed as previously described (22). The protein-staining sulforodamine B (SRB) assay was used for measurement of cell proliferation. The test is based on the estimation of cell number indirectly by providing a sensitive index of total cellular protein content which is linear to cell density. The cells were trypsinized, counted and placed in 96-well plates at optimal plating density of each cell line was determined over a range from $5x10^4$ to $15x10^4$ ensuring exponential growth throughout the experimental period and a linear relationship between absorbance at 490 nm and cell number where analysed by the SRB assay, and incubated to allow for cell attachment. After 24 h, the cells were treated with serial dilutions of the samples. Each sample was initially dissolved in DMSO and further diluted in medium to produce different concentrations. One hundred microliters/well of each dilution were added to the plates in six replicates to obtain the final concentrations ranging from 5 to 400 μg/ml for the essential oils, and from 2 to 50 μg/ml for the commercially available identified constituents (linalool, limonene, 1,8-cineole, *trans*-caryophyllene, and α-humulene). The final mixture used for treating the cells contained not more than 0.5% of the solvent (DMSO), the same as in the solvent-control wells. After 48 h of exposure, 100 μl of ice-cold 40% trichloroacetic acid (TCA) was added to each well, left for 1 h at 4˚C, and washed with distilled water. The TCA-fixed cells were stained for 30 min with 50 μl of 0.4 (w/v)% SRB in 1% acetic acid. The plates were washed with 1% HOAc and air dried overnight. For reading of the plate, the bound dye was solubilised with 100 μl of 10 mM tris base (tris[hydroxymethyl]aminomethane). All products were purchased from Sigma-Aldrich. The absorbance of each well was read on a Molecular Devices SpectraMax Plus Plate

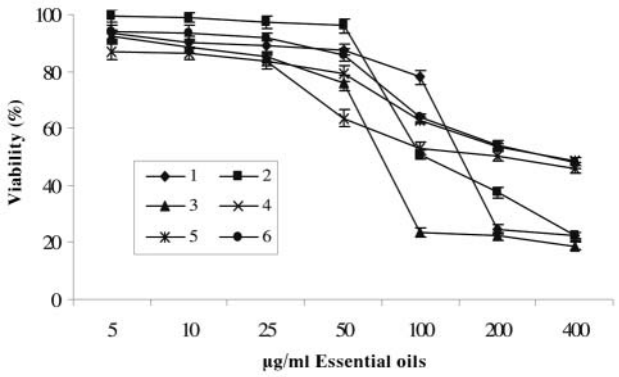

Figure 1. *The dose-dependent cytotoxic effect on C32 cell line of the essential oils (EO) of S. thymbra (1), S. perfoliata (2), L. nobilis fruits (3), L. nobilis leaves (4), P. palestina (5) and S. officinalis (6). Cytotoxicity was measured as the reduced change in absorbance in cultures containing EO at 490 nm as compared with control untreated cultures. Each point represents the average from three separate measurements, each done in triplicate, and the standard deviation of the mean.*

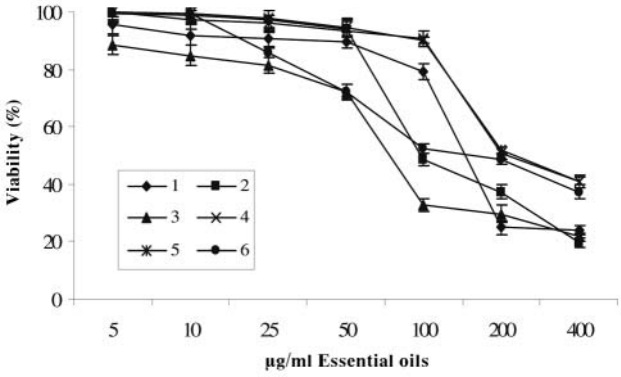

Figure 2. *The dose-dependent cytotoxic effect on ACHN cell line of essential oils (EO) of S. thymbra (1), S. perfoliata (2), L. nobilis fruits (3), L. nobilis leaves (4), P. palestina (5) and S. officinalis (6). Cytotoxicity was measured as the reduced change in absorbance in cultures containing EO at 490 nm as compared with control untreated cultures. Each point represents the average from three separate measurements, each done in triplicate, and the standard deviation of the mean.*

Reader (Molecular Devices, CELBIO, Milan, Italy) at 490 nm. Cell survival was measured as the percentage absorbance compared to the untreated control. Vinblastine sulfate salt and Taxol were used as positive control.

Statistical analysis. All experiments were carried out in triplicate. Data were expressed as means±S.D. Differences were evaluated by the one-way analysis of variance (ANOVA) test completed by a multicomparison Dunnett's test. Differences were considered significant at $^{**}p<0.01$. The inhibitory concentration 50% (IC_{50}) was calculated by a nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows, GraphPad Software, San Diego, CA, USA (www.graphpad.com). The dose–response curve was obtained by plotting the percentage of inhibition *versus* the concentrations.

Results

Essential oil composition. The yields of essential oils ranged from 1.5% to 3% (Table I). In order to identify putative active compounds present within the essential oils, gas chromatography systems were employed. The chemical composition of the oils is shown in Table II. *S. thymbra* essential oil was characterized by 40 compounds (80.91% of the total oil) of which 24 were monoterpenes, 14 sesquiterpenes and 2 diterpenes. The GC/MS analysis revealed the presence of α-pinene, sabinene, *p*-cymene, linalool (2.81%), and *trans*-caryophyllene (3.67%). Fifteen monoterpenes, 17 sesquiterpenes, and 1 diterpene were identified in *S. perfoliata* essential oil, representing 86.67% of the total oil. In the sesquiterpene fraction the major component was *trans*-caryophyllene (4.11%). Twenty-six components were identified in the essential oil of *L. nobilis* leaves, which represented 97.21% of the total oil which consisted of 1,8-cineole (35.15%) as the main component, and α-pinene, sabinene, β-pinene, limonene, linalool, 1-*p*-menthen-8-yl acetate as dominant constituents in the monoterpene fraction. A high amount of 1,8-cineole (9.43%) was also found in *L. nobilis* fruit oil. This monoterpene was the second most abundant compound with β-ocimene being the most abundant. The GC/MS analysis of *P. palestina* essential oil was characterized by 29 components, representing 79.26% of the total oil. In this oil sabinene (17.08%) and limonene (8.56%) were the major constituents. Monoterpenes were the major fraction of *S. officinalis* oil, of which 1,8-cineole was the most abundant compound (43.62%). In the sesquiterpene fraction *trans*-caryophyllene and α-humulene were found in the highest quantities (1.05% and 3.41%, respectively).

Cytotoxicity. The cytotoxicity of the oils on human cancer cell lines is shown in Figures 1 and 2, and the IC_{50} values are given in Table III. *L. nobilis* fruit oil was the most active on the amelanotic melanoma cells (IC_{50} 75.45 µg/ml) and the renal adenocarcinoma cells (IC_{50} 78.24 µg/ml). Less activity was found when *L. nobilis* leaf oil was applied to both cell cultures (IC_{50} 202.62 and 209.69 µg/ml for ACHN and C32, respectively). *S. perfoliata* essential oil also exerted high activity, with IC_{50} values of 98.58 and 100.90 µg/ml for C32 and ACHN cells, respectively. A comparable IC_{50} value was found for renal cell adenocarcinoma growth when *S. officinalis* oil was used (IC_{50} 100.70 µg/ml). *P. palestina* oil was able to inhibit the growth of ACHN and C32 with IC_{50} of 204.70 and 356.98 µg/ml, respectively. The oils were unable to react with human breast cancer cell (MCF-7) and hormone dependent prostate carcinoma cell (LNCaP).

Table II. *Composition of S. thymbra, S. perfoliata, L. nobilis, P. palestina and S. officinalis essential oils.*

		Essential oils					
t_R	Compound	1	2	3	4	5	6
6.97	α-Thujene	0.89±0.11	0.50±0.06	0.10±0.01	0.44±0.09	0.92±0.05	0.16±0.01
7.13	α-Pinene	10.15±0.32	8.66±0.14	3. 67±0.03	5.72±0.12	6.81±0.12	4.72±0.11
7.37	Camphene	1.08±0.09	0.11±0.08	1.69±0.04	0.14±0.06	0.39±0.01	2.55±0.08
7.95	Sabinene	8.64±0.15	12.76±0.34	1.64±0.03	6.17±0.14	17.08±0.25	6.97±0.21
8.02	β-Pinene	2.90±0.18	8.90±0.23	2.14±0.01	3.46±0.08	6.48±0.09	3.01±0.14
8.22	β-Myrcene	0.68±0.03	1.48±0.09	0.56±0.01	0.45±0.10	-	tr
8.47	α-Phellandrene	tr	0.72±0.07	0.11±0.07	0.12±0.03	1.13±0.03	-
8.62	δ-3-Carene	-	-	-	-	tr	-
8.71	α-Terpinene	1.10±0.12	0.44±0.06	0.15±0.01	0.86±0.05	3.60±0.06	0.17±0.01
8.85	*p*-Cymene	10.76±0.53	-	0.12±0.05	2.23±0.11	6.01±0.09	1.08±0.04
8.90	β-Phellandrene	-	32.85±0.64	-	-	-	
8.94	Limonene*	0.57±0.09	-	0.10±0.01	1.10±0.08	8.56±0.11	1.20±0.05
9.07	1,8 Cineole*	0.28±0.06	0.18±0.09	9.43±0.07	35.15±0.36	-	43.62±0.44
9.09	β-Ocimene	-	-	21.83±0.18	0.08±0.005	-	-
9.47	γ-Terpinene	7.56±0.11	1.11±0.11	0.10±0.01	1.50±0.09	6.33±0.12	0.39±0.08
9.93	α-Terpinolene	0.62±0.15	0.22±0.05	-	0.49±0.07	2.86±0.06	tr
9.96	Fenchone	-	-	0.12±0.02	-	-	-
10.13	Linalool*	2.81±0.11	-	-	7.08±0.14	-	-
10.32	α-Thujone	0.08±0.11	-	-	-	-	12.99±0.13
10.39	δ-Isothujone	-	-	-	-	-	1.48±0.02
10.45	*cis*-p-2-Menthen-1-ol	-	-	-	-	0.32±0.05	-
10.52	α-Campholene aldehyde	0.25±0.11	-	-	-	-	-
10.83	Camphor	-	-	0.35±0.04	-	-	5.71±0.12
11.24	Terpinen-4-ol	-	0.72±0.13	-	4.42±0.07	-	-
11.46	α-Terpineol	1.53±0.16	0.27±0.11	0.40±0.02	2.42±0.04	2.43±0.08	3.18±0.32
11.58	Isoborneol	-	-	0.31±0.01	-	-	-
11.65	*cis*-Piperitol	0.10±0.06	-	-	-	-	-
11.82	Isopulegone	0.10±0.02	-	-	-	-	-
12.05	Methyl thymylether	-	-	-	-	0.38±0.02	-
12.07	Pulegone	tr	-	-	-	-	-
12.57	Neryl acetate	0.26±0.03	0.14±0.04	-	-	-	-
12.59	Bornyl acetate	-	-	0.23±0.01	-	-	0.24±0.06
12.62	α-Terpinyl acetate	-	-	-	4.43±0.10	-	-
12.63	Thymol	9.92±0.07	-	-	-	-	-
12.65	α-Fenchyl acetate	-		-	-	2.05±0.05	-
12.90	Carvacrol	4.98±0.08	-	-	-	-	-
13.31	1-*p*-Menthen-8-yl acetate	0.40±0.08	-	-	13.52±0.25	-	-
13.35	α-Cubebene	-	-	-	-	0.52±0.03	-
13.42	Eugenol	-	-	-	3.73±0.16	-	-
13.67	α-Ylangene	-	-	0.23±0.08	0.17±0.06	0.22±0.01	-
13.71	α-Copaene	1.67±0.13	0.12±0.07	0.17±0.01	-	0.20±0.01	-
13.75	α-Bergamotene	-	0.67±0.07	0.10±0.04	-	-	-
13.79	β-Bourbonene	0.24±0.05	0.92±0.06	-	-	-	-
13.82	β-Elemene	0.21±0.01	-	1.0±0.06	0.10±0.08	0.10±0.01	-
13.86	Methyl eugenol	-	-	-	2.52±0.04	-	-
13.90	Zingiberene	-	-	-	-	0.48±0.01	-
14.06	α-Gurjunene	0.51±0.06	-	-	-	-	-
14.20	*trans*-Caryophyllene*	3.67±0.11	4.11±0.22	0.32±0.04	0.38±0.08	0.63±0.01	1.05±0.07
14.30	Aromadendrene	-	-	-	-	3.99±0.01	0.99±0.04
14.39	β-Farnesene	-	0.30±0.05	-	-	-	-
14.43	α-Humulene*	0.34±0.03	0.49±0.12	0.10±0.01	-	0.29±0.01	3.41±0.45
14.52	*trans*-β-Farnesene	-	-	0.13±0.01	-	-	-
14.64	Epi-bicyclosesquiphellandrene	1.68±0.14	1.15±0.18	-	-	2.40±0.03	-
14.70	Ar-curcumene	-	0.47±0.09	-	-	-	-
14.78	β-Cubebene	-	1.76±0.13	-	-	-	tr
14.82	α-Zingibirene	-	0.35±0.06	-	-	-	-
14.85	α-Guaiene	-	-	-	0.22±0.03	-	-

Table II continued

Table II continued

t_R	Compound	Essential oils 1	2	3	4	5	6
14.89	Germacrene D	-	2.16±0.32	-	-	-	-
15.01	β-Selinene	0.11±0.02	-	-	-	-	-
15.09	α-Muurolene	0.37±0.04	-	-	-	-	
15.15	γ-Cadinene	-	-	0.36±0.02	-	-	tr
15.21	δ-Cadinene	3.11±0.12	0.57±0.14	0.14±0.01	-	1.51±0.07	0.1±0.01
15.54	δ-Guaiene	-	0.57±0.13	-	-	-	-
15.67	Palustrol	0.99±0.08	-	-	-	-	-
15.76	Spathulenol	0.61±0.07	0.88±0.11	-	0.31±0.02	-	-
15.88	γ-Gurjunene	-	-	-	-	0.33±0.11	1.16±0.05
15.90	Viridiflorol	0.78±0.04	1.17±0.15	-	-	-	0.11±0.37
16.14	β-Guaiene	-	-	-	-	0.16±0.03	-
16.27	Junipene	-	0.81±0.11	-	-	-	-
16.44	β-Maaliene	-	-	-	-	3.08±0.04	-
16.61	α-Bisabolol	-	1.01±0.35	-	-	-	-
17.37	Oxo-α-Ylangene	0.15±0.07	-	-	-	-	-
17.76	Neophytadiene	-	0.10±0.08	-	-	-	-
18.64	Biformene	0.10±0.05	-	-	-	-	-
19.31	Eremanthin	-	-	3.65±0.09	-	-	-
19.39	Rimuene	0.71±0.24	-	-	-	-	-
20.82	Dehydrocostus lactone	-	-	7.57±0.12	-	-	-
%	Identification	80.91	86.67	56.82	97.21	79.26	94.29

(1) *Satureia thymbra,* (2) *Sideritis perfoliata, Laurus nobilis* (3) fruits and (4) leaves (5) *Pistacia palestina,* and (6) *Salvia officinalis.* tR: Retention time (as min). *Identification confirmed with authentic standard. % identification: peak area identified relative to total peak area %). tr: <0.05%.

Commercially available samples of some of the identified compounds (1,8-cineole, limonene, linalool, *trans*-caryophyllene, and α-humulene) were tested for their cytotoxic activity on human tumor *in vitro* models, in order to identify the active oil constituents. Interestingly, linalool exerted cytotoxic activity on amelanotic melanoma (IC_{50} 23.16 μg/ml) and renal cell adenocarcinoma (IC_{50} of 23.77 μg/ml). The results also indicated that *trans*-caryophyllene was active in a similar manner against ACHN and C32 cell lines with IC_{50} values of 21.81 μg/mL and 20.10 μg/mL, respectively. The highest cytotoxic activity (IC_{50} 11.24 μg/ml) was found when α-humulene was tested on the LNCaP cells, but this sesquiterpene was inactive against the MCF-7, C32, and ACHN cell lines (IC_{50} >50 μg/ml).

Table III. *Cytotoxic activity of essential oil samples. IC_{50} in μg/ml.*

Essential oils	Cell line C32	ACHN	MCF-7	LNCaP
Satureja thymbra L.	154.30±1.2**	155.88±1.1**	>400	>400
Sideritis perfoliata L.	100.90±1.1**	98.58±0.5**	>400	>400
Laurus nobilis L. (fruits)	75.45±1.2**	78.24±1.5**	>400	>400
Laurus nobilis L. (leaves)	209.69±1.4**	202.62±1.7**	>400	>400
Pistacia palestina Boiss.	356.98±1.3**	204.70±1.8**	>400	>400
Salvia officinalis L.	367.43±1.5**	108.70±1.2**	>400	>400

Vinblastine (for ACHN, C32, and LNCaP,) and Taxol (for MCF-7) were used as positive control. Data are given as the mean of at least three independent experiments±S.D. **p<0.01 *vs.* control.

Discussion

L. nobilis fruit oil exhibited the most interesting biological activity on the ACHN and C32 cell growth inhibition, while the activity of leaf oil was considerably less, which can be attributed to the different composition with regards to the predominant compounds of the oils. This result is of some importance, since data on the cytotoxic activity of *L. nobilis* are scarce. Costunolide and zaluzanin D, two of the sesquiterpenes isolated from laurel, induced cell death and morphological change indicative of apoptotic chromatin condensation in leukemia HL-60 cells (23). The fruit oil contained 7.57% of the dehydrocostus lactone, which was absent in the leaf oil. Moreover, 1,8-cineole, the main component of the *L. nobilis* leaf oil, induced fragmentation, with oligonucleosomal-sized fragments, which is characteristic

of apoptosis in a concentration- and time-dependent manner in Molt 4B and HL-60 cells, but not in KATO III cells (24). However, this monoterpene was inactive against the human tumor *in vitro* models in the present study.

The *in vitro* antimicrobial activity and significant differences in chemical compositions have previously been reported of the essential oils from five taxa of *Sideritis* (25). Moreover, the diterpenes, and the coumarin siderin isolated from Canary Island endemic *Sideritis* species exerted cytostatic activity (26).

In our study *S. officinalis* essential oil inhibited renal adenocarcinoma cell growth. This species has been extensively investigated for its chemical composition and pharmacological profile. Some diterpenoid quinones (royleanone- SAR 3, horminone- SAR 26, and acetyl horminone- SAR 43) isolated from the roots of *S. officinalis* are able to exert cytotoxic and DNA-damaging activity in human colon carcinoma Caco-2 cells and human hepatoma HepG2 cells cultured *in vitro* (27).

Of the five identified components evaluated in the present study, linalool and *trans*-caryophyllene exhibited high cytotoxic activity against the amelanotic melanoma and renal adenocarcinoma cells. Linalool was found to be concentrated in *L. nobilis* and *S. thymbra* leaf oils, while *trans*-caryophyllene was the main constituent of the sesquiterpene fraction in *S. thymbra*, *S. perfoliata* and *S. officinalis*.

The antiproliferative effect of caryophyllene oxide against SK-MEL-28, MDA-MB-231, Hs 578T and 5,637 tumor cell lines has recently been demonstrated (28). These authors also reported the cytotoxic activity of caryophyllene oxide (100 μg/ml) against breast cancer (MCF-7) and prostate cancer (PC-3) cells with 89.67% and 96.75% of cells killed, respectively, while linalool was inactive when it was tested at 100 μg/ml. A recent study has demonstrated that *trans*-caryophyllene was inactive against human lung carcinoma A-549 and human colon adenocarcinoma DLD-1 cells (GI_{50} >20 μg/ml) (29). These data together with our results suggest that the mechanism of action should be investigated.

The other commercially available compound tested in this study was 1,8-cineole, the most abundant constituent in *L. nobilis* and in *S. officinalis* leaf oils. Interestingly, the cytotoxic activity of these oils could not be explained in terms of the 1,8-cineole content. In fact, this monoterpene was inactive against the cell lines used in this study. The sesquiterpene fraction of *S. officinalis* was characterized by the presence of α-humulene, which demonstrated a strong cytotoxic activity on the human prostate carcinoma LNCaP cells. A previous study has reported the cytotoxic activity of α-humulene on PC-3, A-549, DLD-1 and M4BEU tumor cells through glutathione (GSH) depletion and reactive oxygen species (ROS) production (30).

Complete correlation between the activity of the oils and their commercially available components on human tumor models was not found. A possible explanation is that some components act together, synergistically or cumulatively. This possibility has previously been discussed in the literature, where it was pointed out that different components have been shown to affect cell growth in a different manner (31).

Conclusion

The six essential oils and some of their mono- and sesquiterpenes had the ability to inhibit tumor cell growth. Further evaluation is warranted to clarify the mechanism of action and their potential use.

In Memoriam

This work is dedicated to the memory of Professor Dr. C.F. Pollera, who was an inspiration to scientists and played a key role in Clinical Oncology, not only in Italy, but also in the international arena.

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