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ScienceDirect
Nutrition Research 30 (2010) 271–278
Nutrition Research
www.nrjournal.com

Volatile oil composition and antiproliferative activity of *Laurus nobilis*, *Origanum syriacum*, *Origanum vulgare*, and *Salvia triloba* against human breast adenocarcinoma cells

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Received 11 November 2009; revised 25 March 2010; accepted 5 April 2010

Abstract

Medicinal plants and culinary herbs have gained importance in the last decade as cytotoxic and antitumor agents. We hypothesized that some of the commonly used spices with reported antimicrobial activity might have antiproliferative activity. In the present study, selected spices used in Jordan were chemically analyzed and investigated for their antiproliferative activity to the adenocarcinoma of breast cell line (MCF7). The composition of the essential oils of *Laurus nobilis* L, *Origanum syriacum* L, *Origanum vulgare* L, and *Salvia triloba* L was analyzed by gas chromatography-mass spectrometry. The antiproliferative activities of the hydrodistilled volatile oils and the crude ethanol and water extracts were evaluated using the sulphorhodamine B assay. 1,8-Cineol was the major constituent in the hydrodistilled oils of both plants, *L nobilis* and *S triloba*, with concentrations of 40.91% and 45.16%, respectively. The major constituent of *O syriacum* was the carvacrol (47.10%), whereas that of *O vulgare* was *trans*-sabinene hydrate (27.19%). The ethanol crude extracts of *O syriacum*, *L nobilis*, and *S triloba* showed antiproliferative activity to MCF7 with IC_{50} values 6.40, 24.49, and 25.25 μg/mL, respectively. However, none of the hydrodistilled essential oils of the tested plant species or their aqueous extracts demonstrated cytotoxic activity.

Keywords: Culinary herbs; Essential oils; GC-MS; Antiproliferative activity; MCF7; Jordan
Abbreviations: ECACC, European Collection of Animal Cell Culture; GC-MS, gas chromatography-mass spectrometry; MCF7, adenocarcinoma of breast cell line; NCI, National Cancer Institute; RI, retention index; SRB, sulphorhodamine B.

1. Introduction

Plants have been used as food, spices, and medicines for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. In more recent history, the use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century [1]. Since then, natural products have been the origin of many important compounds in drug discoveries.

Different culinary herbs have been screened for their biological activities in management of chronic diseases such as diabetes and hypercholesterolemia, as well as for their antibacterial activities [2-7]. Reports have stated, and it is a common practice among scientists, that one should test the biological activity from more than one perspective, such as the antiinflammatory effect, antimicrobial effect, and anticancer activity [8,9]. Furthermore, for several volatile oil containing plants (monoterpenoids and sesquiterpenoids), antimicrobial as well as anticancer activity has been reported [10].

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0271-5317/$ – see front matter
doi:10.1016/j.nutres.2010.04.001

There are at least 250 000 species of vascular plants existing worldwide out of which more than 1000 plants have been found to possess anticancer properties [11]. The plant kingdom provides an enormous potential for discovery of new drugs for the treatment and prevention of diseases. In fact, plants have long been viewed as a potential cure for cancer, which is projected to become the major cause of death in this century. It is estimated that around 60% of antitumor and antiinfectious drugs, already on the market or under clinical trial, are of natural origin. However, most of these compounds cannot be synthesized economically and are still obtained from wild or cultivated plants [12].

Various active compounds derived from medicinal plants have been assessed for their efficacy and tolerability in the treatment of breast cancer. Some of these plant species, including *Taxus baccata*, *Podophyllum peltatum*, *Camptotecha accuminata*, and *Vinca rosea* have well-recognized anticancer activity in breast cancer, and several isolated pure compounds and their semisynthetic derivatives have been evaluated in clinical trials and marketed [13,14].

In 2008, according to the Jordan National Registry, 855 new cases of breast cancer were registered. Currently, breast cancer is the most commonly diagnosed cancer in women and the second ranking cause of cancer death in the Eastern Mediterranean Region and Jordan. In an effort to address this, we hypothesized that specific herbs exert an inhibitory effect on cancer cells. To test our hypothesis, commonly used spices with reported antimicrobial activity were studied in cell culture. Thus, in the present study, the volatile fractions and the crude aqueous and ethanol extracts of 4 widely used culinary herbs, namely *Laurus nobilis* L, *Origanum syriacum* L, *Origanum vulgare* L, and *Salvia fruticosa* Mill (*Salvia triloba* L) were tested for their antiproliferative activity using MCF7 breast cancer cell line with the expectation that there would be benefits from these plants, not only as spices but also as a source of new anticancer agents. The plants selected for this study are very popular in Jordan. They are used as spices, food ingredients, and in beverages; and, they are well-known as home remedies in the treatment of different ailments [15].

2. Methods and materials

2.1. Plant material

Leaves of *L nobilis*, *O syriacum*, *O vulgare*, and *S triloba* were collected from Amman in spring 2007. All plants were taxonomically identified by one of the authors (F. U. Afifi) and authenticated by direct comparison with the herbarium specimens of the Faculty of Science, University of Jordan (Amman, Jordan). Voucher specimens were deposited in the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan. The plant samples were cut into small pieces and air dried at room temperature.

2.2. Preparation of ethanol extracts

For the preparation of the ethanol extracts, each 2.5 g of dried leaves of *O syriacum* and *O vulgare* and fresh leaves of *L nobilis* and *S triloba* were refluxed by boiling each with 50 mL of ethanol (70%), filtered, and the solvent evaporated until dry. For the preparation of aqueous extracts, the same amount of plant materials were boiled with 50 mL of tap water, kept overnight, and filtered. After filtration, the extracts were frozen at −80°C then freeze dried.

2.3. Volatile oil preparation

Each 100 g of dried plant material were subjected to hydrodistillation with 1 L of water using a Clevenger-type apparatus for 3 hours. Distillation was carried out twice for each plant, and the oils obtained for each were pooled, dried over anhydrous sodium sulfate, and stored at 4°C in amber glass vials until analysis.

2.4. Gas chromatography-mass spectrometry analysis of the volatile oils

About 1 μL of aliquot of each oil sample, appropriately diluted in hexane, was subjected to gas chromatography-mass spectrometry (GC-MS) analysis. The GC-MS analysis was performed using a Varian GC-MS Saturn 2200 with a DP-5MS column (30 cm × 0.25 mm; film thickness, 0.25 mm). The column temperature was kept at 100°C for 3 minutes then programmed to 250°C at a rate of 10°C/min and kept constant at 250°C for 1 minute. Flow rate of helium as a carrier gas was 1 mL/min. Each sample was analyzed twice.

2.5. In vitro assay for antiproliferative activity

The cell line under investigation was human breast adenocarcinoma (MCF7). It was purchased from the European Collection of Animal Cell Culture (ECACC no. 86012803). The cells were cultured in Roswell Park Memorial Institute 1640 medium supplement with 10% heated fetal bovine serum, 1% of 2 mmol/L l-glutamine, 50 IU/mL penicillin, and 50 μg/mL streptomycin.

2.6. Antiproliferative assay

According to the cells' growth profile, cells were seeded with a density of 5000 cell/well (15 000 cell/cm^2) and incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide. After 24 hours, the cells were treated with the extracts, volatile oils, or pure compounds. Each extract/volatile oil initially dissolved in dimethylsulfoxide was diluted with the medium and passed through a 0.2-μm filter. Of each extract, 50 μg/mL was tested initially. The active extracts were considered to be those that gave less than 50% survival at an exposure time of 72 hours. The active extracts were further diluted in the medium to produce 8 concentrations (0.1, 0.5, 1, 5, 10, 25, 50, 100 μg/mL) of each extract, and the oils to give the concentrations 400, 200, 100, 50, 25, and 12.5 μg/mL. Of each concentration, 100 μL/well

was added to the plates in 6 replicates. The final dilution used for treating the cells contained not more than 1% of the initial solvent, this concentration being used in the solvent control wells. This concentration of dimethylsulfoxide did not significantly influence the proliferation rate as compared to media alone. The plates were incubated for 72 hours. At the end of the exposure time, cell growth was analyzed using the sulphorhodamine B (SRB) assay. Three replicate plates were used to determine the cytotoxicity of each sample. A positive control vincristine sulfate was used (lot no. 34H0447; Sigma, Buchs, Switzerland).

2.7. Sulphorhodamine B assay

After incubation for 72 hours, adherent cell cultures were fixed in situ by adding 50 μL of cold 40% (wt/vol) trichloroacetic acid and incubated for 60 minutes at 4°C. The supernatant was then discarded, and the plates were washed 5 times with deionized water and then dried. Of SRB solution (0.4% wt/vol in 1% acetic acid), 50 μL was added to each well and incubated for 30 minutes at room temperature. Unbound SRB was removed by washing 5 times with 1% acetic acid. Then, the plates were air dried and 100 μL of 10 mmol/L Tris base pH 10.5 (Sigma) were added to each well to solubilize the dye. The plates were shaken gently for 20 minutes on a plate shaker, and the absorbance of each well was read on an enzyme-linked immunosorbent assay reader at 570 nm. Cell survival was measured as the percentage absorbance compared to that of the control (nontreated cells) [16].

2.8. Statistical analyses

The percentage of cell survival was calculated as [mean (OD test − OD blank)/mean (OD control − OD blank)] × 100%. The IC_{50} (50% inhibitory concentration) was calculated using the SigmaPlot 9 software program, obtained by plotting the percentage of cell survival against respective concentrations of extracts used in the assay. Values for data collected are expressed as means ± SD of at least 3 independent experiments.

3. Results and discussion

3.1. Volatile oil composition

Volatile oils (0.17%, 0.85%, 0.23%, and 1.52%) were obtained from *L nobilis*, *O syriacum*, *O vulgare*, and *S triloba*, respectively. The composition of the volatile oils extracted from *L nobilis*, *O syriacum*, *O vulgare*, and *S triloba* are listed in Table 1. The table shows the retention indices (RIs) and percentages of the detected compounds. Retention indices of the sample components were calculated on the basis of homologous *n*-alkane hydrocarbons (C_8-C_{20}) under the same conditions. The identities of the separated volatile components of each oil were determined using their recorded mass spectra matched by library searching with the databank spectra (Nist Co., Gaithersburg, MD, and Wiley & Co., Hoboken, NJ) provided by the instrument software and comparing their calculated RI with literature values measured on columns with identical polarities [17]. The identities of several compounds, such as α-and β-pinenes, p-cymene, γ-terpineol, 1,8-cineol, α- and β-thujones, (−)camphor, limonene, linalool, thymol, borneol (Fluka, Buchs, Switzerland) carvacrol, β-caryophyllene, eugenol, humulene, and sabinene hydrate (Sigma-Aldrich, Buchs, Switzerland) were further confirmed by chromatography of their authentic standards under the same GC-MS conditions. The identified volatile constituents in the studied oils represented more than 90%. Each sample was analyzed twice.

In the analysis of volatile oil of fresh leaves of *L nobilis*, 1,8-cineol (eucalyptol) (40.91%) was the major component. Some other detected monoterpenes were α-pinene (5.82%), β-pinene (4.55%), sabinene (6.92%), limonene (2.10%), linalool (1.29%), and α-terpinyl acetate (5.86%). The detected monoterpenes and sesquiterpenes in the bay leaf oil were in accordance with those reported in the literature, although primarily quantitative variations were observed due to the environmental factors, growing conditions, ontogeny, extraction method, and GC-MS conditions carried out in the experiments [18-21]. As in the present study, 1,8-cineol has been consistently detected as the main component of *L nobilis* oil.

Variations in the composition of the volatile oils extracted from *Origanum* species have been the topic in the reports of several researchers. In the composition of the volatile oil of *O syriacum*—depending on the chemotypes—great variations in the major and minor constituents were recorded. Thymol and carvacrol are the 2 major components of this species, whereas their biogenetic precursors γ-terpinene and p-cymene also occur in considerably high amounts [22-28]. Occasionally, γ-terpinene was the major component of *O syriacum* as in the case of the hydrodistilled volatile oils obtained from plants grown in the southern parts of Turkey [29,30]. In the present study, the main components were carvacrol (47.10%) and *p*-cymene (30.22%), indicating that the evaluated *O syriacum* grown in Jordan belongs to the "cymyl" chemotype. Thymol and γ-terpinene were also detected in concentrations of 0.43% and 4.27%, respectively. Also, in several *O syriacum* specimens collected from the neighboring country of Syria, with similar climatic and environmental conditions, high concentrations of carvacrol were recorded [28].

It has been found that *O vulgare* grown in a Mediterranean climate contains higher amount of phenols, whereas *O vulgare* from the inland contains a higher amount of terpenoid alcohols [31,32]. In the present study, monoterpenoid alcohols constitute more than 60% of the *O vulgare* oil, with *trans*-sabinene hydrate (27.19%) and terpineol-4 (19.43%) as the major components. The absence of thymol and carvacrol in the volatile oil of the studied species and the presence of sabinene (5.56%) and *cis*-sabinene hydrate (4.14%) in considerable high amounts in our findings indicate the occurrence of the rare "sabinyl"

Table 1
Chemical composition of the essential oil hydrostilled from the leaves of *L nobilis*, *O syriacum*, *O vulgare*, and *S triloba* based on RI [a] and percentages (%) [b]

Compound	*L nobilis*[1]		*O syriacum*[2]		*O vulgare*[3]		*S triloba*[4]	
	RI	%	RI	%	RI	%	RI	%
α-Thujene	934	0.62	933	0.47	935	0.54	934	0.38
α-Pinene	945	5.82	945	0.38	946	0.64	944	3.35
Camphene	959	0.58	958	0.21	–	–	959	2.88
Octen-3-ol	–	–	980	0.36	–	–	–	–
Sabinene	981	6.92	982	0.35	982	5.56	980	0.24
β-Pinene	984	4.55	985	0.60	985	1.30	984	8.98
Myrcene	–	–	–	–	994	0.28	–	–
3-Octanol	–	–	996	0.33	–	–	–	–
Δ^2-Carene	1007	0.63	–	–	–	–	–	–
α-Terpinene	1026	0.70	1021	0.37	1024	4.46	1020	0.44
p-Cymene	1033	0.74	1030	30.22	1031	3.83	1030	0.34
Δ3-Carene	–	–	1033	0.36	1038	0.59	–	–
Limonene	1036	2.10	–	–	1034	1.68	–	–
β-Phellandrene	–	–	1036	0.35	1036	0.80	–	–
DL-Limonene	–	–	–	–	–	–	1035	1.60
1,8-Cineol	1037	40.91	1038	0.27	–	–	1039	45.16
γ-Terpinene	1064	1.03	1064	4.27	1069	7.83	1067	0.84
Sabinenehydrate (*cis*)	–	–	1078	3.22	1078	4.14	1078	0.39
Terpinolene	–	–	–	–	1092	2.01	–	–
α-Terpinolene	–	–	1092	0.35	–	–	1096	0.43
Sabinenehydrate (*trans*)	–	–	–	–	1100	27.19	–	–
Sabinene hydrate (E)	1097	0.64	1106	0.61	–	–	–	–
Linalool	1105	1.29	1104	0.41	1102	2.17	1104	0.42
α-Thujone	–	–	–	–	–	–	1108	2.16
β-Thujone	–	–	1117	0.38	–	–	1118	2.19
p-Menth-2-en-1-ol	–	–	–	–	1130	0.51	–	–
Terpneol-1	–	–	–	–	1140	0.30	–	–
Camphor	–	–	1150	0.77	–	–	1151	11.53
Borneol	–	–	1172	0.31	1176	0.63	1172	1.18
Terpinene-4 ol	1180	1.55	–	–	–	–	1180	1.09
Terpineol-4	–	–	–	–	1181	19.43	–	–
4-Terpineol	–	–	1191	0.34	–	–	–	–
α-Terpineol	–	–	–	–	–	–	1192	0.62
Dihydrocarvone	–	–	1204	0.36	–	–	–	–
γ-Terpineol	–	–	–	–	1211	6.56	1205	4.40
Sabinene hydrate acetate	1213	1.25	–	–	–	–	–	–
trans-Piperitol	–	–	–	–	1218	0.42	–	–
Carvacrol methylether	–	–	1247	0.23	–	–	–	–
Linalylacetate	1260	0.34	–	–	–	–	–	–
Bornylacetate	–	–	1291	0.34	–	–	1296	2.60
Thymol	–	–	1295	0.43	–	–	–	–
p-Cymene-7-ol	1298	0.61	–	–	–	–	–	–
Carvacrol	–	–	1304	41.10	–	–	–	–
Eugenol	1351	0.92	–	–	–	–	–	–
α-Terpinenylacetate	1356	5.86	–	–	–	–	1355	1.19
Methyleugenol	1409	1.62	–	–	–	–	–	–
Caryophyllene (E)	1425	0.37	1424	2.50	1425	1.79	1420	2.23
Aromadendrene	–	–	–	–	–	–	1443	2.37
α-Humulene	–	–	1461	0.43	–	–	1460	1.07
Bicyclogermacrene	–	–	–	–	1510	1.05	–	–
Spathulenol	–	–	–	–	1581	0.76	–	–
Caryophylleneoxide	–	–	1590	0.36	1586	0.57	–	–
Viridiflorol	–	–	–	–	1595	0.60	1601	0.79
Humulene epoxide II	1610	5.85	–	–	–	–	–	–
Isoeugenolacetate (E)	1616	2.63	–	–	–	–	–	–
Eudesmol γ	1632	1.96	–	–	–	–	–	–
Selina-3,11dien-6-α-ol	1647	0.98	–	–	–	–	–	–
α-Cadinol	1650	0.61	–	–	–	–	–	–
α-Eudesmol α	1653	0.54	–	–	–	–	–	–
α-Bisabolol oxide B α	1657	1.08	–	–	–	–	–	–

Table 1 (*continued*)

Compound	*L nobilis*[1]		*O syriacum*[2]		*O vulgare*[3]		*S triloba*[4]	
	RI	%	RI	%	RI	%	RI	%
Eudesmol 7-epi-α	1665	0.66	–	–	–	–	–	–
Total identified	–	93.36	–	96.68	–	95.64	–	98.87
Monoterpenoids	–	76.14	–	92.7	–	90.87	–	92.41
Monoterpenoid hydrocarbons	–	23.69	–	37.93	–	29.52	–	19.48
Oxygenated monoterpenoids	–	52.45	–	54.77	–	61.35	–	72.93
Sesquiterpenoids	–	12.05	–	3.29	–	4.77	–	6.46
Sesquiterpene hydrocarbons	–	0.37	–	2.93	–	2.84	–	5.67
Oxygenated sesquiterpenes	–	11.68	–	0.36	–	1.93	–	0.79
Phenylpropanoids	–	5.17	–	–	–	–	–	–
Miscellaneous	–	–	–	0.69	–	–	–	–
Unidentified	–	6.64	–	3.32	–	4.36	–	1.13

[1-4]Compounds are generally listed following their elution order.
[a] Retention indices calculated on DP-5MS column.
[b] Percentage is given as the average of 2 independent measurements.

chemotype *O vulgare* in Jordan [33,34]. In addition to the very complex chemotaxonomy of the genus *Origanum*, it has been observed that the variability between commercially produced wild growing plants within the same climate remains high in oil yield and phenolic contents [35].

In the analysis of the volatile oil of the dried leaves of *S triloba*, bicyclic oxygenated monoterpenes represented by 1,8-cineol (45.16%), camphor (11.53%), and γ-terpineol (4.40%), together with the bicyclic hydrocarbon monoterpenes α-pinene (3.35%) and β-pinene (8.98%), were characterized as the major components. In most of the studies on the volatile oil composition of *S triloba*, 1,8-cineol was found to be the main component in the hydrodistilled- and in microwave-assisted hydrodistilled oils as well as in oils obtained by supercritical extraction [36-41].

In comparison of the chemical composition of the volatile oils of the 4 culinary herbs examined and considering the published data, variations were observed as expected. These more qualitative, less quantitative differences in the oil composition are primarily determined by genetic and environmental factors and influenced by the analytical procedures applied [42].

3.2. Antiproliferative activity

The antiproliferative activity of *L nobilis*, *O syriacum*, *O vulgare*, and *S triloba* were determined by cell proliferation assay using the MCF7 cell line. MCF7 is an adenocarcinoma cell line recommended as one of the models for breast cancer tissue by the National Cancer Institute (NCI). The cell line expresses estrogen receptors and has been well studied and documented [43].

Cultures of MCF7 cells were treated with ethanol and water extracts and with the hydrodistilled volatile oils first at one concentration of 50 μg/mL, and the results of this first screening are shown in Table 2. For crude plant extracts that showed less than a 50% survival rate, namely ethanol extracts of *S triloba* (14.89 ± 1.21), *O syriacum* (23.89% ± 2.43), and ethanol extract *L nobilis* (52.53 ± 1.38)—slightly exceeding 50%—further dilutions were made to calculate the exact IC_{50} values. The IC_{50} values for these extracts were calculated as 6.40 ± 3.17 μg/mL for *O syriacum*, 24.49 ± 8.17 μg/mL for *L nobilis*, and 25.25 ± 1.21 μg/mL for *S triloba* (Fig. 1). Control assays were carried out for samples containing only the appropriate volumes of blank solutions and those that showed no effect on cell growth.

Table 2
Antiproliferative effects of ethanol and aqueous extracts and volatile oils of *L nobilis*, *O syriacum*, *O vulgare*, and *S triloba* in MCF7 cell line

	Ethanol extract	Aqueous extract	Volatile oil
L nobilis	52.53 + 1.38	88.32 ± 4.71	150.97 ±.58
O syriacum	23.89 ± 2.43	114.18 ± 7.93	122.80 ± 5.84
O vulgare	88.61 ± 25.53	107.83 ± 5.04	64.09 ± 9.95
S triloba	14.89 ± 1.21	99.71 ± 9.43	83.64 ± 9.28

Data are expressed as percentage ± SD of cell survival of MCF7 cells after 72 hours of exposure to 50 μg/mL fractions of plant extracts. Results present the average and SD of at least 3 readings.

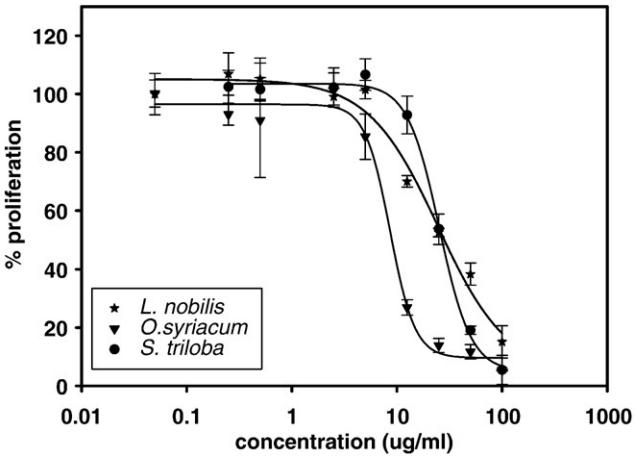

Fig. 1. Percentage proliferation of MCF7 cell line by *L nobilis*, *S triloba*, and *O syriacum* ethanol extracts. Result presents the average and SD of 3 replicates.

For accurate estimation of the antiproliferative activity of the volatile oils, serial dilutions were prepared and the antiproliferative activity was assessed. Tests were done in 3 replicates and repeated on 2 different cell passages. Results are shown in Fig. 2. The IC_{50} values calculated are 30.1 ± 1.14 for *O vulgare*, 130.4 ± 52.2 for *O syriacum*, 101.7 ± 7.91 for *L nobilis*, and 174.3 ± 73.04 for *S triloba*.

In the determination of the antiproliferative activity against the MCF7 cell line, the ethanol extract of fresh leaves of *L nobilis* showed activity. During preliminary screening at one concentration (50 μg/mL), the ethanol extract of *L nobilis* exhibited an inhibition of 52.53%. Regardless of the United States NCI recommendation that a plant extract should be considered as active if it inhibits less than 50% at 50 μg/mL concentration, in this study, the value of 52.53% was considered as "borderline" activity, and IC_{50} was determined for the ethanol extract of *L nobilis* [44]. Accordingly, an IC_{50} value of 24.49 μg/mL was obtained. National Cancer Institute requires for the active crude extracts an IC_{50} value of less than 30 μg/mL. Although the antibacterial and antioxidant activities of *L nobilis* have been extensively studied and reported, there are limited reports dealing with the cytotoxicity of *L nobilis* [18,45-49]. Barla et al [45] investigated *L nobilis* extracts for ovarian cytotoxic activity and for DNA damaging properties against 3 yeasts. Among the 3 tested extracts, the most cytotoxic active extract against the ovarian cancer cell line was found to be the fruit extract with 98% inhibition. The same extract showed marginal inhibition against one DNA repair-deficient yeast strain. Also, the known sesquiterpene lactones were found to be highly cytotoxic against the A2780 cell line [4]. Loizzo et al [46] reported potent cytotoxic activity for *L nobilis* fruit oil against amelanotic melanoma C32 and renal cell carcinoma ACHN, whereas Kaileh et al [47] did observe cytotoxicity against fibrosarcoma cells but not toward MCF7 cell lines using dichloromethane/methanol extract of the leaves. Recently, Lantto et al [49] determined the cytotoxic effects of lyophilized *L nobilis* leaf extracts in SH-SY5Y neuroblastoma cells.

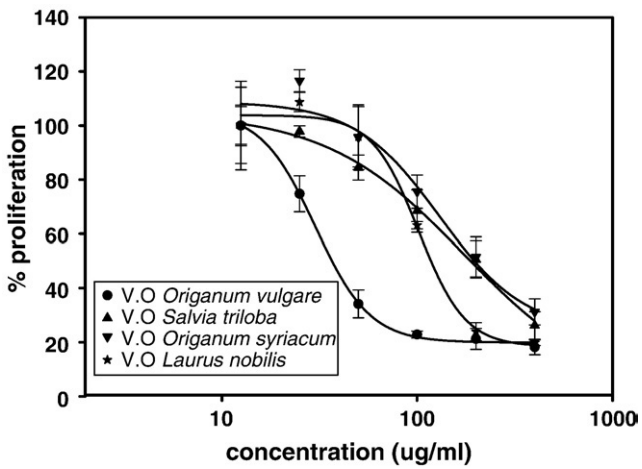

Fig. 2. Percentage proliferation of MCF7 cell line by volatile oils of *L nobilis*, *O syriacum*, *O vulgare*, and *S triloba*. Result presents the average and SD of 4 replicates.

In the analysis of *O syriacum* extracts and the volatile oil, the activity was observed with the ethanol extract. In an earlier study, Al-Kofahi et al [50] could not demonstrate any antiproliferative activity for *O syriacum*. Other species of *Origanum* were also studied for their cytotoxic activity using different cell lines. Bakkali et al [51] studied the genotoxic effects of the essential oil of *O compactum* using the yeast *Saccharomyces cerevisiae* and observed a clear cytotoxic effect, with the cells being more sensitive to the essential oil in the exponential phase rather than in the stationary growth phase. Chinou et al [52] tested the cytotoxic activity of several extracts of *O dictamnus* against murine leukemia (P388) and nonsmall cell lung cancer (NSCLC-N6) cell lines. Both, the initial dichloromethane extract of *O compactum* and the isolated ursolic acid were found to have cytotoxic activity. None of the tested extracts and the volatile oil of the second *Origanum* species (*O vulgare*) used in the present study showed antiproliferative activity against MCF7 cell line.

Cytotoxic and antiproliferative activities are reported for several *Salvia* species. In the present study, only the ethanol extract of *S triloba* exhibited antiproliferative activity to the MCF7 cell line. Similar antiproliferative activity was reported earlier by Alkofahi et al [53]. Extracts of 17 *Salvia* species used in traditional medicine in South Africa were subjected to biological testing for the cytotoxic effects on 3 human cancer cells (MCF7, HT-29, SF-268) and a human kidney epithelial cell line. *S radula* exhibited the most favorable activity, whereas *S africana-lutea* and *S runcinata* were among the least active against the MCF7 cells. Two species, *S dolomitica* and *S garipensis*, showed a degree of selectivity, as they were not active against the HT-29 and SF-268 cell lines but active against the MCF7 cell line [9]. Furthermore, salvicine, a structurally modified diterpenoid quinone derived from *S prionitis*, is considered a novel anticancer drug candidate. The compound showed significant in vitro and in vivo activity against malignant tumor cells and xenografts, especially in some solid human tumor models. This anticancer activity of salvicine is associated with its ability to induce tumor cell apoptosis. Salvicine acted as a topoisomerase II poison through its marked enhancement effect on topo II-mediated DNA double-strand breaks [54]. Studies using MTT assay (3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyltetrazolium) showed that all tanshinones (abietane-type diterpene quinones isolated from the roots of *S miltiorrhiza*) decreased cell viability of HepG2 cells in a concentration-dependent manner [55]. Fiore et al [56] tested 6 *Salvia* species (some collected in Jordan) against prostate carcinoma, colorectal adenocarcinoma, choriocarcinoma, B lymphoblast, glioblastoma, and endometrium adenocarcinoma cell lines and

demonstrated antiproliferative activity for *S dominica*. For the latter species, Abu-Dahab and Afifi [57] reported antiproliferative activity using the MCF7 cell line.

These observations and reports (with regard to the cytotoxicity of the plant extracts) indicate that there are great differences among the antiproliferative activity of the same plant species, depending on the plant parts and extraction solvents used. Furthermore, the different cell lines vary in their sensitivity to the same plant extract.

In conclusion, it is worthwhile to screen the commonly used plants from the local flora for different biological activities because they might present a new alternative source for possible bioactive substances. In this aspect, the culinary herbs and spices have major advantages being inexpensive, safe (used since generations), and easily accessible. Nevertheless, fractionation, purification, and isolation processes are underway with the aim to isolate and chemically modify bioactive natural compounds.

Acknowledgment

This work was supported by the Deanship of Scientific Research at the University of Jordan (grant no. 157/2007-2008). The technical assistance of Lara Majdalawi and Ismail Abaza is appreciated.

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