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Antitumoral and antioxidant effect of essential oils and in vitro antioxidant properties of essential oils and aqueous extracts from Salvia pisidica

ÖZKAN A. , Erdogan A. , Sokmen M., Tugrulay S., ÜNAL O.

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Özet

The aim of the work was to investigate antitumoral effect of essential oils on cancer cells and their possible protective (antioxidant) effects against hydrogen peroxide-induced cytotoxicity. Also, in vitro antioxidant properties of essential oils and aqueous extracts from wild form and cultivated form of Salvia pisidica were compared.

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Antitumoral and antioxidant effect of essential oils and *in vitro* antioxidant properties of essential oils and aqueous extracts from *Salvia pisidica*

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Abstract: The aim of the work was to investigate antitumoral effect of essential oils on cancer cells and their possible protective (antioxidant) effects against hydrogen peroxide-induced cytotoxicity. Also, in vitro antioxidant properties of essential oils and aqueous extracts from wild form and cultivated form of *Salvia pisidica* were compared.
We found out that essential oils from wild form (IC_{50} 128, and 85.17 μg /mL) and cultivated form (IC_{50} 115.00 and 74.83 μg/mL) differed in their antitumoral effects on Hep G2 and H1299 cells respectively. As a very important we consider the finding that both essentials oils significantly reduced the cytotoxicity induced by the strong oxidant in cancer cells. We assume that protective effect of essential oils on cancer cells against H_2O_2 cytotoxicity can be accompanied by their antioxidant action.
Antioxidant properties of essential oils and aqueous extract from wild and cultivated *S. pisidica* were determined by different test systems. In DPPH assay, the weakest radical scavenging activity was exhibited by the aqueous extract from wild form (66.55 ± 1.15 μg/mL). Similarly inhibition of linoleic acid oxidation of aqueous extract from wild and cultivated form (50.93% and 59.66%) was lower than essential oils. In the case of reducing power assay and hydroxyl radical scavenging activity, a similar activity pattern was observed as given in the first two systems. Essential oils from wild and cultivated form had higher reducing power activity (EC_{50}; 100.99 ± 3.0 and 96.87 ± 3.15 μg/mL) and hydroxyl radical scavenging activity (EC_{50}; 15.99 ± 0.95 and 13.01 ± 1.35 μg/mL) than aqueous extracts.

Key words: *Salvia pisidica* L.; antioxidant; antitumoral; aqueous extract; cancer cells; essential oils

Abbreviations: AqE, aqueous extract; CF, cultivated form; EsO, essential oil; EC50, efficient concentration for 50% scavenging of radicals or reducing activity; Hep G2, hepatoma G2; IC50, concentration which inhibits 50% cell growth; TPC, total phenolics content; WF, wild form.

Introduction

Antioxidants play important roles in preventing the diseases induced by reactive oxygen species (ROS) which result in oxidative damage to DNA, proteins and other macromolecules (Ozkan et al. 2007; Fiskin et al. 2006) and are associated with degenerative or pathological events, such as aging (Balaban et al. 2006), asthma (Wilcox et al. 2004) and cancer (Klaunig & Kamendulis 2004; Ozkan et al. 2004). However, there are serious concerns about the carcinogenic potential of synthetic antioxidants widely used in the food industry, for example, butylated hydroxyanisole and butylated hydroxytoluene (Thompson et al. 1988). Therefore, intensive research has been carried out to develop natural alternatives, which may serve as potent candidates in combating carcinogenesis and aging processes.

The safety of synthetic antioxidants used as food preservatives has been studied for years. Their usage has been subjected to strict regulation in many countries after they found that high levels of BHT, BHA, and TBHQ (tert-butylhydroquinone) may cause health problems for this reason they were taken off the list of safe additives in the United States (Kahl & Kappus 1993; US FDA 1997). Concomitantly, public attention to natural antioxidants has been increasing during the last years, and the industry needs to find natural sources of antioxidants that could replace synthetic antioxidants or at least reduce their use as food additives (Shahidi 2000).

Salvia pisidica is the one of the endemic species of *Salvia* genus and widely used as a kind of herbal tea named "ada çayı" in Antalya, Turkey. *Salvia* species have long been used as herbal tea and in a variety of food preparations. *Salvia* species mainly contain essential oil and phenolic compounds such as flavonoids, phenolic acids, and phenolic diterpenes (Baser et al. 1998; Areias et al. 2000). *Salvia* species have received particular attention as a source of natural antioxidants.

According to one study in Spain, the principal com-

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ponents of sage essential oil were camphor (24.95%), 1,8-cineole (24.75%) and camphene (7.63%) (Viuda-Mortas et al. 2007). *Salvia* essential oils were reported as non membrane-toxic (Demirci et al. 2005). In human tumor cells, camphor (BD12) (CC_{50} = 29.7 μg/mL) induced apoptosis and showed cytotoxicity comparable to curcumin and dibenzoylmethane (BD2) (CC_{50} = 22.5 μg/mL) (Nakano et al. 2004).

We decided to evaluate the antitumoral and antioxidant (protective) effect of essentials oils in cancer cells and compare the antioxidant properties of essential oil and aqueous extract from wild and cultivated *S. pisidica*.

Material and methods

Collection of plant material

Wild form (WF) of *Salvia pisidica* Boiss.& Heldr. Ex Benthamwere collected during their flowering seasons from Korkuteli, Antalay-Turkey, July, 6th, 2007. They were identified by a senior taxonomist Dr.Orhan Unal. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Akdeniz Üniversity, Antalya-Turkey (voucher no: SP 302W)

Cultivation of plant material

S. pisidica was collected growing wild from Antalya. All plants with rooting were collected and planted at collection garden in 2007 (voucher no: SP 302C). The propagation of the species by stem cuttings were investigated at 3000 ppm IBA dose. The study was conducted at the experiment green house of Batı Akdeniz Research Institute. Cultivated plant (CF) materials were collected and aerial parts of plants were dried in the shade as wild plants.

Essential oil and aqueous extracts

Aerial parts of plants were dried in the shade and used for extraction. One hundred grams of air-dried plant material, including flowers, leaves and stems from each population (wild and cultivated *S. pisidica* populations) were cut in small pieces, and the essential oils were obtained by hydro distillation in 1500 mL H_2O for 3 h by a Clevenger apparatus (ILDAM Ltd., Ankara, Turkey). The obtained essential oil (EsO) was tested and analyzed. The residual water was used as aqueous extracts (AqE).

Gas Chromatography / Mass Spectrometry (GC/MS) analysis

The oils, dried over anhydrous sodium sulfate, were subsequently analyzed by GC-MS and stored at –20 °C. The composition of the volatile constituents was established by GC-MS/quadruple detector analyses using a Shimadzu QP 5050 system, fitted with an FFAP (50 m 0.32 mm (i.d.), film thickness: 0.25 mm) capillary column. Detector and injector temperature were set at 230 °C. The temperature program for the FFAP column was from 120 °C (1min) to 230 °C at a rate of 6 °C min^{-1}) and than held at 200 °C for 35min. Helium was used as a carrier gas at a flow 14 psi (split 1:10) and injection volume of each sample was 1 mL. The percentage composition was computed from the GC peak areas according to the 100% method without using any correction factors. The identification of the components was based on comparison of their mass spectra with those of Wiley and Nist, Tutore Libraries. The ionization energy was set at 70 eV.

Determination of the total phenolic content

The amount of total phenolics content (TPC) was determined using the Folin–Ciocalteu method (Zheng & Wang 2001). In this method 5 mL of distilled water were added into a 10 mL volumetric flask. A suitable volume of the herbal extract was transferred into the volumetric flask to obtain absorbance in the range of the prepared calibration curve. About 0.2 mL of Folin–Ciocalteu reagent was added and mixed well. After 3 min, 0.4 mL saturated Na_2CO_3 solution was added, mixed well and made up to volume with distilled water. After a 1 h reaction in the dark, the absorbance was measured at 725 nm. A calibration curve of gallic acid was prepared, and the results were expressed as μg GAE (gallic acid equivalents/mL).

2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay

Hydrogen atoms or electrons donation ability of the corresponding samples was measured from the bleaching of purple colored methanol solution of DPPH. This assay was carried out following the same method as reported elsewhere (Hatano et al. 1988). Fifty micro liters of various concentrations of the samples dissolved in methanol was added to 5 mL of a 0.004% methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl, free radicals). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in the following way;

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

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC_{50}) was calculated from the linear regression algorithm of the graph plotted inhibition percentage against extract concentration. For the calculation of these values, Microsoft Excel software was used. Values are presented as means ± SD of five parallel measurements.

β-Carotene–linoleic acid assay

In this assay, antioxidant capacity is determined by indirectly measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al. 1998). A stock solution of β-carotene/linoleic acid mixture was prepared as follows. First, 0.5 mg of β-carotene was dissolved in 1 mL of chloroform (HPLC grade), then 25 μL of linoleic acid and 200 mg of Tween 40 were added. The chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of distilled water saturated with oxygen (30 min at a flow rate of 100 mL/min) was added with vigorous shaking. A 2.5 mL amount of this reaction mixture and 350 μL portion of the sample (2 mg/mL) in ethanol were dispensed each test tube, which was incubated up to 48 hour at room temperature. After this incubation period, the absorbance of the mixture was measured at 490 nm. Same procedure was repeated with synthetic antioxidants, butylated hydroxytoluene (BHT), ascorbic acid and α-tocopherol as positive controls, and a blank

Antioxidative capacities of the oils were compared with those positive controls and blank (contains ethanol instead of essential oil). Values are presented as means ± SD of five parallel measurements.

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Reducing power assay
The reducing power was determined according to Oyaizu (1986). Each sample (10–500 μg/mL) in methanol (1 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium, and the mix was incubated at 50 °C for 20min. Then 2.5 mL of 10% trichloroacetic acid was added, the mixture was centrifuged at 20 g for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of de-ionized water and 0.5 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. EC_{50} value μg/mL is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. BHT, ascorbic acid and α-tocopherol were used as positive control. Values are presented as mean of five parallel measurements.

Hydroxyl radical scavenging activity
The hydroxyl radical scavenging activities of the samples were assayed by using the 2-deoxyribose oxidation method with slight modification (Halliwell et al. 1987). The reaction mixture contained 0.4 mL of 0.2 M sodium phosphate buffer (pH 7.4), 0.1 mL sample solution at different concentrations, 0.1 mL of 1 mM EDTA, 0.1 mL of 1 mM $FeCl_3$, 0.1 mL of 12 mM hydrogen peroxide, 0.1 mL of 60 mM 2-deoxyribose, and 0.1 mL of ascorbic acid. After incubation at 37 °C for 1 h, the reaction was stopped by adding 1 mL of 2% trichloroacetic acid and 1 mL of 1.0% thiobarbituric acid. The mixture was boiled for 15min, cooled in ice and extracted by *n*-butanol and the absorbance was measured at 532 nm against *n*-butanol (as blank). The reaction mixture without test sample was used as control and BHT, ascorbic acid and α-tocopherol were used as positive control.

Cancer cell culture
The hepatoma G2 cells (Hep G2) line and H1299 cell line were purchased from American Type Culture Collection (Rockville, MD). Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium) supplemented with10% fetal calf serum, 1% antibiotic-antimycotic solution in a humidified atmosphere containing 5% CO_2 at 37 °C. For subculturing, cells were harvested after trypsin/EDTA treatment at 37 °C. Cells were used when monolayer confluence had reached 75%.

Cell viability assay
The cancer cells (500 cells/well, monolayer) were plated in a 96-well plate. The next day the cells were treated with different concentrations of essential oil (EsO) and aqueous extract (AqE) (20–300 μg/mL) in the medium for 24 hours. At the end of the incubation periods (24h), the cytotoxicity of EsO and AqE on cancer cells was determined by the The CellTiter-Blue® Cell Viability Assay. The assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Nonviable cells rapidly lose metabolic capacity and thus do not generate a fluorescent signal (Gloeckner et al. 2001). Following cellular reduction, fluorescence is recorded at 560 nm excitation/590 nm emissions. The data were expressed as average values obtained from eight wells for each concentration. The IC_{50} value calculated from equation of graph. H_2O_2 cytotoxicity on cancer cells was measured with same way. For measuring antioxidant effect of EsO against H_2O_2 cytotoxicity, the cells were preincubated different EsO concentrations (10–150 μg/mL) for 1 hour, before hydrogen peroxide treatment (IC_{10} IC_{20} IC_{50} IC_{70}) for 24 hour.

Table 1. Chemical composition of wild and cultured *S. pisidica* essential oil determined by GC/MS.

Components	Composition (%) *S. pisidica* (WF)†	Composition (%) *S. pisidica* (CF)†
α-pinene	1.57	1.69
Camphene	1.09	1.94
β-pinene	3.31	0.24
Sabinene	0.22	–
Limonene	0.46	–
Eucalyptol (1,8-Cineole)	11.37	18.45
Cymene	1.44	–
3-octanol acetate	0.37	0..39
3-octanol	0.77	1.49
Linalool oxide	0.49	–
Thujone	11.84	2.40
Camphor	26.87	32.17
Pinocarvone	0.84	0.17
Bornyl acetate	1.49	3.37
Norinone/Norpinone	1.03	–
Terpineol-4	0.75	–
Sabinol/Sabinyl Acetate	20.21	17.76
Pinacarveol	1.50	0.69
Verbenol	0.72	2.55
Cryptone	1.65	–
Borneol	2.84	2.21
Cuminal	1.40	–
Cymene-8-ol	0.39	0.31
Caryophyllene Oxide	1.29	0.92
3-oxo-beta ionone/ isomethyl beta iononone	3.71	7.62
Eudesmal	0.68	0.58
Spathulenol	–	2.43
Eugenol	–	0.55
Total	98.3	97.89

†CF; cultured form of *S. pisidica,* WF; wild form of *S. pisidica.*

Data analysis
The results of the replicates were pooled and expressed as mean± standard deviation. Analysis of variance and Student's *t*-test were carried out. Significance was accepted at $P \leq 0.05$ (Kirkman 1996).

Results and discussion

GS-MS analysis of the crude oil isolated from dried aerial parts of wild form (WF) and cultivated form (CF) of *S. pisidica* resulted in the identification of some compounds as shown in Table 1. According to gas chromatography (GS/MS) analysis results; 26 (98.3%) and 20 (97.89%) compounds were identified respectively. While camphor, sabinol/sabinyl acetate, thujene and eucalyptol (1,8-Cineole) are the major compounds for EsO-WF, 3-oxo-beta ionone/isomethyl beta iononone, was the main constituent for EsO-CF with camphor, eucalyptol (1,8-Cineole) and sabinol/sabinyl acetate.

To the best of our knowledge, there are many reports on the chemical composition of the oils isolated from the plants from the plant belonging to the genus *Salvia* (Baser et al. 1993, 1995, 1996, 1997). Most of these reports indicate that 1,8-cineole is the main characteristic constituents of *Salvia* oil (Demirci et al. 2002, 2003). As reported elsewhere, the principal components

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Table 2. Antioxidant capacities of wild and cultivated form of *S. pisidica* essential oil and aqueous extract and positive controls.

Samples	DPPH[b]	β-Carotene/linoleic acid[c]	Reducing power[d]	Hydroxyl radical Scv. Act.[e]	TPC[f]
EsO-WF	9.13 ± 0.65 bc	63.20 ± 1.51 y	100.99 ± 3.00 c	15.99 ± 0.95 a	–
EsO-CF	7.83 ± 0.93 b	72.65 ± 2.12 z	96.87 ± 3.15 c	13.01 ± 1.35 a	–
AqE-WF	66.55 ± 1.15 ef	50.93 ± 1.31 x	198.55 ± 3.78 e	89.11 ± 2.05 d	495 ± 3.6 a
AqE-CF	61.47 ± 0.94 e	59.66 ± 1.44 xy	183.99 ± 3.78 d	87.88 ± 1.11 d	515 ± 4.3 b
BHT	18.35 ± 1.27 d	95.92 ± 1.61 w	51.55 ± 2.78 a	31.66 ± 1.55 b	–
Ascorbic acid	3.81 ± 0.26a	93.95 ± 1.82 w	61.66 ± 1.65 b	51.66 ± 2.75 c	–
α-Tocopherol	6.91 ± 0.35b	96.16 ± 1.53 w	57.22 ± 2.80 ab	12.99 ± 0.33 a	–

[a]Results are means of five different experiments. Values that are fallowed by different letters within each column ara significantly different ($p \leq 0.05$); [b]EC_{50} value of DPPH assay (as µg/mL); [c]Given as percentage of % inhibition of the linoleic acid; [d]EC_{50} values of reducing power assay (as µg/mL); [e]EC_{50} values of hydroxyl radical Scavenging activity (as µg/mL) ; [f]Total phenolic content (as µg GA/mL)

of sage essential oil were camphor (24.95%), 1,8-cineole (24.75%) and camphene (7.63%) (Viuda-Martos et al. 2007). In another works, camphor and 1,8-cineole were found main components for most of the other salvia species (Baser et al. 1995, 1997). α-thujene was found main component for *S. pilifera* oil (Kelen & Tepe 2008). Our results show a strong similarity with these reports.

The percentage composition of EsO components of the WF and CF of *S. pisidica* were different. The EsO-WF have lower amount of camphor and 1,8-cineole but higher amount of β-pinene, thujone, sabinol than EsO-CF. Contras to literature spathulenol and eugonel in EsO-WF were not identified. Sabinene, limonone, cymene, linalool oxide, norinone/norpinone, cryptone and cuminal were also not determined in EsO-CF.

In addition to intrinsic factors in the plant, other considerations, such as the pre-extraction processing and extraction conditions directly affect the concentration of total phenolic compounds (TPC) in the final extract. The TPC of ethanol extracts from *S. hortensis* and *S. fruticosa* was found higher their acetone extracts (Georgios & Georgios 2006). On the other hand, TPC of leaves extracts were higher than flowers extracts of *S. fruticosa* (Urek et al. 2008). In one study, sage, *S. fruticosa* infusions had almost same amount of TPC (124 ± 1.57 mg GA/cup) with our results (Atoui et al. 2005). TPC of *S. fruticosa* infusions was found around our AqE-CF, but higher than AqE-WF. Similarly, our TPC amount of AqE-WF and AqE-CF was found higher than *S. glutinose, S. pratensis* and *S. halophila* and lower than *S. officinalis* and *S. sclare* (Kalantzaki & Blekas 2006; Atoui et al. 2005; Miliauskasa et al. 2004). As shown in Table 2, the TPC of AqE-WF (495 ± 3.6 µg GA/mL) was found slightly lower than AqE-CF (515 ± 4.3 µg GA/mL). Amount of TPC is very important for antioxidant capacity.

Antioxidant activity

The chemical complexity of oils and extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behaviour, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable (Sacchetti et al. 2005). Taking into account of identifying hydrophilic and liphophilic species, essential oils and extracts were individually assessed for their possible antioxidative activities by employing complementary tests; free radical-scavenging activity (DPPH), β-carotene/linoleic acid antioxidant assay, reducing power and hydroxyl radical scavenging assay. The antioxidative capacities of essential oils and aqueous extracts from wild and cultivated form of *S. pisidica* were determined by comparing with the activities of known antioxidants, such as BHT, ascorbic acid and α-tocopherol.

Free radical scavenging capacities of the corresponding oils and aqueous extract were measured by DPPH assay and the results are shown in Table 2. According to the results obtained, EsO-CF was found the slightly active one with an EC_{50} value of 7.83 ± 0.93 µg/mL than EsO-WF. This result can come from essential oil composition of them. EsO-CF have slightly higher amount of camphor and eucalyptol than EsO-WF. Both essential oils are strongly active than positive control BHT. The DPPH radical scavenging capacities of essential oils were found more effective than aqueous extracts. When compare to other Salvia species, the DPPH radical scavenging activity of essential oils from *S. pisidica* was foundhigher than *S. aucheri var. aucheri, S. aramiensis, S. pilifera* and lower than *S. cryptantha, S. multicaulis* and *S. officinalis.* This activity of aqueous extract from *S. pisidica* was found more effective than sage and *S. friticosa* leaf and flowers extract (Bozin et al. 2007; Kelen & Tepe 2007; Tepe et al. 2004; Atoui et al. 2005). The scavenging activity increased with increasing concentration of the essential oil and extracts from *S. pisidica*.

Oxidation of the linoleic acid was effectively inhibited higher by the EsO-WF and EsO-CF compare to AqE-WF and AqE-CF of *S. pisidica* (Table 2). On the other hand, inhibition of linoleic acid oxidation capacity of essential oil was not superior as positive controls. Both, essential oils and aqueous extract have linoleic acid inhibition activity against some Salvia species extracts such as *S. multicaulis* and *S. cryptantha* (Tepe et al. 2004).

The reducing power of the EsO-WF, EsO-CF, AqE-WF and AqE-CFof *S. pisidica* was shown in Table 2. Like the other antioxidant activities, the reductive potential of the EsO-WF, EsO-CF, AqE-WF and AqE-CF exhibited a dose-dependent activity within a concentration range of 10–500 µg/mL. On the other hand,

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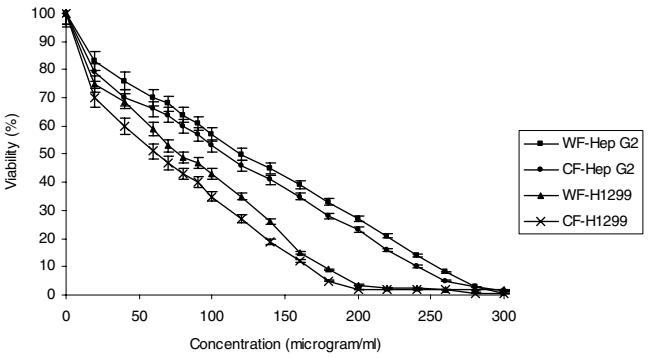


Fig. 1. Dose-dependent cytotoxicity of essential oil from wild and cultivated form of *S. pisidica* on Hep G2 and H1299 cancer cells.

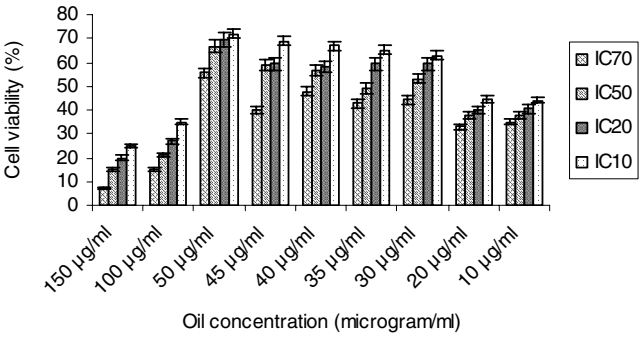


Fig. 2. Antioxidant effect of essential oil from wild form of *S. pisidica* on Hep G2 cancer cells against H_2O_2 cytotoxicity.

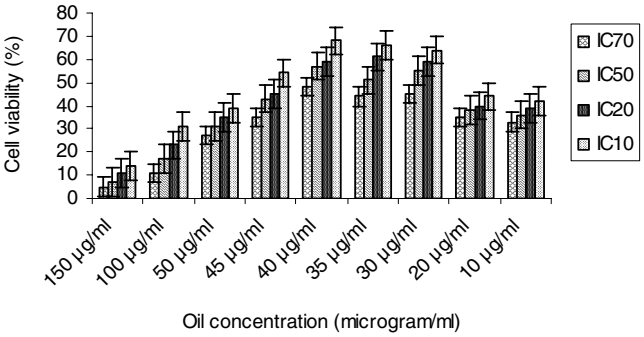


Fig. 3. Antioxidant effect of essential oil from wild form of *S. pisidica* on H1299 cancer cells against H_2O_2 cytotoxicity.

EsO of *S. pisidica* have had higher reducing power activity than AqE of *S. pisidica.* The reducing capacity of S. *pisidica* extracts was found higher than flowers extract while lower than leaves extract of *S. fruticosa* (Urek et al. 2008).

There is considerable interest in the possibility that O_2^- and H_2O_2 mediate some of their toxicity by becoming converted into the highly reactive hydroxyl radical, $^{\bullet}OH$, in reactions that need metal ions. Various "hydroxyl radical scavengers" are often used to study the role of $^{\bullet}OH$ in biological system (Halliwell 1978). As shown in Table 2, the results of in vitro assays show that the CF of *S. pisidica* possessed slightly greater activity than WF. When compared to the positive controls, BHT and ascorbic acid, the EsO had greater activity, whereas the AqE were found to be less efficient in hydroxyl radical scavenging activity.

Cytotoxic and antioxidant effect of essential oils on Hep G2 and H1299 cells

Aqueous extract from WF and CF form of *S. pisidica* didn't show any antitumoral effect on cancer cells at 1000 µg/mL or lower concentrations. So AqE has not effective antitumoral effect on cancer cells.

The viability of Hep G2 cells decreased when the cells were exposed to essential oil at the increasing concentrations of 20, 40, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280 and 300 µg/mL and did not changed at the concentration of 300 µg/mL or higher (Fig. 1). The IC_{50} value of 24 h incubation was calculated as 128 µg/mL for EsO-WF and 120 µg/mL for EsO-CF. EsO exhibited a dose-dependent antitumoral activity within a concentration range of 20–300 µg/mL. H1299 cells are found more sensitive to EsO than Hep G2 cells. The IC_{50} value of H1299 cells for 24 h incubation was calculated as 85.17 µg/mL for EsO-WF and 74.83 µg/mL for EsO-CF. Viability of H1299 cells did not changed at the concentration of 200 µg/mL or higher.

In some cases, cytotoxic activity was attributed to specific components of the oil. Eucalyptol and camphor, components of some essential oils, have been shown to inhibit the proliferation of human leukemic cells (Horvathova et al. 2007) and SK-MEL-28 human melonama cells (Dormanin et al. 2009). The carvacrol and thymol, components of plant essential oils, differed in their cytotoxic and genotoxic effects on K562 cells, and reduced the level of DNA damages induced in this cell by the strong oxidation of H_2O_2. ACCE (the antrodia camphorata crude extract) can be rather effective and beneficial in suppression of both the superficial cancer cell line RT4 and the metastatic cell lines (TSGH-8301 and T24) through different mechanisms (Peng at al. 2007). The antitumoral activity of EsO can came from eucalyptol and camphor which are main components of EsO from *S. pisidica.* The differences of antitumoral effects of WF-EsO and CF-EsO can come from their oil compositions. CF-EsO have higher amount of eucalyptol and camphor thanWF-EsO.

The essential oils from wild and cultivated *S. pisidica* significantly reduced the cytotoxic effects induced in HepG2 and H1299 cells by the strong oxidant H_2O_2 (Figs 2,3,4,5,). We assume that decreasing cytotoxic effect of essential oils can be accompanied by their antioxidant action with lower concentration. The maximum protective concentration was found 50 µg/mL for WF-EsO and 45 µg/mL for CF-EsO on Hep G2 cells (Figs 2, 4). On the other hand WF-EsO with 40 µg/mL and CF-EsO with 35 µg/mL demonstrated maximum protective activity against H_2O_2 on H1299 cells (Figs 3, 5). Essential oil showed cytotoxic effect and protective (antioxidant) effect on Hep G2 cells and H1299 cells depending on concentrations.

The findings above show the presence of natural

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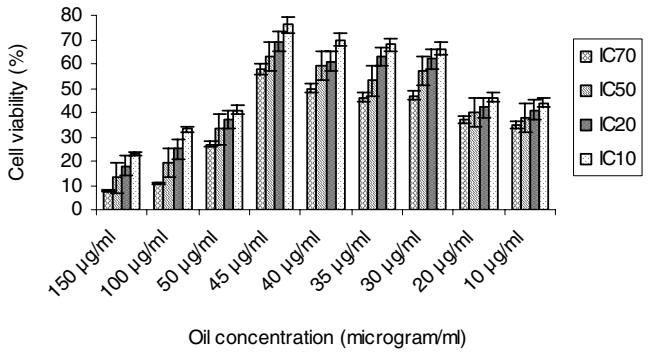


Fig. 4. Antioxidant effect of essential oil from cultivated form of *S. pisidica* on Hep G2 cancer cells against H_2O_2 cytotoxicity.

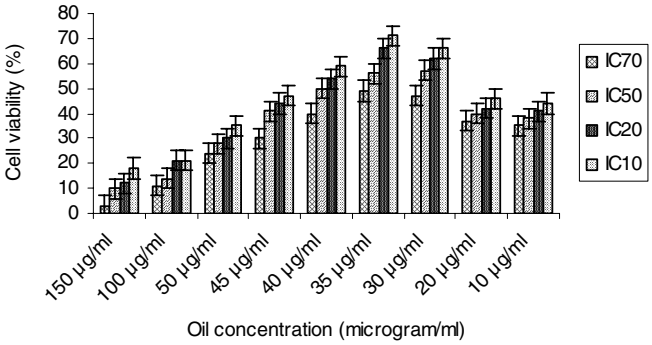


Fig. 5. Antioxidant effect of essential oil from cultivated form of *S. pisidica* on H1299 cancer cells against H_2O_2 cytotoxicity.

antioxidant phenolic compounds in these plants, with better performance than BHT known as a very efficient synthetic antioxidant agent and widely used in food technology (Potterat 1997). Indeed, the major components of the both essential oils, α-pinene, eucalyptol and camphor, have been reported to have high antioxidative activity (Ruberto & Baratta 2000; Tepe et al. 2004). The slight quantitative differences in the amounts of these components might also explain the minor differences between the activities of the two oils. Our results were strongly supported with these reports.

Antioxidant properties of the essential oils and various extracts from many plants are of great interest in both academia and the food, cosmetic and pharmaceutical industries, since their possible use as natural additives emerged from a growing tendency to replace synthetic preservatives by natural ones. In this respect, studying with the endemic species maybe of great interest since their bioactive properties and secrets could be lost forever without being tapped. Owing to their protective features exhibited in antioxidant tests, the essential oil of *S. pisidica*, of which is endemic, could be concluded as a natural source that can be freely used in the food industry as a culinary herb, but, firstly, immediate and necessary measurements should be taken for the protection of these plant species. Extinction of endemic species must be prevented by their cultivation.

In conclusion, our study can be considered as the detailed document on the comparison *in vitro* antioxidant features of EsO-WF, EsO-CF, AqE-WF and AqE-CF and demonstration of antitumoral and anti/pro-oxidant effects of EsO from *S. pisidica*.. The oil and extract from CF of *S. pisidica* showed higher antioxidant capacity than from WF. Many people widely consume the aqueous extract as a hot drink and it can be use of as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. But, EsO is more effective for antioxidant capacity than aqueous extract. The essential oils showed antitumor effect by higher concentrations. Also, essential oils provided protective effect (antioxidant) by lower concentrations against H_2O_2 cytotoxicity on Hep G2 cells and H1299 cells. As very important we consider the finding that, EsO from *S. pisidica* showed antitumoral and anti/pro-oxidant effects on Hep G2 and H1299 cells, depending on the concentrations.

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