



ORIGINAL ARTICLE

Composition, antimicrobial, antioxidant, and antiproliferative activity of *Origanum dictamnus* (*dittany*) essential oil

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Background: Nowadays, there has been an increased interest in essential oils from various plant origins as potential antimicrobial, antioxidant, and antiproliferative agents. This trend can be mainly attributed to the rising number and severity of food poisoning outbreaks worldwide along with the recent negative consumer perception against artificial food additives and the demand for novel functional foods with possible health benefits. *Origanum dictamnus* (*dittany*) is an aromatic, tender perennial plant that only grows wild on the mountainsides and gorges of the island of Crete in Greece.

Objective: The aim of the present study was to investigate the antimicrobial, antioxidant, and antiproliferative properties of *O. dictamnus* essential oil and its main components and assess its commercial potential in the food industry.

Design: *O. dictamnus* essential oil was initially analyzed by gas chromatography–mass spectrometry (GC–MS) to determine semi-quantitative chemical composition of the essential oils. Subsequently, the antimicrobial properties were assayed and the minimum inhibitory and non-inhibitory concentration values were determined. The antioxidant activity and cytotoxic action against the hepatoma adenocarcinoma cell line HepG2 of the essential oil and its main components were further evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and by the sulforhodamine B (SRB) assay, respectively.

Results: The main constituents of *O. dictamnus* essential oil identified by GC–MS analysis were carvacrol (52.2%), γ -terpinene (8.4%), *p*-cymene (6.1%), linalool (1.4%), and caryophyllene (1.3%). *O. dictamnus* essential oil and its main components were effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Enteritidis, *Salmonella typhimurium*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. In addition, the estimated IC₅₀ value for the DPPH radical scavenging activity for *O. dictamnus* essential oil was $0.045 \pm 0.0042\%$ (v/v) and was mainly attributed to carvacrol. The EC₅₀ value for the essential oil in the 72h SRB assay in HepG2 cells was estimated to be $0.0069 \pm 0.00014\%$ (v/v). Among the individual constituents tested, carvacrol was the most bioactive compound and accounted for the observed antiproliferative activity of the essential oil.

Conclusions: The results revealed that *O. dictamnus* essential oil is a noteworthy growth inhibitor against the microbes studied. It also possesses significant antioxidant activity and demonstrated excellent cytotoxicity against HepG2 cells. Taken together, *O. dictamnus* essential oil may represent an effective and inexpensive source of potent natural antimicrobial agents with health-promoting properties, which may be incorporated in food systems.

Keywords: *O. dictamnus* essential oil; antimicrobial; antioxidant; antiproliferative; GC–MS analysis

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Nowadays, there is substantial research interest in essential oils and extracts from various plant origins as potential antimicrobial, antioxidant, and anticancer agents. This trend can be mainly attributed (a) to the need of ensuring food quality and safety against pathogenic and spoilage microorganisms, but at the same time, remedying problems arising by the extensive use of chemical preservatives which are considered responsible for many teratogenic attributes and residual toxicity, (b) to consumer awareness with regards to possible long-term effects of foods and finally (c) to existing reports documenting useful bioactive properties of plant extracts that can effectively substitute synthetic food additives with inexplicit impact. Hence, the aim of the present study was to investigate the antimicrobial, antioxidant, and antiproliferative properties of *Origanum dictamnus* essential oil and assess its commercial potential in the food industry.

Essential oils are complex mixtures of volatile secondary metabolites, isolated from plants, the main constituents of which are responsible for their biological activities. Since ancient times, spices and herbs have been added to foods, not only as flavorings, but also as preservatives and healing agents, but only recently their use as food supplements has attracted considerable attention.

Although there is a considerable number of scientific reports documenting a variety of biological actions of different essential oils from various plant sources (1–3), the use of essential oils of edible and medicinal plants, herbs, and spices still presents an intriguing case, since they constitute a class of potent natural antimicrobial agents. Their incorporation in food systems may be considered as an additional inherent determinant to prevent the growth of pathogens, such as members of *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus* spp., *Aspergillus niger*, etc. (4–6) or to delay the onset of food spoilage usually caused by yeasts and fungi (7, 8).

Similarly, considerable research interest has been grown toward nutritional agents (fruits, vegetables, spices, etc.) with the ability to inhibit cell proliferation and, thus, reduce the spread of cancer (9). Essential oils are considered promising substances toward this direction, as they appear to contain interesting dietary phytochemicals capable of suppressing the initiation of carcinogenesis, cellular hyperproliferation, inflammatory processes, and malignant transformation (10, 11). Thus, dietary essential oils emerge as prominent natural compounds that could be recruited in the battle against cancer. However, the antioxidant and antiproliferative properties of essential oil fractions have been sparsely described in the literature using various *in vitro* systems (12).

O. dictamnus or *dittany* is an aromatic, tender perennial plant that only grows wild in the mountainsides and gorges of the island of Crete in Greece. As a medicinal plant, the herb has been not only used to heal wounds, soothe pain,

and ease childbirth, but also used as an antirheumatic, oxytocic, stomachic, and vulnerary. Its properties are basically attributed to the phenol carvacrol, the main constituent of its essential oil. Although there are published data about the antioxidant activity of various extracts of *O. dictamnus* (13–16), this is the first report describing the *in vitro* antioxidant and antiproliferative properties of *O. dictamnus* essential oil.

Materials and methods

Plant material, isolation of the essential oil, and standard compounds

The air-dried plant material *O. dictamnus* (or *dittany*) of the *Lamiaceae* family was commercially purchased from Crete (Greece). Only the leaves and flowers were used for the preparation of the essential oil.

The essential oil was recovered by hydrodistillation, using 40 g of dry material and 500 mL of water. The duration of the hydrodistillation was 6 h, and the resulting oil was dried over anhydrous sodium sulfate and stored at 4°C in a dark glass vial. A yield of 2.53 (% w/w) was obtained.

All standard compounds were kindly provided by Vioryl Chemical and Agricultural Industry, Research S.A. (Afidnes, Greece).

Microbial strains

Salmonella enterica subsp. *enterica* ser. Enteritidis FMCC B56 PT4 (kindly provided by Prof. Nychas G.J.E., Agricultural University of Athens, Athens, Greece), *S. enterica* subsp. *enterica* ser. *typhimurium* DSMZ 554, *L. monocytogenes* NCTC 10527 serotype 4b, *E. coli* ATCC 25922, *Staphylococcus epidermidis* FMCC B-202 C5M6 (kindly provided by Dr. Nisiotou A., Athens Wine Institute, ELGO-DIMITRA, Greece), and *Staphylococcus aureus* ATCC 25923 were grown in brain heart infusion (BHI) broth (LAB M, Heywood, UK) at 37°C for 24 h. *Saccharomyces cerevisiae* uvaferm NEM (Lallemand, Montreal, Canada) was grown in yeast extract–peptone–dextrose (YPD) broth (yeast extract 10 g/L, glucose 20 g/L, and peptone 20 g/L) at 28°C for 3 days. *A. niger* 19111 (kindly provided by Prof. Nychas G.J.E.) was grown on malt extract agar (LAB M) for 7 days at 37°C.

Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectrometry (GC–MS) analysis was carried out in a gas chromatography–mass spectrometer (GC: 6890A, Agilent Technologies, Santa Clara, California, USA; MSD: 5973, Agilent Technologies) using a Factor Four VF 1 ms column (25 m, 0.2 mm i.d., 0.33- μ m film thickness, Agilent Technologies). A volume of 0.1 μ L of essential oil was directly injected and a 1:100 split ratio was applied. The oven temperature was set at 50°C for 1 min, followed by a temperature gradient of 2.5°C/min to 160°C for 2 min and then 50°C/min to

250°C for 15 min. Helium was used as the carrier gas (flow 1 mL/min). Injector and transfer line were set to 200 and 250°C, respectively. The mass spectrometer was operated in the electron impact mode with the electron energy set to 70 eV. Identification was carried out by comparing the retention times and mass spectra of volatiles with Wiley/NIST 0.5 and in-house created libraries using authentic compounds.

Antimicrobial assays

The antimicrobial activity of the tested essential oil was monitored using the following methods:

Disk diffusion assay

For the antibacterial screening, the disk diffusion assay was performed (17). The bacterial suspensions were 10-fold diluted in ¼ strength Ringer's solution (LAB M). A 0.1-mL portion from the appropriate dilution was spread on BHI agar (LAB M), in order to provide initial inoculums of 10^5 or 10^7 cfu/mL. Subsequently, sterile paper disks (Whatman no. 2) of 5-mm diameter were placed onto the inoculated agar surface containing 5 µL of the essential oil or of its main components (carvacrol, *p*-cymene, γ -terpinene, or linalool). Petri dishes were incubated at 37°C for 24 h. After incubation, the inhibition zones were measured in mm.

The same procedure was also followed for screening the activity against yeasts, using *S. cerevisiae* suspensions 10-fold diluted in ¼ strength Ringer's solution (LAB M) and spread on YPD agar. Sterile paper disks were placed onto the inoculated agar surface containing 5 µL of the essential oil, as described above. The Petri dishes were incubated at 28°C for 3 days and, then the inhibition zones were measured in mm.

For the antifungal activity, 100 fungal spores/plate from *A. niger* were spread on malt extract agar (LAB M). Sterile paper disks were placed onto the inoculated agar surface containing 5 µL of the essential oil, as described above. The Petri dishes were incubated at 37°C for 10 days, and the inhibition zone was measured daily in mm.

All experiments were carried out at least in triplicates and the mean values are presented.

Determination of minimum inhibitory concentration and non-inhibitory concentration

For the determination of minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values, bacterial growth in BHI broth (LAB M) was monitored through changes in optical density (OD) of bacterial suspensions in the presence of multiple concentrations of *O. dictamnus* essential oil or its main components (carvacrol, *p*-cymene, γ -terpinene, or linalool). Stock solutions of the essential oil or of its main constituents were prepared by mixing the essential oil or the standard compounds directly with BHI broth. Aliquots (0.180 mL) of growth medium mixed with the essential oil or with

the standard compounds were transferred to the wells of a 96-well microplate. The bacterial suspensions were diluted 10-fold in ¼ strength Ringer's solution, and a 0.070-mL portion from the appropriate dilution was added to the wells containing the growth medium (final volume 0.250 mL), in order to result in a population of approximately 10^3 cfu/mL. Microplates were incubated in Microplate Reader (Molecular Devices, VERSAmax, Softmaxpro v. 5.0 software, Sunnyvale, California, USA) at 37°C for 24 h. OD measurements were carried out every 10 min at 610 nm. BHI broths with no inoculum and inoculated BHI broths with no essential oil were used as controls. Each experiment was performed at least four times.

MIC and NIC calculation

The calculation of MIC and NIC values was based on the Lambert–Pearson model (LPM) (18, 19). In brief, the effect of the growth, measured by the OD method, is manifested by a reduction in the area under the OD/time or curve relative to a control well at any specified time. By calculating the area using the trapezoidal rule, the relative amount of growth was obtained using the ratio of the test area to that of the control, termed the fractional area, *fa*. Data were fitted to the LPM using non-linear least-squares regression analysis assuming equal variance.

Cell lines and cell cultures

The human hepatocellular carcinoma HepG2 cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown and maintained in Dulbecco's modified Eagle's medium (Biochrom AG, Germany) supplemented with 10% fetal bovine serum (Biochrom AG), penicillin (100 U/mL), and streptomycin (100 µg/mL) (Biochrom AG) and were incubated at 37°C in a humidified atmosphere of 95% O₂ and 5% CO₂. Stock cultures were passaged at 2- to 3-day intervals. Cells were seeded at a density of 5.0×10^3 cells/well in 96-well plates for the sulforhodamine B (SRB) assay.

Antioxidant activity (DPPH assay)

The radical scavenging activity of the essential oil and its major components was estimated using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described previously with few modifications (20). Different concentrations of carvacrol, γ -terpinene, *p*-cymene (0.1–10 mM), linalool (0.028–5.76 mM), and the oil (0.0005–5% v/v) were prepared using dimethyl sulfoxide (DMSO) (Sigma–Aldrich Co., St. Louis, Missouri, USA) as the solvent. Ten microliters of each concentration was placed in a 96-well plate and 190 µL of 300 µM methanolic solution of DPPH (Calbiochem®) was added. Ten microliters of DMSO with 190 µL DPPH was used as the control. The plate was left in darkness for 30 min and then the absorbance was measured at 517 nm using an Elisa plate reader. All determinations were performed in triplicates.

The % inhibition of the DPPH radical for each concentration was determined by making use of the following formula:

$$\% \text{ DPPH radical scavenging activity} = \left[\frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})}{\text{OD}_{\text{control}}} \right] \times 100.$$

The concentration of essential oil that causes a 50% reduction in the DPPH free radical (50% inhibition concentration, IC_{50} value) was calculated from the sigmoidal inhibition curves by regression analysis using the equation of the four-parameter logistic curve (Sigma Plot Software, v.10).

Sulforhodamine B assay

The viability of HepG2 cells after treatment with the essential oil was determined using the SRB assay. SRB is a dye that binds to basic amino acids of cellular proteins and, then, the number of viable cells is estimated with colorimetric evaluation (21). Cells were plated in 96-well plates and treated with different concentrations of carvacrol, γ -terpinene, *p*-cymene (62.5–1,000 μM), linalool (0.004–5.77 mM), the essential oil (0.00007–0.1% v/v), and etoposide (0.07–100 μM) for 72 h. Then, the cells were fixed with the addition of 25 μL of 50% (w/v) cold trichloroacetic acid (Applichem, Darmstadt, Germany) to the growth medium and incubation of the plates at 4°C for 1 h. The cells were washed five times with tap water and then stained with 50 μL of 0.4 (w/v) SRB (Sigma–Aldrich) in 1% (v/v) acetic acid (Scharlau, Barcelona, Spain) for 30 min at room temperature. Then, the cells were rinsed five times with 1% (v/v) acetic acid to remove the unbound dye. The fixed, stained plates were allowed to air-dry and afterwards the bound dye was solubilized by adding 100 μL of 10 mM Trizma base (Sigma–Aldrich) for at least 5 min. Absorbance was measured at 570 nm using an Elisa plate reader, and the percent cellular survival was calculated using the formula:

$$\left[\frac{(\text{Sample OD}_{570} - \text{media blank OD}_{570})}{(\text{mean control OD}_{570} - \text{media blank OD}_{570})} \right] \times 100.$$

The EC_{50} values (efficient concentration that causes a 50% decrease in cancer cells viability) were calculated from the respective dose–response curves by regression analysis using a four-parameter logistic curve through the Sigma Plot Software (v.10).

Results and discussion

The use of essential oils as natural antimicrobial, antioxidant and anticancer agents is less explored compared with their utilization as food flavorings and, thus, their application in the industry is limited. The strategy adopted in the present study was to determine the antimicrobial, antioxidant, and antiproliferative properties of

Table 1. GC–MS analysis of *Origanum dictamnus* essential oil

RT	Compound	% Area
5.602	<i>trans</i> -2-hexenal	0.014
6.003	<i>cis</i> -3-hexenal	0.005
6.868	Ethylbutyl ketone	0.007
9.093	Thujene	0.210
9.415	α -Pinene	0.119
9.983	Camphene	0.018
11.013	Ethyl amyl ketone	0.200
11.015	Sabinene	0.022
11.217	β -Pinene	0.048
11.827	Myrcene	0.583
12.481	α -Phellandrene	0.101
12.889	δ -3-Carene	0.030
13.105	α -Terpinene	1.148
13.315	<i>p</i> -Cymene	6.078
13.657	β -Phellandrene	0.119
13.729	Limonene	0.117
14.053	<i>trans</i> -Ocimene	0.002
14.069	<i>cis</i> -Ocimene	0.029
15.292	γ -Terpinene	8.365
15.426	Thujanol	0.403
16.756	Terpinolene	0.071
16.970	Sabinene hydrate	0.126
17.122	Linalool	1.414
20.576	Borneol	0.192
21.191	Terpinen-4-ol	0.883
21.788	α -Terpineol	0.086
24.612	Carvone	0.100
24.983	Isopiperitone	0.010
26.572	Indol	0.015
27.403	Thymol	0.513
28.273	Carvacrol	52.186
30.893	α -Cubebene	0.119
31.802	Carvacryl acetate	0.120
32.223	α -Copaene	0.684
32.790	β -Cubebene	0.136
34.265	Caryophyllene	1.319
34.654	Thymohydroquinone	0.383
35.437	Epi bicyclosesquiphellandrene	0.051
35.841	Humulene	0.076
37.075	D-Germacrene	0.108
37.667	Bicyclogermacrene	0.071
38.400	β -Bisabolene	0.322
41.444	Caryophyllene oxide	0.410
44.593	α -Cubenol	0.245

O. dictamnus essential oil in correlation to its composition and highlight its possible use in the production of functional foods with potential health benefits.

GC–MS analysis

The results of the GC–MS analysis are presented in Table 1. In total, 44 compounds were identified and the main

Table 2. MIC and NIC (% v/v) of *Origanum dictamnus* essential oil main components against common pathogens

Microbial species	Carvacrol		γ -Terpinene		<i>p</i> -Cymene		Linalool	
	MIC	NIC	MIC	NIC	MIC	NIC	MIC	NIC
<i>Salmonella</i> Enteritidis	0.131	0.079	0.504	0.307	0.527	0.412	0.377	0.287
<i>Salmonella</i> typhimurium	0.080	0.052	0.521	0.320	0.513	0.399	0.465	0.376
<i>Escherichia coli</i>	0.062	0.042	0.454	0.396	0.492	0.328	0.380	0.281
<i>Listeria monocytogenes</i>	0.131	0.079	0.334	0.066	0.266	0.141	0.368	0.302
<i>Staphylococcus epidermidis</i>	0.069	0.009	0.354	0.123	0.598	0.436	0.453	0.398
<i>Staphylococcus aureus</i>	0.059	0.015	0.286	0.116	0.608	0.425	0.563	0.513

constituents detected were carvacrol (52.2%), followed by γ -terpinene (8.4%), *p*-cymene (6.1%), linalool (1.4%), and caryophyllene (1.3%). Noticeably, the main *O. dictamnus* essential oil constituents showed significant antimicrobial activity against common food spoilage and pathogenic bacteria (Table 2) (17, 22–26). However, correlation of the MIC and NIC values with the GC–MS results should be avoided, as GC–MS analysis provided data about the % content of the volatile compounds and not their actual concentration. Among the essential oil constituents, only carvacrol exhibited considerable antioxidant (Fig. 1) and antiproliferative (Fig. 2) activity (23, 27).

Antimicrobial activity

The antimicrobial activity of the essential oil and its main constituents was evaluated against six common food spoilage and pathogenic bacteria, as well as against *S. cerevisiae* and *A. niger*. Initially, the disk diffusion method was applied, according to which the radius or diameter of the inhibition zone of microbial growth around paper disks impregnated with an antimicrobial compound is determined. Subsequently, MIC and NIC values were assessed using an established OD method, which combines the absorbance measurements with the common dilution method, and non-linear regression analysis was used to fit the data using a previously published model (18, 19). The results are presented in Tables 2–4.

The data obtained from the disk diffusion method indicated that all bacteria tested were sensitive to the essential oil. The strongest inhibition zone was observed in *S. aureus* ATCC 25923, whereas the weakest in *S. typhimurium* DSMZ 554 (Table 3).

O. dictamnus essential oil also showed considerable activity against yeasts and fungi. *S. cerevisiae* uvaferm NEM and *A. niger* 19111 were used as model systems in food spoilage (28, 29). Large inhibition zones were observed in both *S. cerevisiae* uvaferm NEM (inhibition zones of 40 ± 0.7 and 35 ± 0.5 for 5 and 7 cfu/mL initial inoculums, respectively) and *A. niger* 19111 (inhibition zones of 50 ± 1 , 40 ± 0.5 , and 15 ± 0.4 for the first 3 days,

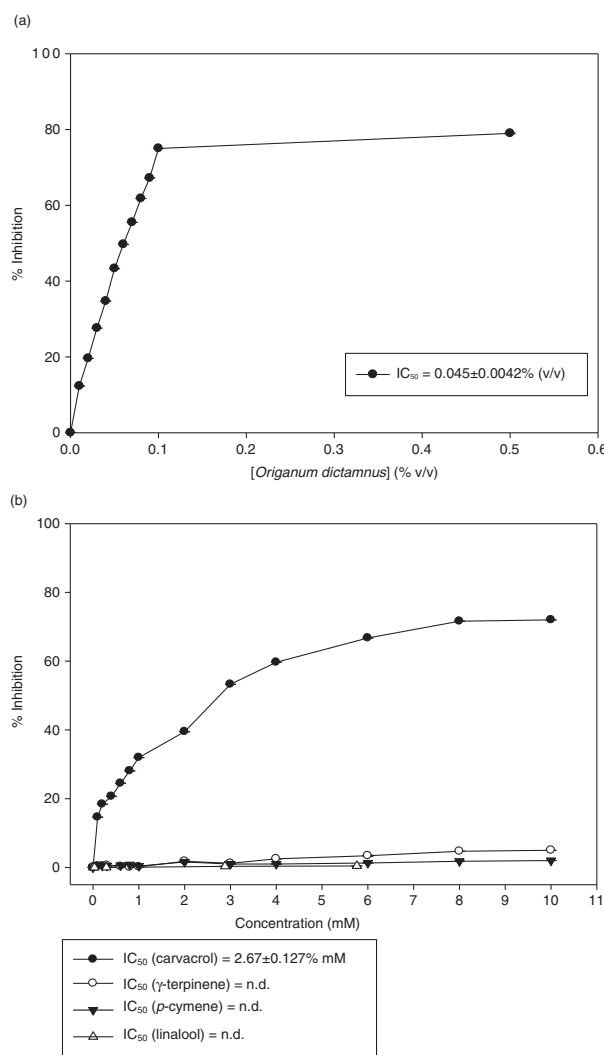


Fig. 1. DPPH radical scavenging activity of (a) *O. dictamnus* essential oil and (b) its major components: carvacrol, γ -terpinene, *p*-cymene, and linalool. Increasing concentrations of the essential oil (0.0005–5% v/v), as well as its major components, carvacrol, γ -terpinene, *p*-cymene (0.1–10 mM), and linalool (0.028–5.76 mM) were incubated in the presence of DPPH for 30 min. The results are shown as mean \pm SD of three independent experiments.

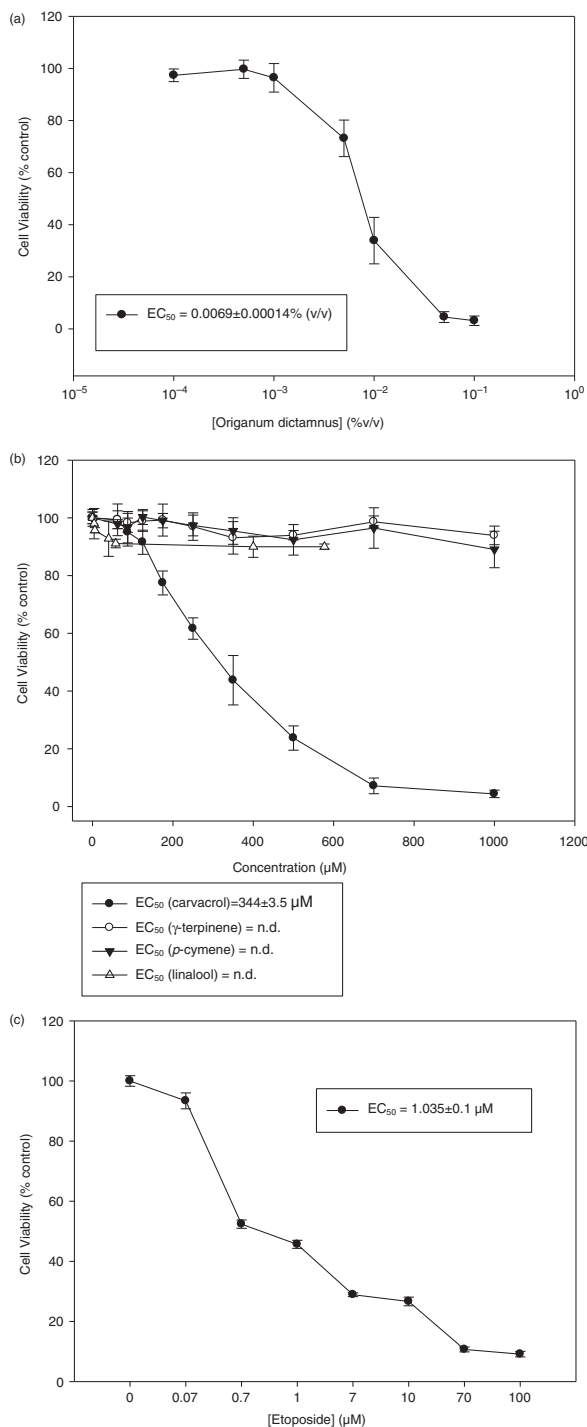


Fig. 2. Antiproliferative activity of (a) *O. dictamnus* essential oil; (b) major components: carvacrol, γ -terpinene, *p*-cymene, and linalool; and (c) etoposide. HepG2 cells were incubated with increasing concentrations of *O. dictamnus* essential oil (0.0001–0.1% v/v), carvacrol, γ -terpinene, *p*-cymene (62.5–1,000 μM), linalool (4–577 μM), and etoposide (0.07–100 μM) for 72 h. Estimation of cell viability was determined by the SRB assay. The results are shown as mean \pm SD of three independent experiments.

Table 3. Antibacterial activity of *Origanum dictamnus* essential oil against common pathogens monitored by disk diffusion assay

Microbial species	Inoculum	
	5 log cfu/mL	7 log cfu/mL
<i>Salmonella</i> Enteritidis	25 \pm 0.5	22 \pm 0.3
<i>Salmonella</i> typhimurium	20 \pm 0.7	15 \pm 0.5
<i>Escherichia coli</i>	30 \pm 1.0	24 \pm 1.0
<i>Listeria monocytogenes</i>	25 \pm 0.5	23 \pm 0.5
<i>Staphylococcus epidermidis</i>	25 \pm 0.5	17 \pm 0.5
<i>Staphylococcus aureus</i>	34 \pm 0.5	23 \pm 0.7

The inoculums tested were 5 and 7 log cfu/mL. The diameter (mm) of the inhibition zone is the mean of six observations from three different experiments.

respectively; the inhibition zones disappeared during incubation for longer time periods).

Although the inhibition zone method is widely used for the evaluation of the antimicrobial activity of essential oils, there are various factors that influence the outcome of the results, such as the composition of the sample tested (type of plant, geographical location, and time of the year), inoculum size, the ability of the essential oil to diffuse uniformly through the agar, etc. (17).

The results of the MIC and NIC determination indicated that *O. dictamnus* essential oil, as well as its main constituents, inhibited all bacteria tested. In accordance to the results of the disk diffusion method, *S. aureus* ATCC 25923 had the lowest MIC and NIC values [0.127 and 0.054 (v/v), respectively], whereas the highest values were determined in *E. coli* ATCC 25922 [0.273 and 0.122 (v/v), respectively] (Table 4). Similar results reporting high sensitivity of *S. aureus* in various essential oils were also previously published (17, 30). Importantly, the antimicrobial activity of *O. dictamnus* essential oil could be attributed to the action of its main constituents (Table 2), although possible synergistic effects should not be excluded. Such effects must be further studied using model systems.

Table 4. MIC and NIC (% v/v) of *Origanum dictamnus* essential oil against common pathogens

Microbial species	MIC	NIC
<i>Salmonella</i> Enteritidis	0.220	0.105
<i>Salmonella</i> typhimurium	0.210	0.070
<i>Escherichia coli</i>	0.273	0.122
<i>Listeria monocytogenes</i>	0.125	0.055
<i>Staphylococcus epidermidis</i>	0.140	0.059
<i>Staphylococcus aureus</i>	0.127	0.054

Antioxidant activity

In a following step, the *in vitro* antioxidant capacity of *O. dictamnus* essential oil and its major components was investigated using the DPPH scavenging assay. The DPPH assay is a reliable test for the estimation of the ability of studied compounds to scavenge free radicals (31). The principal of the assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant. In our experiments, different concentrations of *O. dictamnus* essential oil were incubated in the presence of DPPH for 30 min. The essential oil demonstrated a considerable dose-dependent inhibition of DPPH activity (Fig. 1a), with $IC_{50} = 0.045 \pm 0.0042\%$ (v/v), indicating significant antioxidant activity. Carvacrol was the only constituent to record antioxidant capacity among the essential oil components with an estimated IC_{50} value of 2.67 ± 0.127 mM (Fig. 1b), whereas γ -terpinene, *p*-cymene, and linalool showed no antioxidant activity, causing only up to 4% inhibition of DPPH in the highest concentration tested (Fig. 1b). These results suggest that the observed antioxidant activity of the *O. dictamnus* essential oil is mainly attributed to its main constituent carvacrol.

Apart from inhibiting the generation of reactive oxygen species (ROS) or direct scavenging of free radicals, antioxidant action also extends to numerous other cellular physiological processes, including modulation of signal transduction and regulation of gene expression of detoxifying and antioxidant enzymes, leading to upregulation of cellular antioxidant defenses (32). Most bioactive compounds with antioxidant properties exert their health beneficial effects through modulating cell signaling function. Quercetin, as an example, which is the most abundant flavonoid in fruits and vegetables, is not only a potent direct antioxidant by scavenging ROS, but also exerts antioxidant activity by enhancing the ARE (antioxidant responsive element)-mediated binding activity via increase in NRF2 expression levels, thus leading to upregulation of antioxidant enzymes (33). Quite interestingly, in a study that used an ethylnitrosamine-induced hepatocellular carcinoma experimental model, it was shown that carvacrol supplementation significantly attenuated alterations associated with a decrease in tissue antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase), thereby showing potent anticancer effect in liver cancer (34). Similarly, in another study, carvacrol administration increased the activities of xenobiotic-metabolizing enzymes in mouse liver (35). It would be worth investigating in future studies whether the *O. dictamnus* essential oil and/or its individual constituents exert such antioxidant capacity *via* modulating cell signaling function resulting in the upregulation of cellular antioxidant potential.

Antiproliferative activity

The antiproliferative potential of *O. dictamnus* essential oil and its major components was further determined using the SRB assay against the HepG2 human experimental model of hepatocellular carcinoma. As indicated in Fig. 2a, the essential oil demonstrated a promising result, with cell inhibition observed as soon as 24 h of incubation (data not shown). The results suggested that *O. dictamnus* essential oil inhibits the proliferation of HepG2 cells in a time- and dose-dependent manner. Seventy-two-hour incubation of HepG2 cells with *O. dictamnus* essential oil resulted in an EC_{50} value of $0.0069 \pm 0.00014\%$ (v/v). Similar results were obtained when other experimental models of different cancer types were used (e.g. breast, colon, and lung adenocarcinomas) (data not shown). Although it remains to be determined whether the observed cytotoxicity of *O. dictamnus* essential is specific toward cancer cells, our initial results supported this notion. Nevertheless, this is the first report describing the potential antiproliferative properties of the essential oil of *O. dictamnus*.

The essential oil of *O. dictamnus* is a source of the monoterpenoid phenol, carvacrol, which has been described as a chemopreventive agent. Various studies have demonstrated that carvacrol inhibits cell proliferation and promotes apoptosis, two key properties for cancer therapeutics (36–39). In our study, carvacrol caused a dose-dependent reduction in the viability of HepG2 cells with an estimated EC_{50} value of 344 ± 3.5 μ M (Fig. 2b). The rest of the essential oil components did not appear to cause significant inhibition of cell proliferation. Linalool caused a maximum of 30% cell death, while *p*-cymene and γ -terpinene did not reduce the viability more than 10%, even in the highest concentration (Fig. 2b). These results further supported that the observed antiproliferative activity of the essential oil is mainly attributed to carvacrol. Compared with etoposide, a known chemotherapeutic agent, carvacrol appears to possess moderate activity (Fig. 2c).

Conclusions

The results revealed that *O. dictamnus* essential oil is a noteworthy growth inhibitor of food spoilage and pathogenic microbes, indicating that it represents an effective and inexpensive source of potent natural antimicrobial agent, which may be incorporated in food systems to assure microbial safety. Furthermore, *O. dictamnus* essential oil possesses antioxidant and antiproliferative properties and emerges as a promising chemopreventive agent. However, further research to elucidate the exact mode of action of essential oils, as well to determine the safety of the long-term use of high doses, is required in order to expand their use in industrial practice.

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