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Original Research

Bioactive chemical compounds in *Eremurus persicus* (Joub. & Spach) Boiss. essential oil and their health implications

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Abstract: The genus *Eremurus* is native to Eastern Europe and temperate Asia. Particularly, *Eremurus persicus* (Joub. & Spach) Boiss. is highly valued in traditional foods and medicine. Scientific knowledge about *E. persicus* chemical composition and bioactivity is required. Therefore, the present study is aimed to determine the volatile composition of *E. persicus* essential oil (EO) by means of gas chromatography coupled to flame ionization/mass spectrometry detection. Moreover, the antioxidant, antimicrobial, anticancer, and acetylcholinesterase inhibitory activities of the EO were tested. Interestingly, the anti-dermatophyte potency was close to that of the drug griseofulvin, with minimum fungicidal concentration ranging between 0.7 and 4.5% depending on the fungi strain. The EO was also effective against hepatocellular carcinoma (Hep-G2) and breast adenocarcinoma (MCF-7) human cancer cell lines in a concentration (200-1500 ng/mL)-dependent manner, with a decrease of the cell viability up to 65% and 52%, respectively. The *E. persicus* EO was rich in terpenes and oxygenated terpene derivatives. Individually, limonene (16.25%), geranylgeraniol (15.23%), *n*-nonanal (9.48%), geranyl acetone (9.12%), benzene acetaldehyde (8.51%), linalool (7.93%), α-pinene (6.89%), and 1,8-cineol (5.22%) were the most abundant volatile compounds and could be chosen as analytical markers of this essential oil. In conclusion, our results suggested that this EO possesses a wide range of bioactive properties that could be useful in nutraceutical, functional foods and cosmeceutical formulations.

Key words: Antimicrobial; Antifungal; *Eremurus persicus*; Essential oil; Natural antioxidant; Terpenes.

Introduction

Medicinal plants can be a promising alternative for many diseases and conditions (1-9). Sometimes, these plants are also valued to flavor foods, giving the food a dual role, *i.e.* flavor and bioactive compounds. Moreover, medicinal plants are low cost and tend to have fewer side effects than synthetic drugs. Medicinal plants and their derived products can be obtained in health food shops, pharmacies and on-line without the need for a prescription, and thus they should be properly characterized, standardized, and labeled.

Among bioactive compounds, those contained in essential oils (EOs) are increasingly attracting the attention of different segments of industry not only because of their aroma, but also due to their multiple functions (antimicrobial, antioxidant, anticancer, anti-inflammatory activities, etc.) (10-17). In this sense, essential oils (EOs) are complex mixtures of volatile and lipophilic substances, formed by the secondary metabolism of plants and characterized by a pleasant odor in most of the cases. Common analytical methods used to characterize essential oils include UV-Vis spectroscopy, infrared spectroscopy, gas chromatography (GC) coupled to several detectors and thermal analysis.

In this work, we focused on the study of *Eremurus persicus* (Joub. & Spach) Boiss, a little known plant from the genus *Eremurus* (subfamily Asphodeloideae, Xanthorrhoeaceae). This plant grows in arid and semi-arid, rocky mountains in central Asia and the Middle East (18-21). The leaves of *E. persicus* are traditionally cooked with rice in Iran (22). This species is also used in folk medicine with a wide range of uses, such as diuretic (23), for the treatment of arteriosclerosis, disorders of the liver, inflammation-related diseases, as well as against fungi skin diseases (24-27) (Table 1). In this work the constituents from the aerial parts of *E. persicus* (Joub. & Spach) Boiss. EO were characterized by GC coupled to flame ionization (FID) and mass spectrometry (MS) detection. Besides, the present investigation deals with a screening of *E. persicus* EO bioactivity: antioxidant, antibacterial, anti-dermatophyte, anticancer, and acetyl-

Table 1. Medicinal traditional uses of *Eremurus persicus*.

Plant part	Folk medicine use	Reference
Leaves	Diuretic	(23)
Seeds	Arteriosclerosis	(24)
Leaves	Jaundice, liver disorders, and infectious diseases	(24)
Leaves	Genitourinary and nutritional disorders	(25)
Aerial parts	Skin diseases caused by fungi infections	(26)
Roots	Inflammation-related diseases and skin disorders	(27)

cholinesterase (AChE) inhibitory activity.

Materials and Methods

Plant preparation and procedure

Aerial parts (stems, leaves and flowers) at the flowering stage of *E. persicus* (Joub. & Spach) were collected in April 2014 from Khorasan Razavi, Iran. The plant was taxonomically identified by a botanist at the herbarium of Pharmacognosy, Department of the Faculty of Pharmacy affiliated to Zabol University of Medical Sciences of Iran.

Extraction and isolation of the essential oil

Plant parts were air-dried in the shade at ambient temperature (18-25 °C) for 3 days. Dried aerial parts (100 g) were cut into small pieces and hydro-distilled for 4 hours using Clevenger-type apparatus. The resulting EO was dried over anhydrous sodium sulfate and stored at 4 °C until analysis and bioassays.

Gas chromatography analyses

GC analysis was carried out using an Agilent HP 6890 gas chromatograph (Hewlett-Packard, Cambridge, UK), equipped with a HP-5MS fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature gradient was: 60 °C for 3 minutes and then 250 °C at a rate of 3 °C/minute.The injector and FID temperature was set at 290 °C. Helium was used as a carrier gas with a linear velocity of 1 mm/minute.

The analyses by GC coupled to mass spectrometry (MS) were carried out using an Agilent HP 5973 equipped with a HP-5MS fused silica column, as before. The oven temperature gradient was: 60 °C for 3 minutes and then 250 °C at a rate of 3 °C/minute. The transfer line temperature was 290 °C and the linear velocity of the carrier gas (helium) was 1 mm/minutes. The other parameters were: split ratio, 1/60; ionization energy, 70 eV; scan time, 1 s; mass range up to 150 amu.

Retention indices (RI) were determined using retention times of *n*-alkanes that were injected after the EO samples and under the same chromatographic conditions (Table S1). Compounds were identified by comparison of mass spectral fragmentation patterns and RI with those found in Wiley 7n.L Mass Spectral Library (Wiley, New York, NY, USA), Adams Library and Mass Finder 2.1.The relative percentages of the components of the EO were determined in function of their peak areas in the chromatogram (28).

Determination of the DPPH radical scavenging activity

The antioxidant activity of *E. persicus* EO was measured using the stable radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH). For that, 50 µL of a methanolic stock solution of *E. persicus* EO at different concentrations (5 to 50 g/L) was put into a cuvette, and 2 mL (6M) methanolic solution of DPPH was added. Ascorbic acid and butylated hydroxyl toluene (BHT) were used as controls (in the same concentration). The mixtures were well shaken in a vortex (2500 rpm) for 1 minute and then placed in a dark room. The decrease in absorbance at 517 nm (A_{517}) was determined by a Shimadzu UV 1601 spectrophotometer (Kyoto, Japan) after 60 minutes for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the radical without antioxidant (control) was measured. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically. Inhibition (%) of the DPPH radical was plotted against the sample concentration in the reaction system and calculated as follows:

Inhibition (%) = ((ΔA_{517} of control - ΔA_{517} of sample)/ ΔA_{517} of control) × 100

The IC_{50} was calculated by using the formula described by (1):

EXP (LN (conc>50%) - ((signal >50%-50)/(signal >50%-signal <50%)*LN (conc>50%/conc<50%)))

Determination of the antibacterial activity

The disc diffusion test was performed in triplicate to assess the antibacterial activity of *E. persicus* EO. This semi-quantitative, highly reliable and drug-specific method is based on agar diffusion; it comprise of discs (6 mm in diameter) applied onto an agar plate inoculated with standard strains of *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739) and, then, impregnated with 20 µL of EO or gentamicin as reference compound. Plates were incubated overnight at 37 °C. After 24 h, zone of inhibition of bacterial growth was measured by the following scale: no inhibition, resistant strain (-); < 10 mm, mildly sensitive strain (+); 10 to 20 mm, moderately sensitive (++); > 20 mm, highly sensitive (++).

Determination of the anti-dermatophyte assay

Dermatophyte strains included *Microsporum gypseum* PTCC (Persian Type Culture Collection) (5070), *Microsporum canis* PTCC (5060), *Trichophyton rubrum* PTCC (5069), and *Trichophyton schoenleinii* PTCC (5143). These strains were obtained from Iranian Scientific and Industrial Institute and their identity were confirmed by slide culture and urease test (29). All strains were maintained in 20% glycerol and 10% lactose at -180 ºC in liquid nitrogen. Inocula were prepared by growing isolates on Sabouraud's dextrose agar (SDA) slopes according to Clinical and Laboratory Standards Institute (30) with some modifications. The slopes were flooded with 0.85% saline. Dermatophyte

growth was gently probed and the resulting suspension was removed and mixed thoroughly with the use of a vortex mixer. After the settling of the larger particles, suspensions were adjusted by nephelometry and diluted as necessary until final inoculum concentrations of 2.5 × 10^3 - 2.5 × 10^4 cfu/mL (31).Broth microdilution testing was based on (30) with some modifications. Briefly, a series of doubling dilution of *E. persicus* EOranging from 8% to 0.004% (v/v) was prepared in a 96 well micro dilution tray, with a final concentration of 0.001% (v/v) Tween 80 (Sigma-Aldrich) to enhance *E. persicus* EO solubility. After the addition of inocula, trays were incubated for 96 h at 30°C and the minimum inhibitory concentration (MIC) was determined visually with the aid of a reading mirror, according to CLSI guidelines. The minimum fungicidal concentrations (MFCs) of *E. persicus* EO were determined by sub-culturing 10 µL from wells not visibly turbid and then spots were inoculating on SDA plates. The MFCs were determined as the lowest concentration resulting in no growth on subculture. Isolates were tested on at least two separate occasions, and were retested if resultant MIC or MFC values differed. Griseofulvin (Sigma-Aldrich) was supplied as a powder and stock solution were prepared in dimethyl sulphoxide (DMSO; Merck) to be used as positive control.

Determination of the anticancer activity

Anticancer activity of *E. persicus* EO was assayed on Hep-G2 (human hepatocellular carcinoma) cell lines (ATCC® HB8065™) and MCF-7 (human breast adenocarcinoma) cells (ATCC® HTB22™), which were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The following concentrations were used: 200, 250, 500, 1000, and 1500 ng/mL. Cells were cultivated in Dulbecco's modified medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% antibiotics (Penicillin/Streptomycin, Sigma-Aldrich) at 37 °C in a 5% CO_2 atmosphere. Once cells reached 80-90% confluence, they were detached by trypsin-EDTA solution (0.25% in PBS, Sigma-Aldrich), collected and used for tests. Cells (5×10^4/well) were seeded into 12 wells plates and cultivated for 3 and 7 days at 37 °C, in humidified 5% CO_2 atmosphere. The DMEM with not any EO dilution was used as control. At each assay time, cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) colorimetric assay. Briefly, 100 µL of MTT solution (3 mg/mL in PBS) were added to each sample and incubated for 4 h in the dark at 37 °C; subsequently, formazan crystals were dissolved with 100 µL of DMSO (Sigma-Aldrich) and 50 µL were collected and centrifuged to remove any debris. Optical density (OD) of supernatant was evaluated at 570 nm using a spectrophotometer (Shimadzu UV 1601). OD of control samples was considered as 100% viability.

The cell viability was measured as follow: (EO OD/control OD) ×100.

Determination of the acetylcholinesterase inhibitory (AChE) activity

The AChE activity was determined by using the spectrophotometric Ellman's method (32). Briefly, 360 µL of 1 mM Tris-HCl buffer (pH 7.4), 25 µL of 0.28 U/mL enzyme solution and 100 µL of different EO dilutions (25, 50, 100, 150, and 300 µg/mL) in DMSO were incubated in test tubes, for 15 minutes at room temperature. Then, 10 µL of 6.67 U/mL AChE and 200 µL of 3 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) in buffer were added. Galanthamine (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control. It was prepared in serial concentrations, the same as EO, in 50 mM Tris-HCl buffer pH 7.4. The mixture was incubated for 20 minutes at 37 °C. Then, 10 µL of acetylthiocholine iodide (200 mM) in buffer were added to the mixture and the absorbance was measured at 405 nm (A_{405}) with a Hitachi U-2001 spectrophotometer (Tokyo, Japan) every 10 sec for 3 minutes. Buffer was used as blank. The enzyme hydrolysis of the substrate, acetylthiocholine, produces thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 405 nm. The enzyme inhibition (%) was calculated from the rate of absorbance change with time (V=Abs/Δt) as follows:

Inhibition (%) = 100 - (Change of sample absorbance/Change of blank absorbance) × 100

The experiment was carried out in triplicate. Moreover, the concentration of the EO that inhibited the hydrolysis of the substrate by 50% (IC_{50}) was determined by linear regression analysis of the inhibition percentages *versus* the EO concentrations.

Statistical analysis

All experiments were carried out in triplicate. The statistical analyses were performed using IBM Statistical Package for Social Sciences v. 11.5 software (SPSS, IBM). Data are expressed as mean ± standard deviation (SD); the results were analyzed using one-way analysis of variance (ANOVA) followed by Scheffe's test, and considered to be statistically significant at $P < 0.05$.

Results

Chemical composition of *E. persicus* EO

The chemical composition of *E. persicus* EO is shown in Table 2 and Table S1 (supplementary material). In total, 42 compounds were characterized by GC-FID and GC-MS, representing 98.19% of the EO. In general, terpenes and their oxygenated derivatives (terpenoids) were the main class, with a relative abundance of 68.52%. Among them, the most abundant subclass was monoterpenes (46.13%). Moreover, the main individual constituents were limonene (16.25%), geranylgeraniol (15.23%), *n*-nonanal (9.48%), geranyl acetone (9.12%), benzene acetaldehyde (8.51%), linalool (7.93%), *α*-pinene (6.89%), and 1,8-cineol (5.22%). According to a recent study on *Eremurus persicus* (10), leaves could contribute to the presence of monoterpenes in the extracted EO from the whole aerial parts of this plant, while flowers to sesquiterpenes.

Antioxidant activity of *E. persicus* EO

The radical scavenging capacity of the EO was tested using the 'stable' free radical DPPH, as commented before. Table 3 shows the effective concentrations of EO required to scavenge DPPH radical and the scavenging

Table 2. Chemical constituents of *E. persicus* essential oil.

Compound	RI[a]	Relative %
α-Pinene	**936**	**6.89[b]**
β-Pinene	985	0.09
α-Terpinene	1024	0.03([c]tr)
Limonene	**1035**	**16.25**
1,8-Cineol	**1038**	**5.22**
Benzene acetaldehyde	**1046**	**8.51**
Guaiacol	1085	0.52
Linalool	**1095**	**7.93**
***n*-Nonanal**	**1109**	**9.48**
trans-Pinocarveol	1134	0.01(tr)
trans-Verbenol	1136	0.08
3,7-Dimethyl-6-octen-3-ol	1139	0.01(tr)
cis-Verbenol	1142	0.02(tr)
Nonadienol	1156	**1.57**
1,3-Dimethoxybenzene	1165	0.01(tr)
p-Cymen-8-ol	1194	0.45
α-Terpineol	1199	0.02(tr)
Limonene aldehyde	1329	0.01(tr)
Tetradecane	1403	0.1
Geranyl acetone	**1460**	**9.12**
10-epi-β-acoradiene	1472	0.5
Epizonarene	1486	0.03(tr)
Germacrene-D	1491	0.57
α-Farnesene	**1505**	**3.52**
(*E*)-Octenyl cyclopentanone	1531	0.23
(*Z*)-Nerolidol	1537	0.04(tr)
Apiole	**1677**	**2.52**
Oxopentadecanone	1708	0.02(tr)
n-Heptadecane	1712	0.34
2-Pentandecanol	1719	0.44
Farnesol	1738	0.04(tr)
Octadecane	1813	0.55
7-Hydroxycoumarin	1838	0.03(tr)
(*Z*,*Z*)-Farnesyl acetone	**1862**	**2.44**
***n*-Nonadecane**	**1911**	**3.54**
2- Heptadecanone	1917	0.02(tr)
Methyl hexadecanoate	1940	0.02(tr)
Icosane	2005	0.59
(-)-Falcarinol	2038	0.65
Geranylgeraniol	**2049**	**15.23**
Manool	2051	0.02 (tr)
n-Octadecanol	2065	0.53
Major groups		
Monoterpene hydrocarbons		23.26
Oxygenated monoterpenes		22.87
Sesquiterpene hydrocarbons		4.62
Oxygenated sesquiterpenes		2.52
Oxygenated diterpenes		15.25
Linear alkanes		5.12
Oxygenated alkanes		10.49
Benzene derivatives		11.59
Others		2.47
Total characterized		98.19

[a]RI: retention index; [b]major compounds are shown in bold; [c]tr: traces, concentration less than 0.05%.

values as % of inhibition. The values of IC_{50} were in the following order: ascorbic acid (0.48 g/L) < BHT (0.55 g/L) < *E. persicus* EO (6.20 g/L). In this sense, the DPPH assay measures the ability of the extract to reduce the DPPH radical to its neutral form, resulting in bleaching of the DPPH solution. The greater the bleaching action, the higher the antioxidant activity, which is reflected in a lower IC_{50}.

Antimicrobial activity of *E. persicus* EO

Tables 4 and 5 shown the antimicrobial activity of *E. perisicus* EO against bacteria and fungi spp., respectively. *E. persicus* EO exhibited a slight antibacterial activity against *S. aureus* and *E. coli* (Table 4), but remarkably its anti-dermatophyte potency was close to that of the drug griseofulvin.

In this sense, *E. persicus* EO MICs and MFCs for the studied dermatophytes (*Microsporum gypseum, Microsporum canis, Trichophyton rubrum* and *Trichophyton schoenleinii*) ranged from 0.02 to 0.17% and 0.7 to 4.5%, respectively. In the case of griseofulvin, the MICs ranged from 0.03-0.59% (Table 5). *E. persicus* EO have been more effect on the strain *T. schoenleinii* among all dermatophytes.

Table 3. Antioxidant activity of *E. persicus* essential oil at different concentrations measured by the DPPH method.

Concentration (g/L)	DPPH Inhibition (%)		
	***E. persicus* essential oil**	**Ascorbic acid**	**Butylated hydroxytoluene**
5	48.33 ± 0.03[§aA]	95.61 ± 0.00 [bA]	96.18 ± 0.00 [bA]
10	53.72 ± 0.11 [aB]	98.04 ± 0.01 [bB]	97.33 ± 0.00 [bA]
20	56.31 ± 0.12 [aC]	98.66 ± 0.00 [bB]	98.23 ± 0.00 [bB]
50	64.98 ± 0.22 [aD]	98.66 ± 0.00 [bB]	98.69 ± 0.00 [bB]
*IC_{50}	6.20	0.48	0.55

*IC_{50}: concentration (g/L) for a 50% inhibition; [§]Data are expressed as means ± SD of DPPH Inhibition (%) for different concentration of essential oil and controls. Values followed by the same small letter within the row are not significantly different (P< 0.05). Values followed by the same capital letter within the same column are not significantly different (P < 0.05).

Table 4. Antibacterial activity of different dilutions of *E. persicus* essential oil and gentamicin as reference compounds.

	***E. persicus* essential oil**		**Gentamicin (0.006 mg/L)**	
Dilution	***S. aureus***	***E. coli***	***S. aureus***	***E. coli***
1:1	12 (++)	8 (+)	33 (+++)	24 (+++)
1:2	9 (+)	3 (+)	28 (+++)	20 (++)
1:4	No inhibition (-)	No inhibition (-)	18 (++)	15 (++)
1:8	No inhibition (-)	No inhibition (-)	14 (++)	12 (++)

Data are expressed as inhibition zones (mm) for essential oil and control. Inhibition zones of bacterial growth were measured by the following scales: no inhibition, resistant strain (-); < 10 mm, mildly sensitive strain (+); 10 to 20 mm, moderately sensitive (++); > 20 mm, highly sensitive (+++). *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*.

Table 5. Susceptibilities of dermatophytes to *E. persicus* essential oil and griseofulvin.

Dermatophytes	*E. persicus* essential oil (%, v/v)		Griseofulvin (%, v/v)
	MIC[a]	MFC[a]	MIC[a]
Microsporum gypseum	0.04-0.09	0.9-4.5	0.03-0.52
Microsporum canis	0.03-0.17	0.7-3.8	0.05-0.12
Trichophyton rubrum	0.07-0.12	0.9-4.3	0.09-0.59
Trichophyton schoenleinii	0.02-0.06	0.9-3.5	0.07-0.09

[a]MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration.

Anticancer activity *E. persicus* EO

A dose-response correlation was observed among *E. persicus* EO concentration and the viability of both Hep-G2 and MCF-7 cell lines. In Hep-G2 cells, 200 ng/mL of EO decreased cell viability by 98% and 97% after 3 and 7 days, respectively, compared to untreated cells ($P< 0.05$) (Figure 1a and b). The highest concentration of essential oil, 1500 ng/mL, was the most effective: it decreased cancer cell viability by 77% and 65% after 3 and 7 days, respectively, compared with untreated control cells ($P< 0.05$) (Figure 1a and b).

Similarly, viability of MCF-7 cells dose-dependently decreased from 97% to 58% and from 94% to 52%, compared to untreated cells, 3 and 7 days after treatment with *E. persicus* EO at the tested concentrations, respectively ($P< 0.05$) (Figure 1c and d).

Acetylcholinesterase inhibitory activity

The ability of *E. persicus* EO to inhibit AChE activity is shown in Fig. 2. The EO moderately inhibited AChE activity (IC_{50} = 95.37 µg/mL), compared with galanthamine (IC_{50} = 50.74 µg/mL).

Discussion

Many countries have a documented history of using aromatic and medicinal plants in their healing traditions (33). The scientific documentation of their composition and bioactivity *in vitro* are a first requirement for understanding their folk uses. Therefore, in this work *E. persicus* EO was screened as a potential source of medicinally active compounds by means of *in vitro* tests: antioxidant, antimicrobial, anticancer and acetylcholinesterase inhibitory activity. The reason why we select these tests was primarily based on its folk uses against fungi infections as well as the interest in finding natural products with antimicrobial activity and applicability in chemotherapy. Moreover, EOs and their constituents can also enhance the efficacy of antibiotics and antifungal drugs that also implies the potential reduction of the drug use (34, 35).

Our findings showed that *E. persicus* EO could have more interest to treat fungal than bacteria infections, at least against the gram-positive and gram-negative bacteria tested here. Among the dermatophytes fungi, the most sensitive strain was *T. schoenleinii* (MIC = 0.02-0.06%, v/v). Several species from the genus *Trichophyton* are recognized to causes superficial mycoses commonly known as tinea infections in humans and other animals (35). In fact, *E. persicus* is traditionally used to treat skin disorders caused by fungi infections (Table 1) as commented above.

The *E. persicus* EO was standardized for further uses using GC-FID and GC-MS, one of the commonest analytical choices in this field. Its analysis revealed the presence of several high abundant volatile compounds, which were previously reported as antimicrobial agents, such as limonene, geranylgeraniol, geranyl acetone, linalool, *α*-pinene, and 1,8-cineol (Figure 3) (35-39). As an example, limonene, *α*-pinene, and 1,8-cineol possesses antifungal activities (35, 38,39). A recent study has shown that linalool may enhance the antimicrobial efficacy of essential oils against bacteria and fungi in synergistic or additive manner (37). In addition, the aldehydes *n*-nonanal (9.48%) and benzene acetaldehyde (8.51%) were also abundant compounds in the EO of interest. In this regard, EOs from mosses rich in *n*-nonanal were more effective inhibiting the fungi *Candida albicans* and *Saccharomyces cerevisiae* than several

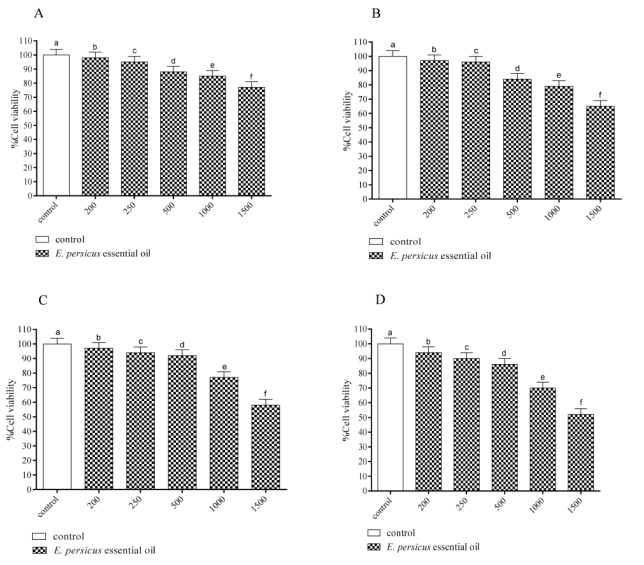

Figure 1. Anticancer activity using different concentrations (ng/mL) of *E. persicus* essential oil: Hep-G2 cells treated for a) 3 days and b) 7 days; MCF-7 cells treated for c) 3 days and d) 7 days. Data are expressed as mean ± SD; different letters indicate statistically significant differences at P<0.05.

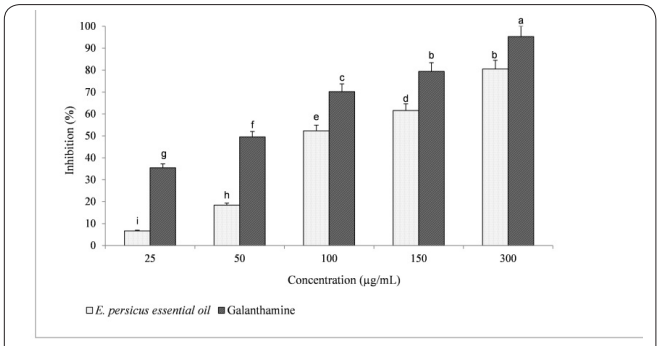

Figure 2. Acetylcholinesterase inhibitory activity of *E. persicus* essential oil. Data are expressed as mean ± SD; different letters indicate statistically significant differences at P<0.05.

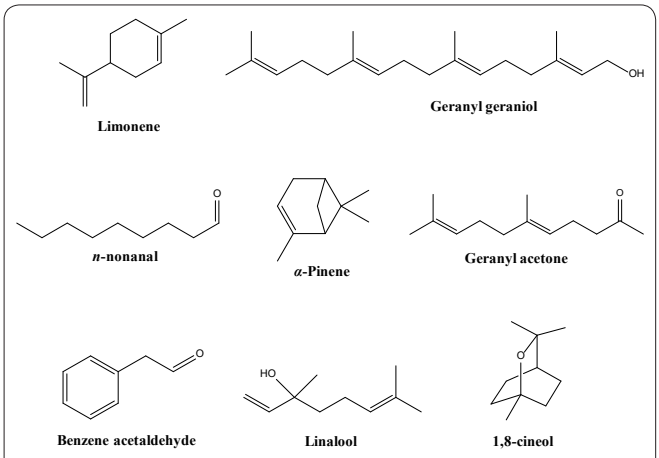

Figure 3. Chemical structures of the main volatile compounds found in *E. persicus* essential oil.

bacteria strains (40). Although the individual efficiency of volatile compounds depends on their structure, functional groups and concentration (36), it remains unclear whether a synergic effect may occur in complex matrices such as essential oils.

The antioxidant properties of EOs are controversial and highly dependent on their composition. Some studies suggest that EOs may effectively counteract intracellular reactive oxygen species (ROS) levels, despite a poor scavenging activity against DPPH and $ABTS^{+}$ radicals (41). Conversely, Paik et al. (42) evidenced that an EO rich in the monoterpene ester geranyl acetate (29.9%) increased the production of ROS and induced the apoptosis of Hep-G2 hepatoma cells, which could explain the inhibition of tumor development in mice treated with this EO. In the present study, *E. persicus* EO showed a slight antioxidant activity (IC_{50} = 6.20 g/L) compared to ascorbic acid (IC_{50} = 0.48 g/L) and the synthetic antioxidant BHT (IC_{50} = 0.55 g/L). Remarkably, *E. persicus* EO was able to decrease the viability of Hep-G2 and MCF-7 cell lines in a concentration-dependent manner (from 200 to 1500 ng/mL). Among the main volatile constituents of *E. persicus* EO, previous studies have shown that limonene exhibited cytotoxicity against MCF-7 and Hep-G2, α-pinene triggered oxidative stress and related signaling pathways in Hep-G2, whereas 1,8-cineol had moderate cytotoxicity in Hep-G2 (43). In addition, Usta et al. (44) showed that linalool decreased Hep-G2 viability by: increasing ROS, inhibiting mitochondrial complexes I and II, and decreasing the levels of ATP and reduced glutathione. Therefore, the mechanism of action of EOs is complex, and their constituents may act through multiple ways in the chemoprevention of cancers, being more selective to cancer cells than normal ones (42).

The characterization results presented here are valuable for improving the knowledge about the phytochemical composition of *E. persicus*, which is poorly known. The GC-MS results showed that *E. persicus* EO is rich in terpenes and derivatives with previously reported antifungal activity. This fact may support the antidermatophyte activities observed in the present study. Moreover, *E. persicus* EO exhibited anticarcinogenic properties, being able to decrease the viability of Hep-G2 and MCF-7 cell lines in a concentration-dependent manner. Therefore, *E. persicus* aerial parts are potential source of promising new cosmeceutical, nutraceutical and functional food formulations. Further purification of *E. persicus* EO constituents is required in future to find the most active constituents, know how they act, and evaluate their toxicity.

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Conflict of Interest

The authors declare no conflict of interest.

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