ORIGINAL ARTICLE

CHEMICAL COMPOSITION AND FUNCTIONAL PROPERTIES OF ESSENTIAL OILS FROM *NEPETA SCHIRAZIANA* BOISS.

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Abstract

Nepeta schiraziana Boiss. (*Lamiaceae*) is a species endemic of Iran used in traditional medicine to treat a number of ailments. This study investigates the chemical composition as well as the cytotoxic, antimicrobial, acetylcholinesterase (AChE) inhibitory and antioxidant activities of *N. schiraziana* essential oil (EO). EO chemical composition was analysed by GC-MS. Cytotoxicity was evaluated against MCF-7 and Hep-G2 cancer cells, while antimicrobial activity was assessed against fungi (*Candida albicans*, *Aspergillus niger*), Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*) bacteria. Forty-four compounds were identified; the main constituents were 1,8-cineole (33.67%), germacrene D (11.45%), β-caryophyllene (9.88%), caryophyllene oxide (7.34%). *N. schiraziana* EO showed a concentration-dependent cytotoxicity against both cancer cell lines. It also significantly inhibited the growth of *A. niger* (MIC 1.85 ± 1.12) and *C. albicans* (MIC 4.94 ± 3.07 μg/mL). MIC for *S. aureus*, *B. subtilis*, *K. pneumoniae* and *P. aeruginosa* were 16.37 ± 1.23, 168.22 ± 2.14, 180.99 ± 4.67 and 248.74 ± 5.12 μg/mL, respectively. Slight antiradical and AChE inhibitory activities were measured. This is the first report on the chemical composition and bioactivities of *N. schiraziana* EO. The antibacterial, antifungal and anticancer properties were confirmed *in vitro*.

Rezumat

Nepeta schiraziana Boiss. (*Lamiaceae*) este o specie endemică a Iranului utilizată în medicina tradițională pentru a trata o serie de afecțiuni. Acest studiu a evaluat compoziția chimică, precum și activitatea antioxidantă, citotoxică, antimicrobiană, anti-acetilcolinesterazică (AChE) a uleiului esențial provenit de la *N. schiraziana* (UE). Compoziția chimică a UE a fost analizată prin GC-MS. Citotoxicitatea a fost evaluată asupra celulelor MCF-7 și Hep-G2, în timp ce activitatea antimicrobiană a fost evaluată asupra fungilor (*Candida albicans*, *Aspergillus niger*), bacteriilor gram-pozitive (*Staphylococcus aureus*, *Bacillus subtilis*) și bacteriilor gram-negative (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*). Au fost identificați patruzeci și patru de compuși, constituenții principali au fost 1,8-cineol (33,67%), germacren D (11,45%), β-cariofilină (9,88%), cariopilen oxid (7,34%). UE din *N. schiraziana* a demonstrat o citotoxicitate dependentă de concentrație asupra ambelor linii de celule. De asemenea, a inhibat semnificativ creșterea *A. niger* (CMI 1,85 ± 1,12) și *C. albicans* (CMI 4,94 ± 3,07 μg/mL). Concentrația minimă inhibitoare pentru *S. aureus*, *B. subtilis*, *K. pneumoniae* și *P. aeruginosa* a fost 16,37 ± 1,23, 168,22 ± 2,14, 180,99 ± 4,67 și, respectiv, 248,74 ± 5,12 μg/mL. Au fost evidențiate activități antioxidante și AChE moderate. Acesta este primul studiu privind compoziția chimică și bioactivitățile uleiului esențial provenit de la *N. schiraziana*. Proprietățile antibacteriene, antifungice și anticanceroase au fost confirmate *in vitro*.

Keywords: *Lamiaceae*, Iranian endemism, anticancer activity, antimicrobial activity, acetylcholinesterase inhibitory activity, antioxidant activity

Introduction

For thousands of years medicinal plants represented an important source of remedies, as well as the basis of traditional or indigenous healing systems still used, in the estimates of the World Health Organization (WHO), by the majority of the population in the developing countries [67]. The interest on herbal medicine and plant-derived compounds is now renewing, in the attempt to reduce drug costs, drug resistance and side effects [4, 14, 30, 31, 53, 58, 64, 66]. Recently, ethno-medicines have been receiving important consideration by researchers and pharmaceutical industries to complement or replace conventional pharmacotherapies.

Plants produce numerous secondary metabolites to attract pollinators or defend themselves against environmental stress, pathogen attacks or other adversities. Essential oils (EOs) are among the most important plant natural products, composed by very complex mixtures of lipophilic, volatile and aromatic molecules, arising from the plant secondary metabolism [4, 57]. EOs are studied for their antioxidant and anticancer properties and used, as natural additives, in different foods to reduce the proliferation of microorganisms and their toxin production in crops and processed foods, due to their antimicrobial activity [4, 54, 57].

Cancer, infections and neuro-degenerative disorders are among the major burdens of diseases, worldwide. Cancer is rapidly increasing in the last decades, and failure of conventional chemotherapy due to chemoresistant cancer cells as well as its side effects on non-target cells and tissues has stimulated the research on suitable, natural alternatives [31]. Similarly, drug resistance of pathogenic microorganisms has impaired the efficacy of conventional antibiotics used to treat several infectious diseases [4, 53, 58, 64]. Alzheimer's disease (AD), one of the most common types of dementia, is a neurodegenerative disorder caused by the progressive neuron cell death and which involves the impairment of the enzyme acetylcholinesterase (AChE), responsible for the hydrolysis of acetylcholine (ACh). In AD, this enzyme forms deposits within the neurofibrillary tangles and amyloid plaques. A wide range of evidence showed that plant-derived AChE inhibitors could reduce the progression of this disease [19].

The use of phytotherapeutics, in general, shows fewer adverse effects than conventional pharmaceuticals, representing a promising treatment strategy.

The *Nepeta* genus (*Lamiaceae*) consists of more than 250 species that are naturally distributed in Asia, Europe, North America and Africa. Thirty-nine out the 67 *Nepeta* species present in Iran, with the common Persian name *Pune-sa*, are endemic. Most species have been reported to possess medicinal properties such as antitussive, diaphoretic, anti-spasmodic, anti-asthmatic, diuretic, emmenagogue and febrifuge [8, 18, 35]. *Nepeta schiraziana* Boiss. is one of the species endemic in Iran. To the best of our knowledge, the cytotoxic, antimicrobial, acetyl-cholinesterase inhibitory and antioxidant activities of the *N. schiraziana* have not been investigated so far. Therefore, the present study investigates the chemical composition as well as cytotoxic, anti-microbial, acetylcholinesterase inhibitory and anti-oxidant activities of the EO obtained from the aerial parts of *N. schiraziana*.

Materials and Methods

Plant collection and essential oil extraction. The aerial parts (stems, leaf and flowers) of *N. schiraziana* were collected in June 2014 from wild plants at the flowering stage in the mountains of Meymand (geographic coordinates: 28°52′04″N 52°45′12″E), Firuzabad County, Fars Province, Iran. The botanist Dr Mohammedreza Joharchi confirmed the taxonomy and a voucher specimen (no. 42317) was deposited in Ferdowsi University Mashhad Herbarium. The aerial parts of *N. schiraziana* were dried in the shade for three days. Then, the dried aerial parts (100 g) were hydro-distilled for 4 h utilizing an all-glass Clevenger-type apparatus in accordance with the method outlined by the British Pharmacopeia [43]. The essential oil (EO) obtained was dried over anhydrous sodium sulphate (Sigma-Aldrich, USA) and stored at 4°C before performing gas chromatography-mass spectrometry (GC-MS) analysis and further assays.

Gas chromatography and gas chromatography coupled to mass spectrometry analyses. Gas chromatography (GC) analysis was carried out using a Shimadzu 15A gas chromatograph (Kyoto, Japan), equipped with a DB-5 fused silica column (50 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was performed as follows: 60°C for 3 min; 220°C at a rate of 5°C/min, injector temperature and detector (FID) temperature 270°C; helium was used as carrier gas with a linear velocity of 1 mL/min. The gas chromatography coupled to mass spectrometry (GC-MS) analysis was carried out using an Agilent (Hewlett Packard) 6890/5973 GC/MS system equipped with a HP-5MS fused silica column (30 m × 0.25 mm i.d.). The oven temperature was 60 - 240°C at a rate of 5°C/min; transfer line temperature 260°C; carrier gas helium with a linear velocity of 1 mL/min; ionization energy 70 eV; scan time 1 sec and mass range of 40 - 300 amu. Retention indices (RI) were determined using retention times of *n*-alkanes that were injected after the essential oil under the same chromatographic conditions. Compounds were identified by comparison of mass spectral fragmentation patterns and retention indices with Wiley 7n.L Mass Spectral Library (Wiley, New York, NY, USA), Adams Library and Mass Finder 2.1 [1, 33]. The relative percentages of the EO components were obtained according to the peak area in the chromatogram [61].

Human cancer cell lines and cytotoxicity assay. The MCF-7 (human breast adenocarcinoma) cell lines (ATCC® HTB22™) and Hep-G2 (human hepato-cellular carcinoma) cells (ATCC® HB8065™) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and used for cyto-toxicity test. The cells were cultivated in Dulbecco's modified Eagle's Medium supplemented with 10%

heat-inactivated foetal bovine serum, 1% L-glutamine, HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid) buffer and 50 µg/mL gentamicin (Sigma-Aldrich, St. Louis, MO, USA). The cells were cultured at 37°C in a humidified atmosphere with 5% CO_2. Cytotoxicity of EO (serial dilutions) was evaluated against cancer cells by the crystal violet staining method, reported by Saotome *et al*. [50] with slight modifications. Absorbance was determined at 595 nm by an automatic microplate reader. The concentration at which the growth of cells was inhibited to 50% of the control (IC_{50}) was calculated. The control cells were incubated without EO, and with or without dimethylsulfoxide (DMSO). Vinblastine sulphate was used as standard anticancer drug.

Antimicrobial activity: microorganism preparation, disk diffusion assay and minimum inhibitory concentration. The *N. schiraziana* EO was assayed against two fungi (*Candida albicans* and *Aspergillus niger*), two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Microorganisms were cultured at 37ºC for 14 - 24 h and the densities were adjusted to 0.5 McFarland standards at A_{530} nm (10^8 CFU/mL). The EO antimicrobial activity was evaluated by the disc diffusion method [6]. Discs were impregnated with different concentrations (10, 20, 40, 60, 80 and 100 µg/mL) of EO; ketoconazole, ampicillin and gentamicin (10 mg/disc) were used as positive control for fungi, Gram-positive and Gram-negative bacteria, respectively. In addition, dimethyl sulfoxide (DMSO) was used as the negative control. Antimicrobial activity was assessed by measuring the zone of inhibition. The minimum inhibitory concentration (MIC) was determined using serial dilutions of the EOs (0 - 100 µg/mL) using microdilution test according to the Clinical and Laboratory Standards Institute [11]. The bacterial and fungal strains were suspended in Luria-Bertani media. Growth control was the medium with bacteria and fungi but without EO. The MICs were visually detected by comparing the growth in each well with the growth in the control wells and defined as the lowest concentration of the EO with > 95% growth inhibitory activity.

Acetylcholinesterase inhibitory activity. The acetylcholinesterase (AChE) activity was determined by using the spectrophotometric Ellman's method [16]. 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, 75, 100, 150 and 300 µg/mL) in DMSO were incubated, in test tubes, for 15 min at room temperature. Then, 10 µL of 6.67 U/mL AChE and 200 µL of 3 mM DTNB (5,5′-dithio-bis[2-nitrobenzoic acid]) in buffer were added. Galanthamine (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control, prepared in serial concentrations as EO, in 50 mM Tris-HCl buffer, pH 7.4. The mixture was incubated for 20 min 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 with a Hitachi U-2001 spectrophotometer (Tokyo, Japan) every 10 sec for 3 min. The buffer was used as blank. The enzyme hydrolysis of the substrate acetylthiocholine produces thiocholine, which reacts with Ellman's reagent (DTNB) and gives 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate both detectable at 405 nm. The enzyme inhibition (%) was calculated from the rate of absorbance change over the time (V = Abs/Δt): Inhibition (%) = 100 - (Change of sample absorbance/Change of blank absorbance) × 100.

EO concentrations that inhibit the hydrolysis of the substrate (acetylcholine) by 50% (IC_{50}) were determined by linear regression between the inhibition percentages vs. EO concentrations.

Antiradical activity. The scavenging activity of the EO on the synthetic 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical (Sigma-Aldrich, St. Louis, MO, USA) was determined as previously reported [45]. A volume of 1 mL of the EO serial dilutions (5, 10, 15, 30, 60, 120 and 240 µg/mL) was mixed with 2 mL of methanolic solution containing DPPH radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously, left to stand for 30 min, in the dark, and the absorbance was read at 517 nm against a blank with a Hitachi U-2001 spectrophotometer (Tokyo, Japan). The α-tocopherol and butylated hydroxyl anisole (BHA) (Sigma-Aldrich, St. Louis, MO, USA) were used as reference standards. The scavenging ability was determined as follows:

$$\text{Inhibition (\%)} = [(A_c - A_s)/A_c] \times 100.$$

Where A_c and A_s are the absorbance of control and sample, respectively. IC_{50} value was defined as the concentration of sample needed to inhibit 50% of DPPH free radicals.

Statistical analysis. The tests were performed in triplicate for chemical characterization as well as cytotoxic, antimicrobial, acetylcholinesterase inhibitory and antioxidant tests. Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's HSD (honestly significant difference) *post-hoc* test at $p < 0.05$ using SPSS v. 11.5.

Results and Discussion

Chemical composition of N. schiraziana essential oil. The chemical composition of *N. schiraziana* EO is shown in Table I. Forty-four compounds were identified, representing 97.9% of *N. schiraziana* EO. Among these compounds, 43.13% were oxygenated

monoterpenes, 16.64% were monoterpene hydrocarbons, 28.57% were sesquiterpene hydrocarbons, 9.52% were oxygenated sesquiterpenes and 0.04% was represented by non-terpenes. The main constituents were 1,8-cineole (33.67%), germacrene D (11.45%), β-caryophyllene (9.88%), caryophyllene oxide (7.34%), *α*-pinene (4.59%), camphor (3.75%), β-pinene (3.67%), *p*-cymene (3.52%), β-elemene (2.63%), sabinene (2.52%), δ-elemene (2.44%), myrtenol (2.17%), β-bourbonene (2.12%), linalool (1.22%), δ-cadinene (1.52%), α-terpineol (1.14%), *cis*-verbenol (1.11%), carvacrol (0.52%) and limonene (0.51%).

Table I

Chemical constituents of *Nepeta schiraziana* essential oil

Compound	RI[a]	Relative %
2-Methyl butyl acetate	853	t[b]
α-Thujene	915	t
α-Pinene	**936**	**4.59**[c]
Camphene	950	t
Dehydro-1,8-cineole	970	0.08
Sabinene	**975**	**2.52**
β-Pinene	**982**	**3.67**
Myrcene	991	0.16
α-Terpinene	1022	0.23
***p*-Cymene**	**1025**	**3.52**
(Z)-*â*-Ocimene	1028	t
Benzene acetaldehyde	1030	t
Limonene	**1035**	**0.51**
1,8-cineole	**1037**	**33.67**
trans-Linalool oxide	1056	t
cis-Sabinene hydrate	1074	0.11
Linalool	**1093**	**1.22**
Terpinolene	1096	0.21
Nopinone	1120	t
trans-Pinocarveol	1130	0.2
***cis*-Verbenol**	**1135**	**1.11**
trans-Verbenol	1140	0.08
Camphor	**1145**	**3.75**
Terpinen-4-ol	1165	0.09
Lavandulol	1177	0.07
***á*-Terpineol**	**1190**	**1.14**
Myrtenol	**1194**	**2.17**
trans-Dihydrocarvone	1210	0.06
carvacrol	**1294**	**0.52**
***ä*-Elemene**	**1330**	**2.44**
α-Copaene	1370	0.09
***â*-Bourbonene**	**1382**	**2.12**
Geranyl acetate	1386	t
***â*-Elemene**	**1389**	**2.63**
***â*-Caryophyllene**	**1420**	**9.88**
(E)-*â*-Farnesene	1432	t
á-Humulene	1450	0.05
Nerylisobutanoate	1477	0.06
Germacrene D	**1482**	**11.45**
Bicyclogermacrene	1495	0.37
ã-Cadinene	1510	0.12
***ä*-Cadinene**	**1521**	**1.52**
Caryophyllene oxide	**1578**	**7.34**
n-Hexadecane	1590	t
Major groups		
Monoterpene hydrocarbons		**16.64**
Oxygenated monoterpenes		**43.13**
Sesquiterpene hydrocarbons		**28.57**
Oxygenated sesquiterpenes		**9.52**
Others		**0.04**
Total identified		**97.9%**

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

Cytotoxic activity. The results of the cytotoxicity assay are summarized in Table II. The EO determined a significant dose-dependent decrease in viability of both cancer cell lines ($p < 0.05$) (Table II). In case of Hep-G2 cells, IC_{50} of *N. schiraziana* EO (85.74 ± 6.23 µg/mL) was higher than that of the vinblastine (6.32 ± 1.07 µg/mL). Similarly, IC_{50} of the *N. schiraziana* EO on MCF-7 (32.56 ± 3.23 µg/mL) was higher than that of the reference drug used (6.47 ± 1.29 µg/mL) (Figure 1).

Table II

Cytotoxic activity of *Nepeta schiraziana* essential oil (EO) on human cancer cell lines

Concentrations (µg/mL)	*N. schiraziana* EO		Vinblastine	
	% Viability			
	Hep-G2[a]	MCF-7[b]	Hep-G2	MCF-7
0	100	100	100	100
1.56	99.47 ± 2.11*	98.44 ± 3.27	75.30 ± 1.04	48.42 ± 3.01
3.125	95.42 ± 2.09	94.23 ± 1.1	52.29 ± 4.09	52.32 ± 4.24
6.25	93.11 ± 3.01	89.62 ± 2.14	50.12 ± 3.07	50.72 ± 2.49
12.5	88.55 ± 4.37	68.92 ± 5.43	42.31 ± 2.03	36.15 ± 3.09
25	77.92 ± 2.7	52.32 ± 2.19	16.19 ± 2.07	18.12 ± 3.09
50	65.29 ± 1.13	46.23 ± 5.09	14.17 ± 0.06	15.55 ± 1.02
100	45.64 ± 2.07	44.54 ± 1.32	10.53 ± 1.07	10.45 ± 3.2
200	40.34 ± 3.25	35.42 ± 3.29	5.34 ± 2.53	7.77 ± 0.01
IC_{50} (µg/mL)	85.74 ± 6.23	32.56 ± 3.23	6.32 ± 1.07	6.47 ± 1.29

[a]Human hepatocellular carcinoma cells; [b]human breast adenocarcinoma cells; *data are expressed as mean ± SD.

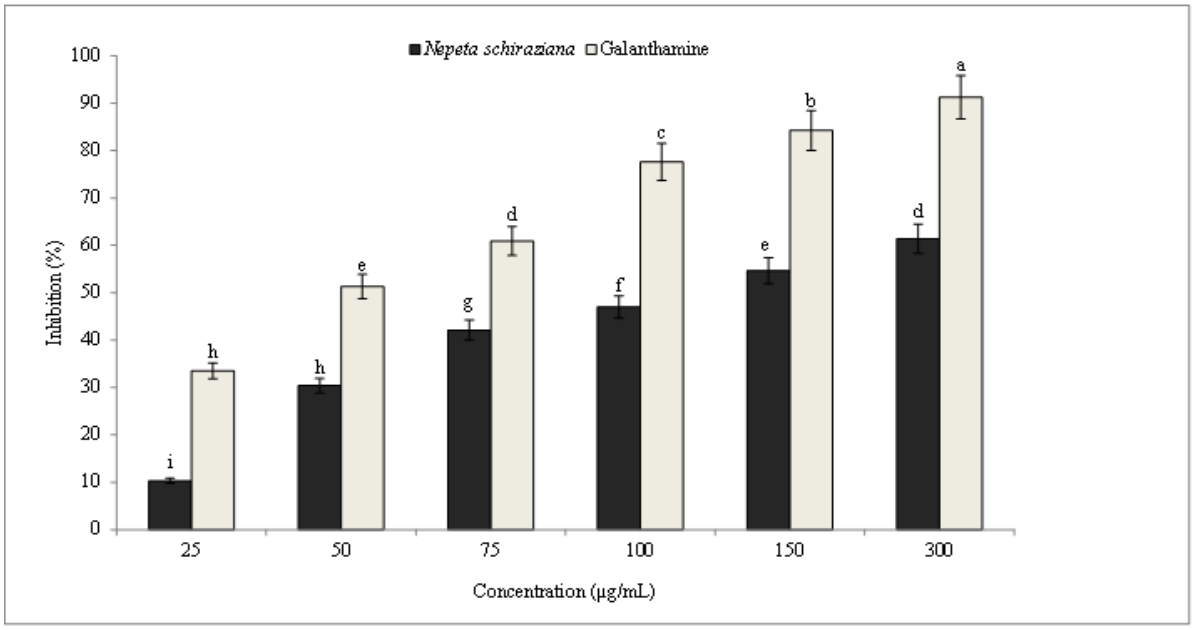

Figure 1.

Acetylcholinesterase inhibitory activity of *Nepeta schiraziana* Boiss. essential oil
Different letters indicate significant differences according to the Tukey's HSD (honestly significant difference) test at $p < 0.05$; all results are expressed as mean ± SD (n = 3).

Antimicrobial activity. Results on the antimicrobial activities are summarized in Table III. The *N. schiraziana* EO significantly inhibited the growth of *A. niger* and *C. albicans* ($p < 0.05$) at the assayed concentrations (Table III). MIC for *A. niger* and *C. albicans* were 1.85 ± 1.12 and 4.94 ± 3.07 µg/mL of EO, respectively (Table III). In addition, the *N. schiraziana* EO significantly inhibited the growth of Gram-positive bacteria (*S. aureus* and *B. subtilis*), while it moderately affected Gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa)* ($p < 0.05$) (Table III). The MIC for *S. aureus*, *B. subtilis*, *K. pneumoniae* and *P. aeruginosa* were 16.37 ± 1.23, 168.22 ± 2.14 µg/mL, 180.99 ± 4.67 µg/mL and 248.74 ± 5.12 µg/mL of EO, respectively. The tested fungi were the most sensitive micro-organisms, with the lowest MIC.

Table III

Antimicrobial activity of *Nepeta schiraziana* essential oil

Essential oil (µg/mL)	*Aspergillus niger*	*Candida albicans*	*Staphylococcus aureus*	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
10	11.41 ± 3.01* f	4.73 ± 2.27 f	14.55 ± 1.22 f	0.0 ± 0.0 g	0.0 ± 0.0 e	0.0 ± 0.0 f
20	25.32 ± 1.24 d	8.99 ± 1.56 e	32.19 ± 2.03 e	6.32 ± 1.04 f	0.0 ± 0.0 e	0.0 ± 0.0 f
40	26.72 ± 3.51 d	16.08 ± 0.01 d	48.15 ± 3.03 d	12.23 ± 1.12 e	7.77 ± 3.09 d	3.35 ± 1.04 e
60	33.49 ± 2.32 c	18.17 ± 2.01 c	53.87 ± 4.1 c	16.56 ± 3.19 c	8.59 ± 2.11 d	5.59 ± 2.02 d
80	55.32 ± 3.11 b	32.52 ± 2.11 b	68.81 ± 2.42 b	20.33 ± 0.23 c	15.55 ± 1.4 c	8.65 ± 2.5 c
100	69.88 ± 2.01 a	59.21 ± 2.05 a	75.31 ± 1.03 a	25.42 ± 1.45 b	17.14 ± 2.03 b	9.12 ± 3.09 b
DMSO	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g	0. 0 ± 0.0 g	0.0 ± 0.0 e	0.0 ± 0.0 f
Ampicillin	-	-	32.53 ± 1.05 e	28.99 ± 0.4 a	-	-
Gentamicin	-	-	-	-	38.99 ± 0.2 a	25.7 ± 0.0 a
Ketoconazole	14.23 ± 2.04 e	18.32 ± 4.03 c	-	-	-	-
MIC (µg/mL)	1.85 ± 1.12	4.94 ± 3.07	16.37 ± 1.23	168.22 ± 2.14	180.99 ± 4.67	248.74 ± 5.12

*Data are expressed as mean ± SD of inhibition zone diameter (mm) for different concentration of essential oil; DMSO: dimethyl sulfoxide; MIC: minimum inhibitory concentration. The values with different letters within a column are significantly different ($p < 0.05$).

Acetylcholinesterase inhibitory activity. The ability of different concentrations of *N. schiraziana* EO to inhibit acetylcholinesterase activity is shown in Figure 1. EO slightly inhibited the acetylcholinesterase activity (IC_{50} = 117.3 µg/mL), compared with galanthamine (IC_{50} = 47.54 µg/mL).

Antioxidant activity. The results of DPPH free radical scavenging assay on *N. schiraziana* EO are shown in Figure 2. EO slightly scavenged the DPPH free radical (IC_{50} = 52.24 µg/mL), compared with positive controls BHA (IC_{50} = 6.5 µg/mL) and α-tocopherol (IC_{50} = 9.17 µg/mL).

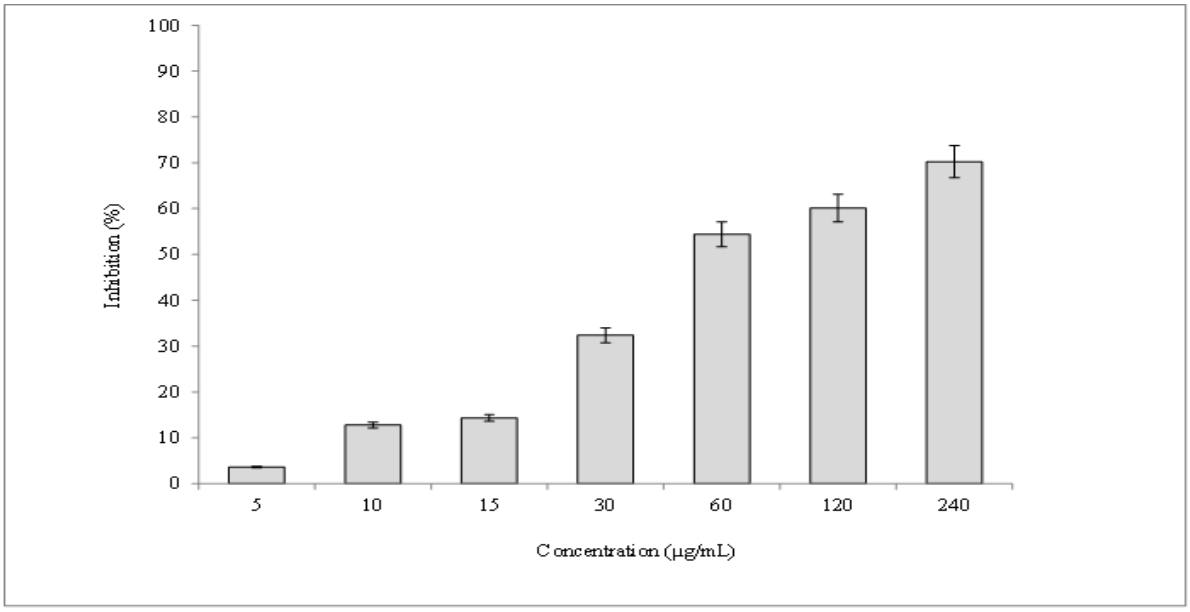

Figure 2.

Free radical scavenging activity of *Nepeta schiraziana* Boiss. essential oil

The chemical composition of *Nepeta* spp. EO has been largely investigated. The major constituents in *Nepeta menthoides* Boiss & Buhse EO were: 4a-α,7β,7a-α-nepetalactone (18.39%), 4a-α,7α,7a-α-nepetalactone (17.57%), 1,8-cineole (16.66%) and geranyl acetate (7.0%) [21]. Sonboli *et al*. [63] documented 1,8-cineole (47.9%) and 4a-α,7α,7a-α-nepetalactone (20.3%) as the main components in *Nepeta crispa* Willd. EO from Iran. In *Nepeta cataria* EOs, 4a-α,7-α,7a-β-nepetalactone (55-58%) and 4a-α,7-β,7a-α-nepetalactone (30-31.2%) were the major compounds at all developmental stages [69]. Rustaiyan *et al*. [47] studied the composition of the EOs of four Iranian *Nepeta* species, showing EOs of *Nepeta prostrata* Benth. and *Nepeta straussii* Hausskn. ex Bornm. were rich in 1,8-cineole (26.1% and 22.1%, respectively) and β-pinene (13.6% and 12.1%, respectively). The other main components included myrtenol (11.8%) and germacrene-D (18.5%) in *N. prostrata* EO and *N. straussii* EO, respectively.

The major constituents of *Nepeta saccharata* Bunge EO were neo-isomenthol (18.6%), hexadecanoic acid (12.1%), 1,8-cinole (11.7%) and

germacrene-D (11.6%). (E)-Sesquilavandulol (39.5%), β-caryophyllene (14.7%) and caryophyllene oxide (11.6%) were the pre-dominant constituents in the *Nepeta pungens* Benth. EO. All EOs consisted mainly of oxygenated mono-terpenes except that of the *N. pungens*, which was rich in oxygenated sesquiterpenes [47].

The main components in the *N. cephalotes* EO were 4a-α,7α,7a-α-nepetalactone (90.1%) and β-pinene (7.5%), while 4a-β,7α,7a-β-nepetalactone (64.0%) and 1,8-cineole (7.1%) were the major compounds in *N. bornmuelleri* EO, as well as 4a-α,7α,7a-α-nepetalactone (61.0%) and caryophyllene oxide (7.8%) in *N. mirzayanii* EO [52]. *N. bracteata* EO, which was rich in spathulenol (14.0%), caryophyllene oxide (12.3%), bicyclogermacrene (11.4%) and β-caryophyllene (11.2%) [52]. The composition of the *N. menthoides* EO from Iran was mainly associated with oxygenated monoterpenes (71.9%), where 1,8-cineole (33.8%) and 4a-*α*,7a-*α*,7a*α*-nepetalactone (23.2%) were the major components [62].

The Iranian *Nepeta racemosa* Lam. EO, instead, was composed of 4a-β,7α,7a-β-nepetalactone (33.6%), 4a-α,7α,7*a*-β-nepetalactone (25.6%), 4a-α,7α,7a-α-nepetalactone (24.4%) and 1,8-cineole (9%) [13]. Overall, 1,8-cineole was the most abundant compound in the EOs of the genus *Nepeta*, at different ranges. Our results are in accordance with these studies, since 1,8-cineole represented the most abundant (33.67%) component determined in *N. schiraziana* EO. This was consistent with the report by Akhgar and Moradalizadeh [2], who at first provided the chemical composition of the essential oil of *N. schiraziana*, also reported by Sharma and Cannoo [60]. 1,8-cineole possesses several biological and pharmacological activities including antimicrobial [51], analgesic [29, 49], antibacterial [41, 10], anti-oxidant [48], antispasmodic [5, 12, 38], antiviral [3], hypotensive [27, 44], neuroprotective [39] and mucolytic [22, 65] properties. Germacrene D was also found in high amounts (11.45%), similarly to β-caryophyllene (9.88%) (Table I).

Natural products represent an important reservoir of putative anticancer agents [7, 30, 31, 66]. The results of our study showed a significant dose-dependent decrease in viability of both cancer cell lines, likely ascribed to 1,8-cineole activity, germacrene D and β-caryophyllene.

The monoterpene 1,8-cineole/ eucalyptol, indeed, induced apoptosis, and not necrosis, on human colon cancer cell lines HCT116 and RKO, with the inactivation of surviving and Akt (key players in cancer cell survival and proliferation), of Mdm2 (which induces overexpression of p53 which, in turn, upregulates p21 expression) and with the activation of p38 [37]. These molecules induce the cleavage of PARP and caspase-3, eventually causing apoptosis. Nonetheless, germacrene D, found at high amount in the *Nepeta ucrainica* L. spp. *Kopetdaghensis* EO, inhibited the growth of MDA-MB-231, MCF7, Hs578T, PC-3 and Hep-G2 cell lines [26]. β-caryophyllene also significantly suppressed constitutive STAT3 activation in multiple myeloma cell line, in a dose- and time-dependent manner [23].

This anti-cancer activity was mediated through the inhibition of kinases c-Src and JAK1/2 [23], besides inducing the expression of tyrosine phosphatase SHP-1 that correlates with the down-regulation of constitutive STAT3 activation.

Furthermore, other minor constituents of the EO (β-pinene, β-elemene and carvacrol) may have exerted an anticancer role. Li *et al*. [20] reported that β-pinene in EO of *Schefflera heptaphylla* L. showed significant anti-proliferative activity against A375 cancer cell line. Edris [35] demonstrated that β-elemene from *Nigella sativa* L. EO inhibited the growth of laryngeal cancer cells by activating caspase-3 cleavage and decreasing the accumulation of eukaryotic initiation factors eIF-4E and 4G, basic fibroblast growth factor (bFGF) and vascular epithelial growth factor (VEGF).

Jiang *et al*. [20] also illustrated that β-elemene arrested the cell cycle and induced apoptosis in lung cancer cells and that carvacrol induced apoptosis and activation of ROS and caspase-3.

In consequence of the high heterogeneous composition of EOs, it is difficult to define a unique mechanism of action. Therefore, a molecule could have an effect on one type of cancer cell line and not on others. In general, cytotoxic properties of EOs can involve various mechanisms of action, including disruption of cell membrane integrity and function *via* depolarization, increasing of its permeability or reduction of membrane-bound enzymes activities [9]; induction of apoptosis [25]; or alteration of mevalonate metabolic pathway [17].

Concerning the antimicrobial activity, *N. crispa* EO was effective against seven Gram-negative and Gram-positive bacteria, particularly *B. subtilis* and *S. aureus*, and four fungi [63]. Sonboli *et al*. [62] also investigated the antibacterial activity of *N. menthoides* EO from Iran, finding high activity not only of the EO but also of its two main constituents, 1,8-cineole and 4a-α,7a-α,7aα-nepetalactone, against all tested bacteria.

In general, the antimicrobial effects of an EO could be due to the interaction between EO compounds and bacterial cell wall, possibly causing alteration of cell membrane and ion leakage, perturbation of proton pumps and many others.

On the other hand, this work showed just a slight antiradical activity of *N. schiraziana* EO when compared with the positive control. In this case, DPPH free radical scavenging assay was applied. There are many reports about the antioxidant

activity of EOs measured by different methods, which hinder result comparison [32]. Therefore, the correlation between the antioxidant ability of EOs and their chemical composition is still controversial.

Finally, in the perspective of preventing and managing neurodegenerative disorders, *N. schiraziana* EO showed a certain AChE inhibitory activity, possibly due to low molecular weight mono- and sesquiterpenes, i.e. α-pinene, α- and β-caryophyllene, able to cross easily the blood-brain barrier [36].

AChE inhibitory activity of *N. schiraziana* EO may be attributed to its α-pinene content (4.59%), as previously demonstrated [34, 40, 42].

Intriguingly, Miyazawa and Yamafuji [34] reported that the presence of oxygenated functional groups in bicyclic terpenes could decrease their capacity to inhibit AChE. Anti-AChE activity of EOs is strongly dependent on the interaction of numerous terpenoids compounds, and different interactions involving synergistic, additive or antagonistic effects among mono- and sesquiterpenes may maximize the EO efficacy [68].

Conclusions

In the present study, we investigated, to the best of our knowledge for the first time, the biological activities of the EO obtained from the aerial parts of *N. schiraziana*, a plant endemic in Iran with documented uses in traditional medicine and diet.

Biological assays revealed that the EO possesses antimicrobial activity, particularly against Gram-positive bacteria and fungi, as well as a significant dose-dependent cytotoxic activity against MCF-7 and Hep-G2 cancer cell lines. *N. schiraziana* EO also exhibited a slight DPPH radical scavenging and an AChE inhibitory activity. These preliminary results suggest that *N. schiraziana* EO deserves attention for further mechanistic studies as well as *in vivo* investigations on its efficacy and safety.

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