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INVESTIGATION ON CHEMICAL COMPOSITION, ANTIMICROBIAL, ANTIOXIDANT, AND CYTOTOXIC PROPERTIES OF ESSENTIAL OIL FROM *DRACOCEPHALUM KOTSCHYI* BOISS.

Behnam Ashrafi [1, 3], Parvin Ramak [2*], Behrouz Ezatpour [3], Gholam Reza Talei [3]

[1]Department of Biology, Borujerd Branch, Islamic Azad University, Borujerd, Iran
[2]Research Division of Natural Resources, Lorestan Agricultural and Natural Resources Research and Education Center, AREEO, Khorramabad, Iran.
[3]Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences,

***Corresponding author E-mail:** researchherb175@gmail.com

Abstract

Background: *Dracocephalum kotschyi* Boiss is a herb with wide-spread applications. Lorestan traditional healers have applied it for the treatment of rheumatoid diseases and stomach disorders.
Materials and methods: Hydrodistillation process was used for essential oil extraction, the extracted essential oil was then analyzed through combination of capillary GC–FID, GC–MS and RI. The *in vitro* antimicrobial, antioxidant and cytotoxic activities of this essential oil were examined. Results indicate that the essential oil has a broad range of anti-microbial activity against all of the tested microorganisms.
Results: The 50% of cytotoxic concentrations was 26.4 µg/ml and 4266.7 µg/ml for Hela cells and human lymphocytes, respectively. The oil cytotoxicity against the human tumor cell line was far higher than the amount required for human healthy cells. Conversely, the essential oil's IC_{50} value of 49.2 µg/ml in the DPPH assay, could be regarded as its strong antioxidant potential.
Conclusion: According to the data obtained, it can be concluded that *D. kotschyi* essential oil could be applied as a safe antibacterial and antioxidant agent for food and pharmaceutical purposes.

Key words: Antimicrobial, Antioxidant, *Dracocephalum kotschyi* Boiss, Cytotoxicity, Essential oil.

Introduction

Aromatic plants' fragrance and biological properties arise from their Essential oils. Availability, fewer side-effects, and reduced toxicity of some aromatic medicinal plants resulted in their application for treating infectious diseases in different phytotherapy manuals (Lee et al., 2007). Today, plant materials play key role in primary healthcare remedies in many developing countries (Zakaria, 1991). Anti-microbial properties of physiologically active principles in medicinal herbs have led to their exploitation in traditional medicine for the treatment of various ailments (Kelmanson et al., 2000; Srinivasan et al., 2001). Throughout history, numerous plant oils have been used for food safety and quality improvement (Yang et al., 2015). *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella spp*., and *Streptococci* are among common foodborne pathogenic bacteria frequently isolated from various foods such as meat, dairy products, seafood and vegetables (Pirbalouti et al., 2010). Food poisoning is caused due to consumption of foods Gram-positive and Gram-negative bacteria contaminated. Essential oils are complex mixtures of volatile secondary metabolites compsed of mono- and sesquiterpenes such as carbohydrates, alcohols, ethers, aldehydes, and ketones, which are responsible for the fragrant and biological properties of aromatic medicinal plants (Senatore, et al. 2004). The genus *Dracocephalum* L. is composed of about 60 species; also, eight species of *Dracocephalum* grow as wild herbs in regions of Iran with high altitude. Several studies have been conducted on identity and biological properties of the mentioned species (Fattahi, et al., 2013; Yousefzadeh, et al., 2013). *Dracocephalum kotschyi* Boiss is native to Iran and grows wild at regions with 2000-3200 m altitude in Lorestan, Fars, Golestan, Hamadan, Mazandaran and Tehran provinces. *D. kotschyi* is a short perennial herb that is woody below, its stems are 10-20 cm long and covered, and has small pubescent leaves that are calyx two-lipped, with upper lip three-toothed, stamens, and flowers in verticillasters in the ails of upper leaves (Mozaffarian, 2008). In traditional medicine, *D.kotschyi* is applied as a warm herbal medicine for rheumatoid diseases and stomach disorders treatment. Its effectiveness for headaches, congestion, and liver disorders has been described as well (Zargari, 1995). Moreover, Antihyperlipidemic (Sajjadi et al., 1998), anti-epimastigote (Saeidnia et al., 2004) and antivisceral (Golshani et al., 2004) effects are among the other properties reported for *D. kotschyi*. *D.kotschyi* herb is known as "Sama" in Lorestan locally and it is traditionally used in cooking meat and fish and dairy processing (Local People Interview) and there is a possibility that the use of this traditional herb could reduce the microbial load of the meat and dairy products.

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In the research that has been already conducted on *D.kotschyi* essential oil, it is made clear that the chemical compounds found in the essential oil of this species presents major changes under different climatic conditions (Morteza-Semnani and Saeedi, 2005; Saeidnia et al., 2007; Yaghmai and Taffazoli 1988). Since the mountainous Lorestan Province climate is different from the points the reports of which are already available, an so far the *D.kotschyi* chemotype oil grows in Lorestan mountains is not reported, and there is no history of research about the antioxidant, cytotoxic and antimicrobial effects of this plant, in this study while addressing the chemical compositions of *D.kotschyi* grown in Garrin Mountain and the comparison its compounds with *D.kotschyi* grown in other regions, the antioxidant, cytotoxic and antimicrobial activity of this species was studied.

Material and methods

Plant material

Flowering, aerial parts of wild *Dracocephalum kotschyi* Boiss were collected in July 2014 from Garin Mountain in the Lorestan province, west of Iran. The region's altitude was *ca*. 3300 m above the sea level. The plant material identification was done by Dr. Mehrnia, and a voucher specimen was deposited at the herbarium of the Lorestan Agricultural Research and Natural Resources Center, Khorramabad, Iran.

Isolation and analysis of the essential oil

A portion (300 g) of the dried and compeletly ground aerial parts of *D. kotschyi* was submitted to water distillation using a Clevenger-type apparatus (British type) for 3 h. The average yield of the extraction was 0.6% w /w according to the dry weight of the sample. The yellow oil of the plant was dried over dry sodium sulfate and stored at +4 ° C after filtration.

Analyses of the essential oil were performed by application of gas chromatography with flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). According to the property of the essential oil extract, the condition of GC or GC–MS was optimized. GC–FID analysis was conducted on a HP 6890 gas chromatograph equipped with a FID detector and an HP-5 fused silica capillary column (30 m×0.25 mm, film thickness 0.25 μm).

Helium (purity 99.999%) with flow rate of 0.9 ml/min was employed as the carrier gas. The column oven temperature was incresed from 50 ◦C (hold 1 min) to 240 ◦C (hold 10 min) with the rate of 5 ◦C/min. The injector and FID detector temperatures were aslo 230 and 300 ◦C, respectively. The injector was operated in the split less mode and programmed to return to the split mode after 1 min from the beginning of the run. Diluted samples (1/10 in ether, v/v) of 0.3 μL were injected manually, and the split ratio was adjusted to 50:1.

GC–MS analysis was carried out in an Agilent 6890 gas chromatograph interfaced with an Agilent 5973 MSD applying helium as the carrier gas (0.9 ml/min, and the same capillary column previously mentioned). The column temperature was raised from 50 (hold 3 min) to 180 ˚C at the rate of 5 ˚C/min and to 240 ˚C at 10 ˚C/min rate; and then was kept stable for an additional 20 min at 280 ◦C. The injector and detector temperatures were 200 and 280 ◦C, respectively. A 0.3-μl sample was injected using the split mode (split ratio 50:1). Oil components were analyzed and identified by comparison of MS fragmentation pattern with that of pure compounds, corresponding data in the literature (Adams 2007) and/or computer mass spectra libraries (Wiley 138K and NIST 98). For gaining the retention indices, a standard solution of *n*-alkanes (C6–C26) was used (Jennings and Shibamoto 1980). Also, the electronic integration of the FID peak areas without using any correction factors was employed for obtaining quantitative data.

Antimicrobial activity

Microbial strains

The antibacterial activity of the essential oils from *D. kotschyi* against Gram-positive and Gram-negative bacteria species was tested (provided by the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran). The studied Gram-positive bacteria were *Staphylococcus aureus* (ATCC 12600), *Staphylococcus epidermidis* (PTCC 1435), *Streptococcus agalactiae* (PTCC 1768), *Streptococcus mutans* (PTCC 1683), *Enterococcus faecalis* (ATCC 29219) and *Listeria monocytogenes* (ATCC 13932), and the group of Gram-negative bacteria including *Escherichia coli* (ATCC 11775), *Salmonella typhi* (PTCC 1609), *Salmonella paratyphi* A (PTCC 1230), *Salmonella enterica* (PTCC 1709), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603), obtained from Persian Type Culture Collection (PTCC), Iran and America Type Culture Collection (ATCC), were examined.

Disk Diffusion Assay

The protocols used in this study were conducted according to the guidelines of CLSI, formerly known as NCCLS (Wayne 2007) with slight modifications. In summary, each sterile Petri dish with a diameter of 9 cm was prepared with 20 ml of Mueller–Hinton medium. Then, 100 μL of a bacterial suspension (10^8 CFU/mL) was spread on

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the plates. After 5 min, a sterile filter paper disc (6 mm) containing 20 µL of *D. kotschyi* essential oil was placed on the surface of the plate. In order to accelerate essential oil diffusion into the agar, the plates were incubated at 4 °C for 1 h followed by incubation at 37 °C, for 24 to 48 h. The diameters of the inhibition zones (mm) were measured, including the diameter of the disks. Gentamycin (30 µg/disk) and vancomycin (30 µg/disk) served as positive controls for Gram-positive and Gram-negative, respectively. All tests were performed in triplicate.

Minimum inhibition concentration (MIC) and minimal bactericidal concentration (MBC)

Since it is an essential oil and not easily dissolved in Mueller-Hinton broth (MHB) nutrient medium, in order to dissolve the essential oil in the nutrient medium, first it is dissolved in the organic solvent DMSO and then added to the nutrient medium MHB located on a shaker (100 rpm) so that the oil is uniformly dissolved in all parts of the medium. Each ml of *D.kotschyi* oil weights 0.68 g. 300 ml oil is dissolved in 300 µl DMSO solution and then using a nutrient solution MHB, the volume is increased to 1000 µl and spin and vortex for 5 minutes (2000 rpm) so that a stock with a concentration of 20480 µg/ml is prepared and then by serial dilution in nutrient medium MHB the concentrations of 20 to 5120 µg/ml oil are prepared in the wells. The final DMSO concentration was 5% (v/v), and this solution was selected as a negative control.

96-well microtiter plates utilizing broth microdilution method was applied for the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations determination. In a typical procedure, the bacterial strains were cultured overnight at 37 °C on MHB, adjusted to a final density of 8 log CFU/mL, and used to inoculate (1-10) 96-well microtiter plates containing serial dilutions of the essential oils (5120-20 µg/ml) on MHB. A positive control containing the bacterial culture and broth without the plant oils was included in each test. The contents of each well were mixed on a plate shaker at 300 r/min for 30 seconds and then incubated at 37 C for 24 h, while *Streptococcus agalactiae* and *Streptococcus mutans* were incubated for 48 h. Then, 2,3,5-triphenyltetrazolium chloride (Sigma, T8877) was used for the visual indicator of bacterial growth. The MIC of the essential oils was regarded as the lowest concentration showing no growth.

All samples exhibiting no turbidity were sub-cultured; however, the lowest concentration from which the microorganisms did not recover was the minimal bactericidal concentration (MBC). Each experiment was performed in triplicate.

Antioxidant activity: Free radical scavenging Capacity

The free radical scavenging capacity of the essential oils and two positive controls, butylated hydroxytoluene (BHT; Sigma, W218405) and ascorbic acid (Sigma A4403), was assessed from bleaching the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Brand-Williams et al. (1995). In such process, various methanolic oil concentrations (5, 10, 20, 30, 40, 50, 60, 80 and 100 µg/ml) were mixed with the same volume of a 0.2 mM methanolic solution of DPPH (Sigma, D9132). After a 30-min incubation at room temperature, the absorbance was recorded at 517 nm using a UV/VIS spectrophotometer. The free radical scavenging capacity was calculated as follows:

% scavenging = 100 - (Abs sample - Abs control)/Abs DPPH × 100 %, where the Abs sample is the absorbance of the sample without DPPH. The oil concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentages against the oil concentrations. The assay was repeated three times.

Cytotoxicity assay

The human cervical carcinoma HeLa cell line (NCBI code No.: 115; ATCC No.: CCL-2) and the human normal healthy lymphocyte cell line (NCBI code No.: 124; ECACC No.: 91112124) were obtained from the Pasteur Institute, Tehran-Iran. The lymphocyte cells were grown in RPMI 1640 supplemented with 10% fetal calf serum and the HeLa cells were grown in RPMI 1640 supplemented with 10% FBS, 1% (w/v) glutamine, 100U/ml penicillin and 100 µg/ml streptomycin. They were cultured in a humid atmosphere at 37°C in 5% CO2.

The cytotoxicity assay detected the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] by mitochondrial dehydrogenase to the formazan blue product, which is a reflection of the normal mitochondrial functioning and cell viability (Lau et al., 2004). Briefly, the cells (5×10^4) were seeded in wells containing 100 µl of the RPMI medium supplemented with 10% FBS in a 96-well plate. After 24 hours of adhesion, different concentrations of the essential oils were added to triplicate wells over the range of 5120 to 20 µg/ml. After 2 days, 10 µl of MTT (5 mg/ml stock solution) was added and the plates were incubated for an additional 4 hours. The medium was discarded, and the formazan blue formed in the cells was dissolved with 100 µl of dimethyl sulphoxide (DMSO). Quantification of formazan was performed using an ELISA microplate reader (SLT, Austria) at 490 nm. The cell viability curves were calculated with regard to the control cells. Cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). All tests and analyses were run in triplicate, and the mean values were recorded.

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Statistical analysis

All experiments were repeated three times. The data were analysis was performed by SPSS computer software version 18 using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

Results and Discussion

Chemical Composition of the Essential Oils

Hydrodistillation process of the aerial parts of *D. kotschyi* resulted in formation of 0.16% (w/w) yellowish fragrant oil distillates. The Mass chromatogram of *D. kotschyi* essential oil is shown in Fig. 1 and chemical composition of the oils is listed in Table 1. The components were mentioned in order from their elution on the HP-5MS column. Forty three components were identified from the leaf essential oil of *D. kotschyi* composing 99.9% of the total oil. 11 of the components were monoterpene hydrocarbons (26.7%), 8 of them were oxygenated monoterpenes (43.28%), 15 were sesquiterpene hydrocarbons (22.4%), 7 were oxygenated sesquiterpenes (6.4%) and 2 were other components (1.1%). The monoterpene (70%) and sesquiterpene (29%) were the dominant forming groups of *D. kotschyi* essential oil. The main monoterpene hydrocarbons were α-pinene (10.34%), Limonene (6.95%), β-Myrcene (3.42%) and β-Pinene (2.18%). The major oxygenated monoterpenes were Geranial (12.08%), Geraniol acetate (10.27%), Geraniol (9.55%) and Neral (8.9%). While, α-Copaene (3.6%), trans-β-Farnesene (3.46%), Germacrene-D (3.38%), δ-Cadinene (2.33%) and trans-Caryophyllene (2.04%) were among the main sesquiterpene hydrocarbons. Representative oxygenated sesquiterpenes were vulgarol B (1.78%), α-Cadinol (1.33%) and β- Turmerone (1.32%).

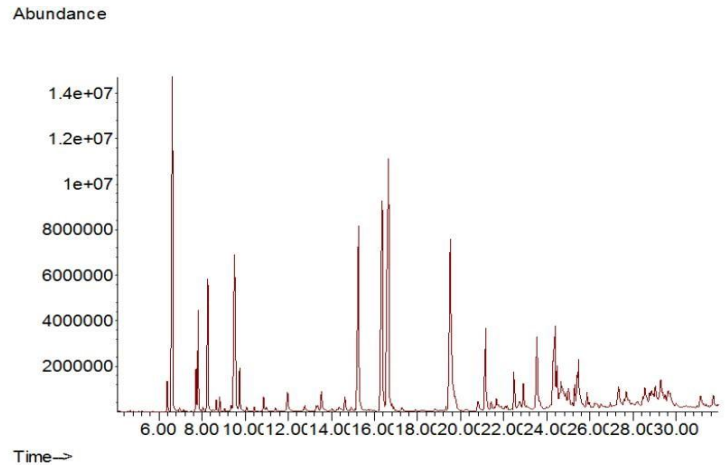

Figure1: *Dracocephalum kotschyi* essential oil chromatogram carried out using a gas chromatographmass spectrometry Agilent 6890 with a capillary column of HP-5MS.

Table 1: Chemical composition of the essential oils from the aerial parts of *D. kotschyi*.

Compounds	RI	Relative content (%)
Monoterpene Hydrocarbons		
α-Thujene	929	0.71
α-Pinene	935	10.34
Sabinene	975	1.01
β-Pinene	978	2.18
β-Myrcene	993	3.42
α-Phellandrene	1006	0.31
Δ-3-Carene		0.34
p-Cymene	1024	0.18
Limonene	1032	6.95
(Z)- β-ocimene	1038	1.13
ɤ-Terpinene	1057	0.15
Oxygenated Monoterpenes		
trans-Sabinene hydrate	1093	0.41
Linalool	1100	0.81
α-Terpineol	1192	0.54
Neral (citral b)	1231	8.90

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Geranial (Citral a)	1236	12.08
Geraniol	1258	9.55
Carvacrol	1297	0.72
Geranyl acetate	1365	10.27
Sesquiterpene Hydrocarbons		
α-Cubebene	1375	0.17
α-Copaene	1377	3.61
β- Bourbonene	1387	0.37
β- Elemene	1411	0.43
trans-Caryophyllene	1415	2.04
β-selinene	1436	0.76
trans-β-Farnesene	1452	3.46
Zingiberene	1476	0.93
Aromadendrene	1479	1.50
Germacrene-D	1481	3.38
β-Bisabolene	1504	1.16
δ-Cadinene	1537	2.33
α-Gurjunene	1557	1.20
valencene	1483	0.66
ɤ-Gurjunene	1587	0.43
Oxygenated Sesquiterpenes		
Caryophyllene oxide	1595	0.36
allo-Aromadendrene epoxide	1623	0.44
Isospathulenol	1642	0.41
α-Cadinol	1651	1.33
T-Muurolol	1660	0.79
β- Turmerone	1680	1.32
Vulgarol B	1747	1.78
Others		
Myristic acid	1763	0.65
Isopropyl myristate	1826	0.49
Total		99.94

Different studies have been made on the chemical composition of essential oils of *D. kotschyi* from other regions. A comparison of the main components of *D. kotschyi* essential oils from this research and other studies could be found in Table 2. In spite of some similarities in reported components of this study and other researches, there are significant quantitative and qualitative differences between the samples obtained different locations in Iran. These differences might be due to different climatic, seasonal and geographic conditions; harvest periods; and distillation techniques (Morteza-Semnani and Saeedi 2005).

Table 2: Comparison of the main components in *D. kotschyi* essential oils from other studies and this study.

Origin	Major constituents	References
Aladagh mountains (3000 m) near Bojnourd in the province of Khorasan in northeastern Iran	Citral (29), Caryophyllene (21.5), terpinyl acetate (12), and myrcene (7), Menthone (6.8)	Yaghmai and Tafazoli, 1988
Bojnord (Khorasan, in Northeast of Iran)	Limonene (14), verbenone (21.4), α-terpineol (8.8), perillyl alcohol (7.9) and Caryophyllene (7)	Golshani et al., 2004
June 2004 from the suburb of Sari, Mazandaran province, North of Iran	δ -3-carene (9.7%), limonene (9.2%), carvacrol (8.3%), 1,8-cineole (6.9%) and carvone (5.1%).	Morteza-Semnani and Saeedi, 2005
Muteh protected region in Isfahan province in May 2001.	α-pinene (10.5%), caryophyllene oxide (9.2%), terpinen- 4-ol (5.7%) and germacrene D (5.6%).	Javidnia et al., 2005
Tochal Mountain (3200 m) near to Tehran during the flowering stage in July 2002.	geranial (35.8%), limonene-10-al (26.6%), limonene (15.8%) and 1,1-dimethoxy decane (14.5%)	Saeidnia et al., 2007
Garin mountains (3200 m) near to Alshtar in the province of Lorestan, during the flowering stage in July 2014	Geranial (12.1), α-Pinene (10.34), Geraniol acetate (10.27), Geraniol (9.55), Neral (8.9) and Limonene (6.95)	Present work

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Antimicrobial activity

The antimicrobial behavior of *D. kotschy* essential oils against a panel of twelve bacteria strains was studied by agar disk diffusion and broth microdilution susceptibility assays. Bacteria selction was made based on their relevance as food contaminants. Their potency was qualitatively and quantitatively determined from the diameters of the inhibition zones (DDs), minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs), as shown in Table 3.

Table 3: Antimicrobial activity of *D. kotschy* essential oils.

Microorganisms	Essential oil			Gentamycin			Vancomycin		
	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC
S. aureus ATCC 12600	21	160	1280				30	16	128
S. epidermidis PTCC 1435	26	80	320				30	16	2
S. agalactiae PTCC 1768	30	80	1280				35	2	64
S.s mutans PTCC 1683	21	80	80				29	4	128
E. faecalis ATCC 29219	16	640	640				23	32	32
L. monocytogenes ATCC 13932	23	160	640				30	160	640
E. coli ATCC 11775	15	640	1280	25	8	64			
S. typhi PTCC 1609	31	80	320	34	5	5			
S. paratyphi A PTCC 1230	30	160	1280	32	1	16			
S. enterica PTCC 1709	22	160	320	30	2	2			
P. aeruginosa ATCC 27853	10	320	1280	31	5	5			
K. pneumoniae ATCC 700603	18	320	320	23	8	128			

DD: diameter of inhibition zone (mm) including disk diameter of 6 mm. MIC, MBC: values given as µg/ml (for the essential oils and antibiotics).

The observed antibacterial properties of the essential oils against various bacteria were different, ranging from strong antibacterial activity (inhibition zone > 20 mm) to moderate activity (inhibition zone < 12–20 mm) and even no inhibition (zone < 12 mm) (Orlanda and Nascimento 2015). Based on the width of the inhibition zone diameter, essential oils exhibits the strongest antibacterial activity against most Gram-positive organisms (except for *E. faecalis*) and a few Gram-negative organisms, including *S. typhi*, *S. paratyphi* and *Salmonella enterica* ($p \leq 0.01$). The weakest antibacterial activity was observed against *P. aeruginosa*, with a 10.40 ± 0.05 mm zone of inhibition ($p \leq 0.01$).

The results of broth micro-dilution method antimicrobial test are listed in Table 3. *D. kotschy* essential oils showed antimicrobial activity against all of the tested strains, with inhibition values varying from 80.0 to 460.0 µg/ml for MIC and 80.0 to 1280.0 µg/ml for MBC in bacteria.

Table 3 shows that the oils in this study act as potential antimicrobial agents since the required Gram-positive organisms corresponding to each MIC were low (from 80 to 160 µg/ml, except for the standard strain *E. faecalis* which needs 640 µg/ml to become sensitive to the oils). Although the essential oils presented a higher MIC for *E. coli* at 640 µg/ml, the analyzed Gram-negative bacteria exhibit strong activity for the essential oils.

The antimicrobial activity of the essential oils could be attributed to the presence of mostly active compounds, such as Geranial (Maksimović et al. 2008; Seth et al., 2012), α-pinene (Dorman and Deans, 2000), Geraniol acetate, Geraniol (Duarte et al. 2007; Singh et al., 2012), Neral (Maksimović et al. 2008; Sartoratto et al. 2004) and Limonene (Inouye et al., 2001). moreover, the components such as trans-Caryophyllene, Germacrene-D, δ-Cadinene, β-Pinene, β-Myrcene and Sabinene which were found in lower levels, could also contribute to the antimicrobial activity of the oils (Aguiar et al. 2013; Dorman and Deans 2000; Oztürk et al. 2009). In fact, the synergistic effects of the diversity between the major and minor constituents present in the essential oils should be taken into consideration in accounting for their biological activity.

Overall, in comparison with Gram-negative bacteria, the antimicrobial effects of *D. kotschy* essential oils were stronger against Gram-positive bacteria which is in accordance with a general observation derived from studies with essential oils from many other plant species (Ballester-Costa et al., 2013; Orlanda and Nascimento, 2015; Shakeri et al., 2014). This generally higher resistance of Gram-negative bacteria could be associated to their outer phospholipidic membrane, which is almost impermeable toward lipophilic compounds (Nikaido and Vaara, 1985). Lack of this barrier in Gram-positive bacteria makes the direct contact of the hydrophobic components of the essential oils with the phospholipid bilayer of the cell membrane possible, such direct contact results in either an increase of ion permeability and leakage of vital intracellular constituents or failure of bacterial enzyme systems (Wendakoon and Sakaguchi, 1993).

Although tests on foods are necessary, the present study shows the potential of *D. kotschy* essential oils as an alternative to traditional food preservatives which removes or reduces the growth of important food borne pathogens and spoilage bacteria, therefore contributes in e food safety and shelf life enhancement.

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Antioxidant activity and Cytotoxicity

The stable DPPH free radical has been widely approved as a tool for the free radical-scavenging activity assessment in antioxidants (Nagai et al., 2003). In the DPPH test, the antioxidants managed to reduce the stable DPPH radical to yellow-colored diphenylpricryhydrazine. The effects of antioxidants on DPPH radical scavenging are attributed to their hydrogen-donating ability (Ye et al., 2013). In this study, an *in vitro* examination of the free radical scavenging activity of the essential oils is presented. The essential oils showed the highest reduction ability with an IC_{50} of 49.2 µg/ml. BHT and ascorbic acid were employed as positive controls that exhibited antioxidant activity, with IC50 values of 28.7 µg/ml and 87.3 µg/ml, respectively.

The high antioxidant activity of *D. kotschy* essential oils can be attributed to their richness in monoterpenes (70%), especially oxygenated monoterpenes (43%). Previous studies also revealed that essential oils with high percentage of monoterpenes (Ghasemi Pirbalouti et al., 2014; Luís, et al., 2016) exhibit antioxidant activities, which is in good agreement with our results. The current results suggest that synergistic activities of multiform unsaturated compounds of essential oils can be responsible for their antioxidant activity.

D. kotschyi showed good cytotoxic action towards the human tumor cell line. Based on the cytotoxic activity obtained from MTT assay, the IC_{50} values for HeLa and lymphocyte cells were calculated to be 26.4 µg/ml and 4266.7 µg/ml, respectively. The IC_{50} showed higher cytotoxicity of the oil towards the human tumor cell line in comparison with healthy human cells requirement.

The fact that the essential oil of a plant at the concentrations that is has antibacterial properties has no damaging effect on the Lymphocyte cells is accepted as an indicator to measure the safety in oil consumption in foods (Taherkhani, 2014).These results also suggest lower adverse side effects of the *D. kotschy* essential oils.

Geranial (12.08%) and Neral (8.9%) composed over twenty percent of the *D. kotschyi* oil. Neral and geranial are the two stereoisomers of citral. Geranial (also known as citral a) is an (*E*)-isomer and neral (also known as citral b) has a (Z)- configuration. Studies showed anticancer properties of citral (Dubey et al., 1997). Citral was reported to be cytotoxic to P388 mouse leukemia cells at an IC_{50} value of 7.1 µg/ml.

Conclusion

The present study provides, for the first time, important data on the biological behavior of *D. kotschyi* oils. The essential oils in *D. kotschyi* effectively inhibit the growth of all tested food-borne pathogenic bacteria. Monoterpene compounds are predominant components of *D. kotschyi* essential oils. Fractionation allows the separation of oxygenated and non-oxygenated monoterpenes. The high antioxidant activities of *D. kotschyi* essential oils could be partially due to the high presence of monoterpene compounds in its chemical compositions. Natural source-derived safe antioxidants prevent oxidative deterioration of foods and protect the living cells from oxidative damage. The IC50 shows far higher cytotoxicity of *D. kotschyi* oils toward the human tumor cell line when compared with the amount the requirement for healthy human cells. These results confirm the low adverse effects of the oils. The growing tendency of current consumers to maintain a healthy lifestyle resulted in their greater concerns about high quality, natural and safe food products. This has made application of essential oils as natural antimicrobial agents an attractive approach in the field of food preservation. This study showed very positive results indicating high potential of Semsa essential oils for applications in food products; however, more experiments, including elucidation of the mechanism of action and in vivo tests, are also required to further support the advantages and safety of *D. kotschyi* essential oils.

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Conflict of Interest

The authors state that they have no conflict of interest.

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