Extraction of essential oil from *Dracocephalum kotschyi* Boiss. (Lamiaceae), identification of two active compounds and evaluation of the antimicrobial properties

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Abstract

Ethnopharmacological relevance: Dracocephalum kotschyi is a medicinal plant widely used in traditional medicine to treat pain, fever, inflammation, and seizures.

Aim of the study: Due to the importance of this plant and the well-known antibacterial activity of essential oils, the aim of the present study was to investigate the composition of essential oil and evaluate the antimicrobial activity of its main active compounds.

Materials and methods: In order to test its possible application at industrial level the oil was extract from the cultivated and wild plants. The epigean parts were collected in June 2018 from the same region of Daran (Isfahan, Iran). The extraction of essential oil was carried out using a Clevenger apparatus. The composition of the essential oil was assayed by using a gas chromatography/mass spectroscopy apparatus (GC/MS).

Results: Results showed that the predominant compounds of essential oil of cultivated plants were α-pinene (13.66%), (E)-citral (12.89%), neral (11.25%), methyl geranate (8.66%), limonene (8.33%), campholenal (6.22%) and geraniol (5.69%), while those found in naturally grown plants were two main compounds: cyclohexylallene (52.63%) and limonene (35.88%). The antimicrobial properties of the plant were determined against 12 strains of microorganism by evaluating inhibition halo, minimum

inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The highest inhibition halo for both oils from cultivated and wild plants was obtained against *Aspergillus brasiliensis*. The MIC value against Gram-positive *Bacillus subtilis* was 31.25 μg/ml, it was the lowest value provided by the essential oil obtained from the cultivated sample, the MIC was significantly lower than that obtained by treating the same strain with Rifampin. On the other hand, *Candida albicans* had the highest sensitivity (MIC value of 31.25 μg/ml) for the essential oil obtained from wild plants as the inhibitory concentration was lower than that obtained treating the yeast with Nystatin.

Conclusions: Therefore, according to the results of the present study, the use of the essential oil obtained from *D. kotschyi* can be used to protect food and to treat microbial infections.

Key words: Essential oil, GC/MS analysis, Antimicrobial activity, Limonene, *Dracocephalum*, Irano-Turanian vascular flora

1. Introduction

For many years plant-derived remedies have been widely used for the treatment of different diseases and in some cases they were considered the sole remedy capable of counteracting particular infections. More recently, formulations derived from them have been tested and used in the pharmaceutical industry (Bakkali et al., 2008). The use of medicinal and aromatic herbs in the treatment of infectious diseases dates back thousands of years. Many of the secondary metabolites of these plants have been shown to have important biological activities that are desperately needed even now (Brooks and Brooks, 2014). Indeed, the uncontrolled use of antibiotics has been the main cause of the multidrug-resistance of a wide variety of Gram-negative bacilli and Gram-positive cocci such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Staphylococcus*, and *Enterococcus*. The failure of antibiotics causes problems in the treatment of common infections, and encouraged the scientific community to identify and test new and safe antimicrobial molecules of natural origin, especially plant metabolites (e.g. Oussalah et al., 2007; Mitić et al., 2018). Plant derived antimicrobial compounds capable of killing bacteria with a different mechanism than antibiotics, may represent a valid approach especially for the treatment of infections caused by resistant microbe strains (Eloff, 1999).

In this regard, essential oils (hereafter EOs) have attracted special attention due to their complex content of volatile organic compounds, which are synthesized in the aromatic vascular plants as defence mechanism to exert antifungal, anti-parasitic, antiviral and antibacterial activities (Raut and Karuppayil, 2014). Likewise, they can address similar activities in human body (Andrade et al., 2013; Nafis et al., 2019) and they are considered an available source of chemical diversity to be used for a wide range of infectious diseases even against chemical-resistant strains (Andrade-Ochoa et al., 2015) and with low side effects (Al-Sayed and Abdel Daim, 2018). Differently to a single antibiotic, the EOs are a complex mixture of phytochemicals containing different antibacterial compounds, which may affect different parts of the bacteria, thus addressing a large protection and reducing the resistance of bacteria. Many compounds have been shown to be effective in inhibiting the growth and proliferation of bacteria (Hemaiswarya et al., 2008). In addition, some EOs, especially that containing limonene, can counteract or control the formation of free radicals and inflammation, suggesting their potential use as adjuvant in inflammation (Xiang et al., 2017). Considering their chemical and biological diversity, they have been tested to be used in food, pharmaceutical, agricultural and cosmetic industries (Sarikurkcu et al., 2018).

The Lamiaceae Martinov is one of the largest plant families in the world (~252 genera and 6700 taxa) and has the main differentiation center in the Mediterranean and Irano-Turanian biogeographic regions (Cantino et al. 1992, 1997; Kadereit 2004; Bendiksby et al. 2011). The genus *Dracocephalum*, known in the Persian language as “franjmoshk” or “baderekhashboo”, is one of the most important of Lamiaceae, comprising 71 species of which 11 are autochthonous of Iran and five (*D. ghahremanii* Jamzad, *D. kotschyi* Boiss., *D. oligadenium* Bornm. & Gauba, *D. polychaetum* Bornm. and *D. surmandinum* Rech. f.) are endemic (Jamzad, 2012). *Dracocephalum kotschyi* is a half-shrub, 10-20 cm tall (Rechinger, 1982), considered as one of the most endangered endemic species in Iran (Jalili and Jamzad, 1999). In traditional Iranian medicine *D. kotschyi* has been used to treat pain, fever, inflammation, and seizures, the boiled plant was used to relieve rheumatic pain and heal wounds, moreover it seems to play a positive effect in strengthening the immune system (Sajjadi et al., 1998). The whole plant (both vegetative and reproductive organs) contains a very aromatic, penetrating, and fragrant essential oil (Batuli, 2001). Two monoterpene glycosides are considered the most important

bioactives compounds of this plant, seven terpenoids and phytosterols were isolated as well and tested as analgesic in mice (Golshani et al., 2004).

Previous studies have shown that some species of *Dracocephalum* have antibacterial, antitussive, anti-diarrhea, antioxidant, anticancerous, anti-inflammatory, antidiabetic and soothing properties (Amin, 1992; Heydari et al., 2019; and Moradi et al., 2020). The health-promoting properties of EO of *D. kotschyi* collected from natural populations, are due to its main compounds such as limonene, carvacrol, methyl geranate, germinal, α-pinene, γ-terpinene, perilla aldehyde, eucalyptol, caryophyllene oxide, 1,8-cineole, verbenone, perillyl alcohol, neral and geranyl acetate (Golparvar et al., 2017; Najafpour Navai and Mirza, 2007; Ghanbari Hemasi et al., 2011; Sani et al., 2017; Golshani et al., 2004; Morteza-Semnani And Saeedi, 2005, Javidnia et al., 2005, Sonboli et al., 2018; Samadi et al., 2018; and Fallah et al., 2020). In the cultivated plants, limonene germanium (14.3%) has been reported as the main constituent of its EO (Najafpour Navai and Mirza, 2007). In another study α-pinene, neral, geranial, methyl geranate and limonene were identified as the major constituents (Fallah et al., 2020). The antimicrobial properties of this plant have been reported in various studies (e.g. Ashrafi, 2017; Shakib et al., 2018).

Considering the importance of *D. kotschyi* in traditional medicine of Iran, in the present study, two different EO were obtained from the cultivated and wild plants and their composition was assayed along with the antimicrobial activities.

2. Materials and methods

2.1. Plant material

To sample plant, in June 2018, coinciding with the flowering, three points in each site (Meydanak and Dezdak) from Daran region of Iran were randomly selected. In each point aerial parts of *D. kotschyi* from different plants (about 50 plants in each site) were collected (Table 1). The specimens were transferred to the laboratory after being harvested. Two samples of the whole plant were identified and after exsiccation recorded in the University of Kashan Herbarium.

Table1. Geographic coordinates of sites from where the plants were collected.

Site	Longitude E (WGS 84)	Latitude N (WGS 84)	Mean elevation (m a.s.l.)
Cultivated (Meydanak)	50° 33′ 11.1″	33° 02′ 40.3″	2383
Wild (Dezdak)	50° 16′ 40.9″	32° 56′ 27.4″	2895

2.2. Essential oil extraction

After complete drying, the samples were grinded to have small particles and facilitate the extraction process. 70 g of each sample have been subjected to extraction by means of water distillation using a Clevenger apparatus for three hours as recommended by the European Pharmacopoeia (Anonymous, 1996). The weight of EO collected after sodium sulphate dehydration was calculated accurately and the obtained EO was stored in the dark at 4°C until further use (). EO yield was calculated based on weight percent (w/w). This process was repeated three times for each plant. Then, the yield of three repetitions of EO from each site was reported as mean ± standard deviation.

2.3. Essential oils analysis by GC/MS method

The main active compounds of the EO have been determined using a GC-MS apparatus. A chromatograph (model 6890) coupled with an Agilent Mass Spectrometer (model N-5973), an HP-5MS Capillary Column with 5% Methylphenylsiloxane Static Phase (Length 30 m, Internal Diameter 0.25 mm, Layer Static Thickness 0.25 µm) and a ionization energy of 70 eV has been used for the qualitative identification of compounds. Temperature was regulated as follows: 60°C at the beginning and then improved, at a rate of 3°C, up to 246°C. The injector and detector temperature were maintained at 250°C, the injection volume was 1 µl with 1.50 split and the helium carrier gas at a flow rate of 1.5 ml/min. The chemical compounds of the EOs were identified based on the analysis of the chromatograms of each EO by means of comparison between the retention indices and mass spectral data of each peak of the samples and those of standards of n-alkane mixtures (C8-C20) or reported in a computer library (Wiley-14 and NIST-14 Mass Spectral Library) or in literature (Adams, 2007).

2.4. Tested microorganisms

Twelve microorganisms were used to evaluate the antimicrobial and antifungal activity of the EOs, which were provided by the Iranian Research Organization for Science and Technology (IROST). Three Gram-positive bacteria, i.e. *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29737) and *Bacillus subtilis* (ATCC 6633), six Gram-negative bacteria, i.e. *Klebsiella pneumonia* (ATCC 10031), *Shigella dysenteriae* (PTCC 1188), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella paratyphi-A serotype* (ATCC 5702), *Proteus vulgaris* (PTCC 1182) and *Escherichia coli* (ATCC 10536), and three fungal strains, i.e. *Aspergillus niger* (ATCC 16404), *Aspergillus brasiliensis* (PTCC 5011) and *Candida albicans* (ATCC 10231), have been used. Bacterial strains were cultured overnight at 37 °C in nutrient agar and fungi were cultured overnight at 30 °C in Sabouraud dextrose agar.

2.5. Agar well-diffusion method

This procedure was performed according to CLSI (Clinical and Laboratory Standard Institute) standards (CLSI, 2012). Plates containing containing Müller Hinton agar medium for bacteria and sabouraud dextrose agar for fungi were prepared, then 100 µl of bacterial suspensions with a half-McFarland turbidity were cultured. Wells with a diameter of 6 mm and a thickness of 4 mm were created in culture media. The EOs were dissolved in dimethyl sulfoxide at a concentration of 30 mg/ml. 10 µl (equivalent to 300 µg) of each EO was poured into the wells. The plates were incubated at 37°C for 24 h for bacterial strains and 48 h and 72 h at 30°C for yeast and fungi and the antimicrobial activity of samples was measured for each microorganism measuring the diameter of the inhibition zone with the antibiogram ruler (in millimetres). Results were calculated as mean values ± standard deviations of three replicates for each strain. Dimethyl sulfoxide was used as negative control while gentamicin and rifampin for bacteria and nystatin for fungi were used to compare the inhibitory effect of EOs.

2.6. Minimum inhibitory concentration (MIC)

The minimum concentration capable of inhibiting the growth of the tested microorganisms was calculated by using the broth microdilution method (CLSI, 2012) and agar dilution assay (Gul et al., 2002). The EOs (2000 µg/mL) were dissolved in a mixture of dimethyl sulfoxide and broth medium to reach the final concentrations (1000, 500, 250, 125, 62.5, 31.25 and 15.63 µg/mL) used in the tests. For this purpose, sterile 96-well plates were used. Each well was filled with 95 µl of brain heart infusion (BHI) broth medium for bacteria and sabouraud dextrose (SD) broth medium for yeast, 5 µl of bacterial suspension (0.5 McFarland dilution), and 100 µl of the EOs at different concentrations, and then incubated at 37°C for 24 h for bacterial strains and 48 h and at 30°C for yeast. To determine the MIC of fungal strains, first, appropriate amounts of EO with different concentrations were added to sterile liquid agar dextrose agar medium containing Tween 20 (50% by v/v). The culture medium was inoculated with 5 µg (spore/mL10^{4}) of fungal isolates.

Culture medium was used instead of EO for negative control and gentamicin and rifampin antibiotic powder for bacteria and nystatin antibiotic powder for yeast and fungi were used for positive control instead of EO. The inoculation plates were heated at 30° C for 72 h. The experiment was repeated three times for each EO sample and the MIC was the lowest concentration of an antimicrobial capable of inhibiting the visible growth (absence of turbidity).

2.7. Minimum bactericidal concentration (MBC)

The minimum concentration capable of killing the tested microorganisms was evaluated by treating the selected strains with different concentration of EOs, as described above. After 24 hours of incubation 5 µl of the content of each well were inoculated with neutrin agar medium and incubated at 37°C for another 24 h. Finally, the colony-forming units (CFUs) were enumerated. The MBC was the lowest concentration able to effectively kill the microorganisms.

2.8. Statistical analysis

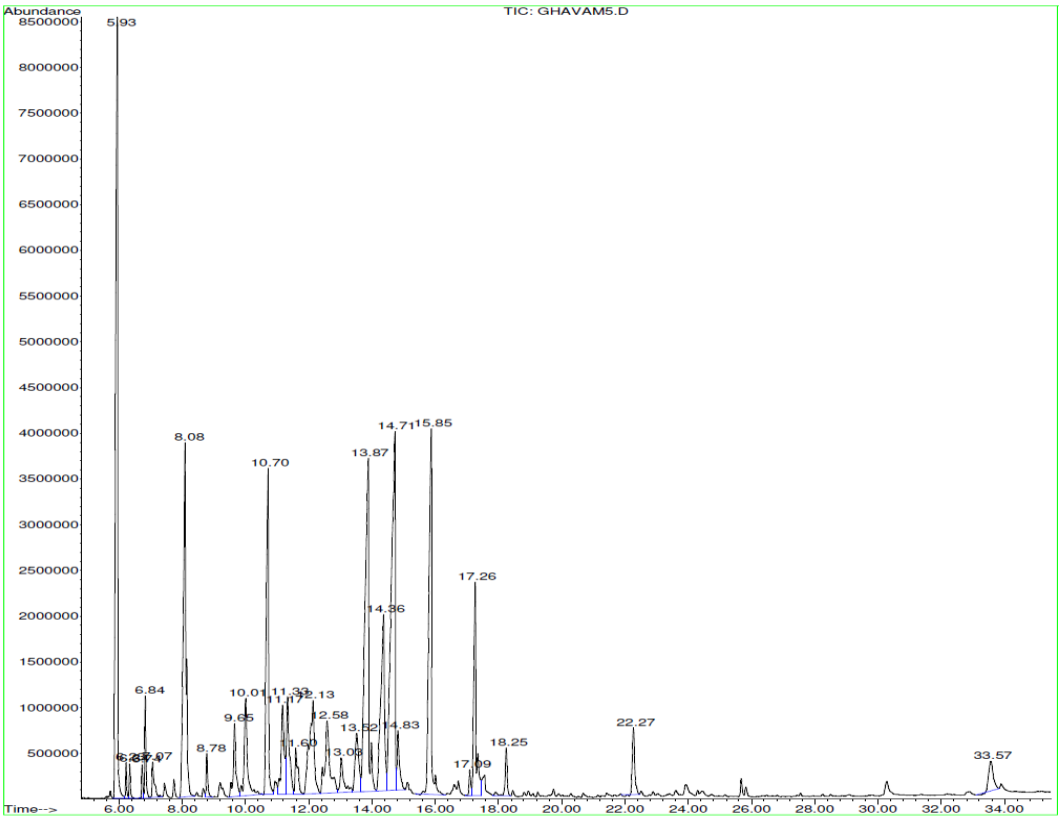
The statistical analysis was performed using SPSS software. First, the normality of the statistical variables was investigated using a Kolmogorov-Smirnov test, and after ensuring the normality of the

data, the variance of the data was analysed using One-Way Analysis of Variance with a probability level was 1 % error was performed

3. Results and discussion

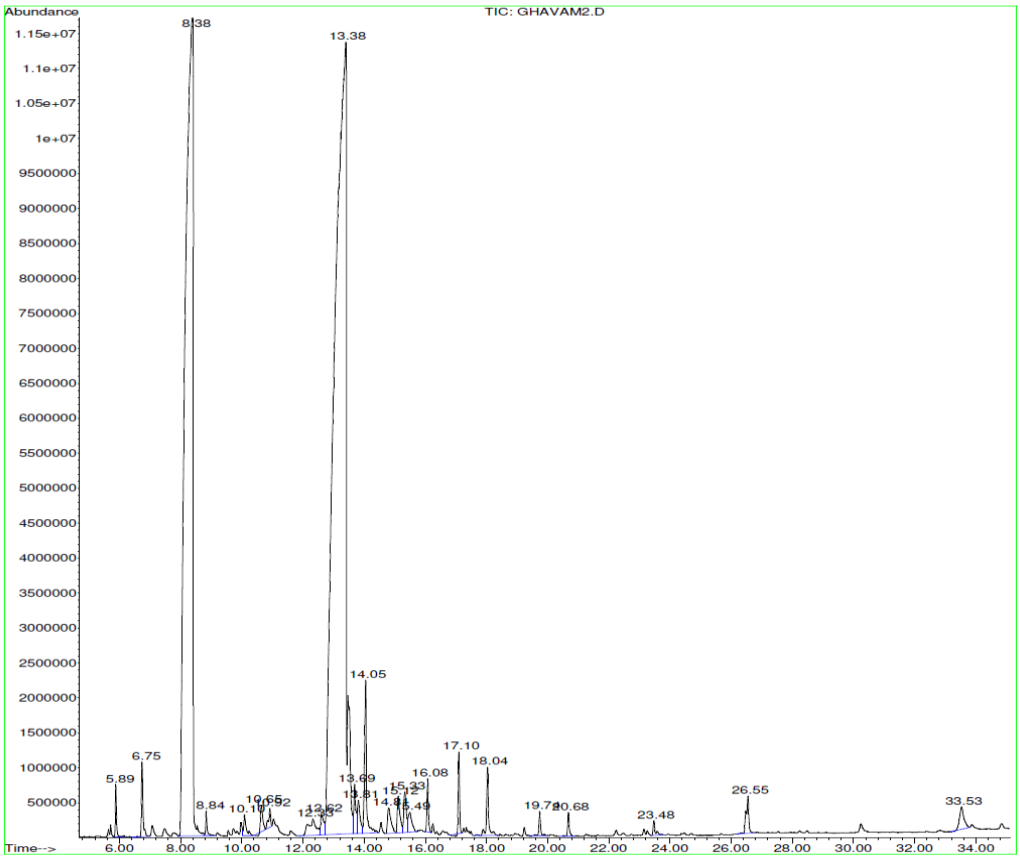
3.1. Chemical composition of the essential oils

The EOs obtained by using cultivated or wild plants of *D. kotschyi* were yellow. The extraction yield was 0.2% (w/w) from the cultivated plant, and 0.97% (w/w) from the naturally grown one. The results are encouraging as the yields were higher than those found by Najafpour Navaii and Mirza (2007) for the cultivated plants and by Ashrafi et al. (2017), Ghanbari Hemasi (2011) and Najafpour Navaei and Mirza (2007) for the wild samples. Previous studies have shown that wild ecotypes are usually more resistant to adverse environmental conditions, pests and diseases and their extractive products are richest in secondary metabolites (Omidbeigy, 1995). The chemical composition of the EOs evaluated by GC/MC system, revealed the presence of 28 active compounds in the oil obtained from the cultivated plants corresponding to 100% of the total mass and only 21 active compounds (corresponding 99.8% of the total mass) in the EO obtained from the wild samples (Table 2, fig 1 and 2). Differently, the number of active compounds found in the EO of *D. kotschyi* by Najafpour Navaei et al. (2007), was 23 from cultivated and 27 from natural ones. These differences can be related to the habitat and plant cultivation conditions. Accordingly, results showed that only six compounds were common in both samples and a large difference regarding the type of compounds was detected between the EOs obtained from the cultivated and naturally grown plants. The influence of the habitat conditions on the secondary metabolites produced by different plants has been studied, and all results obtained agreed that the role of habitat significantly affects the type and amount of secondary metabolites (Sirostava and Shim, 2002; Zubayde et al., 2005; Walker et al., 2001; Donor et al., 2009 and Zargoosh et al., 2019), supporting the results of the present study. Further, the soil composition and the geographical position are other key factors capable of affecting the EO composition Alcaraz-Melendez et al., 2007).



204

205 **Fig. 1.** GC/MS chromatogram of essential oil obtained from cultivated *D. kotschyi*.



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207 **Fig. 2**. GC/MS chromatogram of essential oil obtained from naturally grown *D. kotschyi*.

208 The ANOVA results showed that there was a significant difference between the mean of the
209 components obtained for essential oils of cultivated and wild plants ($P \leq 0.05$).

210 Oxygenated monoterpenes (51.68%) were the main compounds of the EO from cultivated plants,
211 followed by monoterpenes hydrocarbons (28.56%), according to Ashraf (2017) results. Among all α-
212 pinene (13.66%), (E)-citral (12.89%), neral (11.25%), methyl geranate (8.66%), limonene (8.33%), α
213 campholenal (6.22%) and geraniol (5.69%) were the most abundant. Different results, in terms of type
214 and amount of active compounds, were obtained by Najafpour Navaei and Mirza (2007) and by Falah
215 et al. (2020). The first found myrthenol (30.1%), limonene (23.6%), geranial (14.3%), neral (9.3%),
216 and methyl geranate (8.7%) as the main component contained in the EO from cultivated plants, while
217 the main components of the EO cultivated in Fereidoon Shahr (described in the second study) were
218 neral (21.03 to 28.37%), geranial (17.32 to 23.43%), geranyl acetate (7.93 to 20.82%), α-pinene (11 to
219 12.07%), limonene (3.90 to 10.49%) and methyl geranate (4.57 to 6.20%). By comparing previous

220 results with those obtained in this study, it can be stated that methyl geranat is present in both samples
221 in a similar amount and the main compounds found in the present study in the EO from cultivated
222 plants of *D. kotschyi* are the same than that described in the study of Falah et al. (2020), probably
223 because of the similarity of the habitat.
224 Cyclohexylallene (52.63%) and limonene (35.88%), were the main compounds of the EO obtained
225 from the wild plants, and the other compounds were less than 2%, which did not correspond to those
226 detected by Gholshani et al. (2004). Differently to previous studies on secondary metabolites of *D.*
227 *kotschyi*, for the first time in this study, cyclohexylallene has been identified as main component of the
228 EO obtained from wild plants. Further, the amount of limonene detected in the EO prepared in
229 previous studies was always lower: 14.04% (Golshani et al., 2004); 9.2% (Morteza-Semnaniand
230 Saeedi, 2005); 15.8% (Saeidnia et al., 2007); 29.1% (Najafpour Navai and Mirza 2007); 8.75% (
231 Ghanbari Hemsi et al., 2011); 7.2 % (Mansif Esfahani, 2007); 23.56% (Gulparvar et al., 2016); 0.41%
232 (Nejad-Sadeghietal et al., 2015); 6.95% (Shakib et al., 2018); 6.95% (Ashrafi et al., 2017); 8.0%
233 (Sonboli et al., 2018); 15.23% (Sodeifian et al., 2016) and 23.6% (Falah et al., 2020). The high yields
234 and the unique EO composition of the present sample underline the peculiar characteristics and
235 properties of *D. kotschyi* from the Daran region of Iran probably connected with its growth conditions.
236 Indeed, many external sources (geographical location, time of collection, age of the plant, soil
237 composition, etc.) and/or internal factors (genetic characteristics) can affect the production of
238 secondary metabolites and the consequent composition of resulted EOs (Elhidar et al., 2019; Hajdari et
239 al., 2016)). The high amount of limonene can ensure optimal biological properties and industrial
240 applications of this oil. Indeed, limonene is used in beverages, cosmetics, flavours and has been
241 evaluated in alternative medicine, since their antimicrobial, anti-cancer and anti-parasitic properties
242 (Erasto and Viljoen, 2008).

243 **Table 2.** Chemical compositions of the EO obtained from cultivated and wild plants of *D. kotschyi*.

No	Compound (%)	RI[#]	Relative percentage		Molecular formula
			Cultivated	Natural	

1	α-Pinene	873.399	13.66±0.00[a]	0.35±0.00[l]	$C_{10}H_{16}$
2	Camphene	877.684	0.36±0.00[s]	-	$C_{10}H_{16}$
3	Verbenene	893.596	0.37±0.00[s]	-	$C_{10}H_{14}$
4	Sabinene	908.609	0.35±0.00[s]	0.68±0.01[g]	$C_{10}H_{16}$
5	β-Pinene	912.251	1.11±0.00[n]	-	$C_{10}H_{16}$
6	β -Myrcene	919.867	0.68±0.01[q]	-	$C_{10}H_{16}$
7	Limonene	953.311	8.33±0.02[d]	35.88±0.01[b]	$C_{10}H_{16}$
8	γ-Terpinene	976.490	0.57±0.00[r]	0.20±0.00[m]	$C_{10}H_{16}$
9	Fencholenic aldehyde	1004.232	1.68±0.00[k]	-	$C_{10}H_{16}O$
10	Linalool	1013.492	2.67±0.02[i]	-	$C_{10}H_{18}O$
11	Santene	1015.873	-	1.44±0.01[d]	C_9H_{14}
12	trans-p-2,8-Menthadien-1-ol	1031.746	-	0.31±0.02[l]	$C_{10}H_{16}O$
13	α-Campholenal	1032.010	6.22±0.01[e]	-	$C_{10}H_{16}O$
14	trans-Limonene oxide	1037.830	-	0.22±0.00[m]	$C_{10}H_{16}O$
15	Camphor	1044.444	2.36±0.03[i]	-	$C_{10}H_{16}O$
16	1,5-Dimethylbicyclo(3.2.1)octan-8-ol	1048.677	2.51±0.02[i]	-	$C_{10}H_{18}O$
17	cis-Limonene oxide	1055.820	1.13±0.01[n]	-	$C_{10}H_{16}O$
18	(-)-Terpinen-4-ol	1069.841	4.08±0.01[g]	-	$C_{10}H_{18}O$
19	1-Adamantanecarbonitrile	1075.132	-	0.68±0.00[g]	$C_{11}H_{15}N$
20	(+)-trans-Isolimonene	1082.010	3.13±0.01[h]	-	$C_{10}H_{16}$
21	Cyclohexene, 1,5,5-trimethyl-3-methylene-	1082.804	-	0.36±0.01[l]	$C_{10}H_{16}$
22	Verbenone	1093.915	1.08±0.02[n]	-	$C_{10}H_{14}O$

23	Cyclohexylallene	1102.884	-	52.63±0.03[a]	C_9H_{14}
24	Carveol	1106.009	1.93±0.01[j]		$C_{10}H_{16}O$
26	cis-Carveol	1112.980	-	0.50±0.00[i]	$C_{10}H_{16}O$
27	Z-Citral=Neral	1114.423	11.25±0.00[c]	-	$C_{10}H_{16}O$
28	(-)-Carvone	1118.75	-	1.70±0.00[e]	$C_{10}H_{14}O$
29	Geraniol	1126.201	5.69±0.02[f]	-	$C_{10}H_{18}O$
30	(E)-Citral	1134.615	12.89±0.02[b]	-	$C_{10}H_{16}O$
31	Cyclooctene, 3-(1-methylethenyl)-	1137.019	-	0.50±0.00[i]	$C_{11}H_{18}$
32	α-Fenchyl acetate	1137.5	1.21±0.00[l]		$C_{12}H_{20}O_2$
33	Limonen-10-ol	1149.519	-	1.11±0.00[e]	$C_{10}H_{16}O$
34	P-Menth-1-en-9-ol	1153.365	-	0.41±0.00[k]	$C_{10}H_{18}O$
35	Methyl geranate	1162.259	8.66±0.02[d]	-	$C_{11}H_{18}O_2$
36	Perilla alcohol	1167.548	-	0.46±0.01[j]	$C_{10}H_{16}O$
37	α-Copaene	1192.067	0.38±0.02	0.59±0.02[h]	$C_{15}H_{24}$
38	Geranyl acetate	1195.913	4.36±0.03[g]		$C_{12}H_{20}O_2$
39	Limonen-10-yl acetate	1214.454	-	0.77±0.04[f]	$C_{12}H_{18}O_2$
40	trans-Caryophyllene	1219.194	0.89±0.02[p]	-	$C_{15}H_{24}$
41	Germacrene D	1254.739	-	0.25±0.03[m]	$C_{15}H_{24}$
42	δ-Cadinene	1277.251	-	0.22±0.01[m]	$C_{15}H_{24}$
43	(-)-Caryophyllene oxide	1315.012	1.29±0.00[l]	-	$C_{15}H_{24}O$
44	trans-Oleic acid	1600.554	1.16±0.01[m]	0.54±0.00[h]	$C_{18}H_{34}O_2$
	Total		100	99.8	

Monoterpenes hydrocarbons	28.56	37.47
Oxygenated monoterpenes	51.68	4.3
Sesquiterpenes hydrocarbons	1.27	1.06
Oxygenated sesquiterpenes	1.29	-
Others	17.07	36.97

Retention indices (RIs) relative to n-alkanes (C6–C40) on the same methyl silicone capillary column. Values with different letters are statistically different (Duncan, p≤0.05).

3.2. Antimicrobial activity

The antibacterial and antifungal activities of the EOs obtained from cultivated and wild plants have been evaluated (Table 3). The ANOVA results showed that there was a significant difference between the mean inhibition halos obtained by treating different microorganisms with the essential oil of cultivated and wild plants and antibiotics ($P \leq 0.05$). The highest inhibition halo belonged to *Aspergillus brasiliensis*, which was more susceptible to the EO obtained from naturally grown plants (26.00 ± 0.50 mm) than that obtained from cultivated samples (25.00 ± 0.50 mm). Any significant difference was detected by treating the same bacteria with nystatin used as control, as the inhibition halo was similar (30 ± 0.00 mm). On the contrary the lowest inhibition of growth (inhibition halo 9.00 ± 0.00 mm) was observed for the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus epidermidis*, irrespective of the type of EO tested. Differently, Ashraf et al. (2017), observed the highest and the lowest inhibition halos for *Salmonella typhi* (31 mm) and the lowest for *Pseudomonas aeruginosa* (10 mm).

Moreover, the naturally grown samples had an inhibitory effect on the yeast *Candida albicans* (inhibition halo 16.33±0.58 mm), while the essential oil obtained from cultivated samples, was slightly active against *Aspergillus niger* (inhibition halo 15.00 ± 0.50 mm). Both essential oils were active against Gram-negative *Klebsiella pneumonia* as the inhibition halo (10.00 ± 0.00 mm) was higher than that measured by treating the same bacteria with rifampin and gentamicin (inhibition halo 8±0.00 and 7.8±0.00 mm respectively). These results are in agreement with those previously found by Shakib et al. (2018). The same behaviour was observed for the Gram-negative *Shigella dysenteriae*, as the

inhibition halos were slightly higher than those obtained using Rifampin and Gentamicin (9.00 ± 0.00 Mm and 7.80 ± 0.00 mm): 10.00 ± 0.00 mm using the EO from cultivated plants and 9.50 ± 0.50 mm, using the EO from the natural samples. The EO obtained from cultivated plants was more effective against the Gram-negative *Proteus vulgaris* (inhibition halos 9.17 ± 0.29 mm) than rifampin (inhibition halos 8.00 ± 0.00 mm).

Using the EO obtained from wild plants, the most resistant microorganisms were *Staphylococcus aureus* among Gram-positive, *pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella paratyphi* among Gram-negative and *Aspergillus niger* among yeasts. Using the EO obtained from cultivated plants, the most resistant microorganisms were *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella paratyphi* and *Proteus vulgaris* among Gram-negative bacteria, *Staphylococcus aureus* and *Bacillus subtilis* among Gram-positive bacteria and *Candida albicans* among yeasts.

More detailed data on the antimicrobial properties of EOs were obtained by measuring the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC), Table 3. The total values of MIC of the EO obtained from both cultivated and wild samples varied from ~31.25 µg/ml to ~2000 µg/ml as a function of the microorganism used. The Gram-positive *Bacillus subtilis* showed the lowest resistance against the EO from cultivated plants (MIC c31.25 µg/ml), even if the MIC value was significantly higher than that of rifampin. The MIC values provided by the EO obtained from the cultivated sample against *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus brasiliensis* and *Aspergillus niger* were very high (~2000 µg/ml) disclosing the highest resistance of these strains to this essential sample. On the other hand, *Candida albicans* had the highest sensitivity (MIC ~31.25 µg/ml) to the EO obtained from natural sample, which was more effective than the synthetic drug nystatin (MIC ~33.00 µg/ml) used as control. *Escherichia coli* (MIC value of ~1000 µg/ml) had the highest resistance to the EO obtained from the natural sample. Similar results were reported by Ashraf et al. (2017), that using the EO of *D. kotschyi* found against *Escherichia coli* a MIC ~640 µg/ml, confirming the highest resistance of this strain against.

A similar trend was detected by measuring the MBC of essential oils that ranged from ~250 µg/ml to ~2000 µg/ml for that from crops and from ~62.50 µg/ml to ~1000 µg/ml using the oil from naturally grown sample. MBC values detected for both EOs and all microorganisms tested was always higher

than MIC value, in agreement with Ashraf et al. (2017). *Bacillus subtilis*, *Klebsiella pneumonia* (MBC ~125 μg/ml) and *Candida albicans* yeast (MBC ~62.5 μg/ml) were the most sensitive to the EO obtained from cultivated plants. Differently, Ashraf et al. (2017) detected the lowest resistance against the EO for *Streptococcus mutans* (MBC ~80 μg/ml).

Table 3. Mean diameters of inhibition halo, Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of *D. kotschyi* EOs obtained from cultivated and wild plants.

Test microorganisms	EO from cultivated plants			EO from wild plants			Antibiotics					
							Rifampin		Gentamicin		Nystatin	
	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	IZ	MIC	IZ	MIC
Shigella dysenteriae	ND	125	250	9.50±0.50^{b}	500	500	9±0.00^{b}	15.63	17±0.00^{a}	3.90	NA	NA
Pseudomonas aeruginosa	ND	1000	1000	ND	500	500	ND	31.25	20	7.81	NA	NA
Bacillus subtilis	9.00±0.00^{c}	31.25	125	ND	125	125	19±0.00^{b}	31.25	30±0.00^{a}	3.90	NA	NA
Staphylococcus epidermidis	9.67±0.58^{b}	250	1000	9.00±0.00^{b}	125	250	44±0.00^{a}	1.95	39±0.00^{a}	1.95	NA	NA
Escherichia coli	ND	1000	1000	ND	1000	1000	10	15.63	23	31.25	NA	NA
Staphylococcus aureus	ND	500	500	ND	125	250	21	31.25	27	1.95	NA	NA
Klebsiella pneumoniae	10.00±0.00^{b}	125	125	10.00±0.00^{b}	125	250	8±0.00^{c}	15.63	17±0.00^{a}	3.90	NA	NA
Proteus vulgaris	9.17±0.29^{b}	250	250	ND	500	500	8±0.00^{c}	15.63	24±0.00^{a}	15.63	NA	NA
Salmonella paratyphi-A	ND	250	250	ND	125	125	8	15.63	18	3.90	NA	NA
Candida albicans	ND	62.5	250	16.33±0.5	31.2	62.5	NA	NA	NA	NA	33±	125

				8[b]	5						0.00[a]	
Aspergillus niger	15.00±0.50[b]	2000	2000	ND	500	500	NA	NA	NA	NA	27±0.00[a]	31.2
Aspergillus brasiliensis	25.00±0.50[b]	2000	2000	26.00±0.50[b]	250	250	NA	NA	NA	NA	30±0.00[a]	31.2

IZ: The diameters of the inhibition halos includes the diameters of disks (6 mm). Results are expressed as mean values ± standard deviations of three values. Activity is rated as follows: 6–9 mm: no activity; 10–14 mm: low activity; 15–18 mm: good activity; above 18 mm: significant activity. NA: no activity, ND: not determined. MIC: Minimal inhibitory concentration, MBC: Minimal bactericidal concentration. Values with different letters are statistically different (Duncan, $p \leq 0.05$).

Overall results indicated that the EO obtained from cultivated plants had a significant inhibitory and lethal effect on the Gram-positive *Bacillus subtilis*, which is the main cause of food poisoning. While that obtained from wild plants was most effective against *Candida albicans,* wich is the single most important cause of fungal infections worldwide.

Studies on the mechanism of action of EOs have shown that the different compounds contained in the extractive solution increase the permeability of bacterial membrane, thus facilitating their entrance inside the bacteria reducing their activity and leading to cell death. The efficacy of EOs on Gram-positive bacteria was slightly higher confirming that they are more sensitive to the antibacterial effect of the bioactives. The cause of the lower sensitivity of Gram-negative bacteria may be due to the presence of an external membrane capable of limiting the entrance of hydrophobic compounds of the EO at the lipopolysaccharide layer level (Burt, 2004 and Zarali et al. 2016; Calo et al., 2015).

The higher antimicrobial effect of the essential oils on Gram-positive bacteria in comparison with Gram-negative microorganisms has also been underlined by others in previous studies based on other plants (Orlanda and Nascimento, 2015; Ashraf et al., 2015; and Ghavam et al., 2020). It has been previously reported that α-pinene, terpinen-4-ol, and limonene provide good antibacterial activity (Dorman and Deans, 2000; Silva et al., 2012 and Inouye et al., 2001) thanks to their ability to disrupt

the integrity of bacterial membranes. Also, other compounds such as neral and geraniol may be responsible of the antimicrobial activity of essential oils (Maksimović et al. 2008; Sartoratto et al., 2004; Duarte et al., 2007 and Singh et al., 2012). Inactivation of cellular enzymes and proteins by volatile oils has also been reported as a mechanism involved in the antibacterial activity (Raut and Karuppayil, 2014). In general, the synergistic effects of the various constituents of the EO on their biological and antimicrobial activity should be considered.

The effect of EOs as antimicrobials includes their ability to destabilize the membrane. The terpene compounds are lipophilic and can easily penetrate through the membrane of the bacterial cell, resulting in the loss of membrane integrity. Also, the interactions of terpene compounds with polysaccharides, fatty acids, and phospholipids make bacterial membranes more permeable, and causes the loss of ions and cellular content as well as cell death. Other mechanisms include the inactivation of cellular enzymes and the denaturation of proteins by EOs (Raut and Karuppayil, 2014).

On the other hand, natural EO had a significant inhibitory and lethal effect on *Candida albicans*, one of the most common pathogenic fungi that may cause a series of infectious diseases and has become a serious threat to human health. Meanwhile, misuse of antifungal drugs exacerbates *C. albicans*, more and more *C. albicans* infections are becoming common (Delcour, 2009). In the EO obtained from wild samples, 42.83% are terpenes, which passing through the damaged cell wall exhibit significant inhibitory capacity against *C. albicans,* as confirmed by scanning electron microscopy and transmission electron microscopy analyses (Martínez et al., 2014). Therefore, this EO can be considered a promising alternative for the production of a plant-derived antifungal agent.

4. Conclusions

The *D. kotschyi* is an endemic plant from the Daran region of Iran, used in the traditional medicine due to its beneficial properties probably linked to its special growth conditions. In this study, for the first time, its peculiar composition in secondary metabolites, was confirmed by analysing the EOs. Indeed, cyclohexylallene (52.63%) and limonene (35.88%), were the main compounds of the EO obtained from the naturally grown plants. This peculiar composition should be related to geographical location,

hard climatic conditions of the collection area which can affect the production of secondary metabolites and the consequent composition of resulted EOs. The high amount of limonene can ensure optimal biological properties and industrial applications of this oil. Chemical analysis revealed important difference between this essential oil and that obtained from the cultivated plants in the same area. The difference in composition reflected the different biological efficacy. Indeed, oil from wild plants was more effective against *Candida albicans* yeast, while oil from crops had significant inhibitory and lethal effects on the Gram-positive bacteria *Bacillus subtilis*. Therefore, according to the results of the present study, the EO from cultivated plants of *D. kotschyi* can be used to protect foods avoiding their spoilage and against pathogenic microorganisms, as an effective alternative to synthetic preservatives and that from wild plants can be test as natural drug against *Candida* infections.

Competing interests

The authors declare no competing interests.

Author Contribution Statement

Mansureh Ghavam was the supervisor, designer of the hypotheses, and responsible for all the steps (laboratory, statistical analysis, data analysis, etc.) and wrote the text of the article. Maria Letizia Manca helped with statistical analysis of data and to corrected and wrote part of the text. Maria Manconi interpretaded of part of data, substantively revised the text and edited English language. Gianluigi Bacchetta identified and approved the study plant and edited the text

References

Akhani, H., 2006. Flora Iranica : Facts and Figures and a List of Publications By K . H . Rechinger on Iran and Adjacent Areas. Plant Syst. Evol. 7.

Anonymous (1996).European pharmacopoeia (3rd ed., pp. 121–122). Strasburg,France: Council of Europe.

Al-Sayed, E., Abdel-Daim, M.M., 2018. Analgesic and anti-inflammatory activities of epicatechin gallate from Bauhinia hookeri. Drug Dev. Res. 79, 157–164. doi:10.1002/ddr.21430

373 Alcaraz-Meléndez, L., Real-Cosío, S., Suchý, V., Švajdlenka, E., 2007. Differences in essential oil
374 production and leaf structure in phenotypes of damiana (Turnera diffusa Willd.). J. Plant Biol.
375 50, 378–382. doi:10.1007/BF03030671

376 Andrade-Ochoa, S., Nevárez-Moorillón, G.V., Sánchez-Torres, L.E., Villanueva-García, M., Sánchez-
377 Ramírez, B.E., Rodríguez-Valdez, L.M., Rivera-Chavira, B.E., 2015. Quantitative structure-
378 activity relationship of molecules constituent of different essential oils with antimycobacterial
379 activity against Mycobacterium tuberculosis and Mycobacterium bovis. BMC Complement.
380 Altern. Med. 15, 332. doi:10.1186/s12906-015-0858-2

381 Andrade, F., Rafael, D., Videira, M., Ferreira, D., Sosnik, A., Sarmento, B., 2013. Nanotechnology
382 and pulmonary delivery to overcome resistance in infectious diseases. Adv. Drug Deliv. Rev. 65,
383 1816–27. doi:10.1016/j.addr.2013.07.020

384 Ashrafi, B., Ramak, P., Ezatpour, B., Talei, G.R., 2017. Investigation on chemical composition,
385 antimicrobial, antioxidant, and cytotoxic properties of essential oil from *Dracocephalum kotschyi*
386 Boiss. African J. Tradit. Complement. Altern. Med. AJTCAM 14, 209–217.

387 Assadi, M., Khatamsaz, M., Maassoumi, A., Mozaffarian, V., n.d. Flora of Iran. Res. Inst. For.
388 Rangelands 1–77.

389 Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils - A
390 review. Food Chem. Toxicol. doi:10.1016/j.fct.2007.09.106

391 Bendiksby, M., Thorbek, L., Scheen, A.C., Lindqvist, C., Ryding, O., 2011. An updated phylogeny
392 and classification of Lamiaceae subfamily Lamioideae. Taxon 60, 471–484.
393 doi:10.1002/tax.602015

394 Brooks, B.D., Brooks, A.E., 2014. Therapeutic strategies to combat antibiotic resistance. Adv. Drug
395 Deliv. Rev. doi:10.1016/j.addr.2014.10.027

396 Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods - A
397 review. Int. J. Food Microbiol. doi:10.1016/j.ijfoodmicro.2004.03.022

398 Calo, J.R., Crandall, P.G., O'Bryan, C.A., Ricke, S.C., 2015. Essential oils as antimicrobials in food
399 systems - A review. Food Control. doi:10.1016/j.foodcont.2014.12.040

400 Cantino, P.D., Olmstead, R.G., Wagstaff, S.J., 1997. A comparison of phylogenetic nomenclature with
401 the current system: A botanical case study. Syst. Biol. 46, 313–331. doi:10.1093/sysbio/46.2.313

402 Delcour, A.H., 2009. Outer membrane permeability and antibiotic resistance. Biochim. Biophys. Acta
403 - Proteins Proteomics. doi:10.1016/j.bbapap.2008.11.005

404 Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: Antibacterial activity of plant
405 volatile oils. J. Appl. Microbiol. 88, 308–316. doi:10.1046/j.1365-2672.2000.00969.x

406 Duarte, M.C.T., Leme, E.E., Delarmelina, C., Soares, A.A., Figueira, G.M., Sartoratto, A., 2007.
407 Activity of essential oils from Brazilian medicinal plants on Escherichia coli. J. Ethnopharmacol.
408 111, 197–201. doi:10.1016/j.jep.2006.11.034

409 Ebrahim Sajjadi, S., Movahedian Atar, A., Yektaian, A., 1998. Antihyperlipidemic effect of
410 hydroalcoholic extract, and polyphenolic fraction from Dracocephalum kotschyi Boiss. Pharm.
411 Acta Helv. 73, 167–170. doi:10.1016/S0031-6865(98)00016-8

412 Elhidar, N., Nafis, A., Kasrati, A., Goehler, A., Bohnert, J.A., Abbad, A., Hassani, L., Mezrioui, N.E.,
413 2019. Chemical composition, antimicrobial activities and synergistic effects of essential oil from
414 Senecio anteuphorbium, a Moroccan endemic plant. Ind. Crops Prod. 130, 310–315.
415 doi:10.1016/j.indcrop.2018.12.097

416 Eloff, J.N., 1999. It is possible to use herbarium specimens to screen for antibacterial components in
417 some plants. J. Ethnopharmacol. 67, 355–360. doi:10.1016/S0378-8741(99)00053-7

418 Erasto, P., Viljoen, A.M., 2008. Limonene - A review: Biosynthetic, ecological and pharmacological
419 relevance. Nat. Prod. Commun. doi:10.1177/1934578x0800300728

420 Fallah, S., Mouguee, S., Rostaei, M., Adavi, Z., Lorigooini, Z., 2020. Chemical compositions and
421 antioxidant activity of essential oil of wild and cultivated Dracocephalum kotschyi grown in

different ecosystems: A comparative study. Ind. Crops Prod. 143. doi:10.1016/j.indcrop.2019.111885

França Orlanda, J.F., Nascimento, A.R., 2015. Chemical composition and antibacterial activity of Ruta graveolens L. (Rutaceae) volatile oils, from São Luís, Maranhão, Brazil. South African J. Bot. 99, 103–106. doi:10.1016/j.sajb.2015.03.198.

Ghavam, M., Manca, M.L., Manconi, M., Bacchetta, G., 2020. Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of Salvia hydrangea DC. ex Benth.. Sci Rep 10, 15647. https://doi.org/10.1038/s41598-020-73193-y

Golparvar, A.R., Hadipanah, A., Gheisari, M.M., Khaliliazar, R., 2016. Chemical constituents of essential oil of *Dracocephalum moldavica* L. and *Dracocephalum kotschyi* Boiss. from Iran. Acta Agric. Slov. 107, 25–31. doi:10.14720/aas.2016.107.1.03

Golshani, S., Karamkhani, F., Monsef-Esfehani, H.R., Abdollahi, M., 2004. Antinociceptive effects of the essential oil of Dracocephalum kotschyi in the mouse writhing test. J. Pharm. Pharm. Sci. 7, 76–79.

Hajdari, A., Mustafa, B., Nebija, D., Selimi, H., Veselaj, Z., Breznica, P., Quave, C.L., Novak, J., 2016. Essential Oil Composition of Pinus peuce Griseb. Needles and Twigs from Two National Parks of Kosovo. Sci. World J. 2016. doi:10.1155/2016/5393079

Hemaiswarya, S., Kruthiventi, A.K., Doble, M., 2008. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. doi:10.1016/j.phymed.2008.06.008

Inouye, S., Takizawa, T., Yamaguchi, H., 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrob. Chemother. 47, 565–573. doi:10.1093/jac/47.5.565

Jalili, A., Jamzad, Z., 1999. Tehran: Research Institute of Forests and Rangelands; 1999. Red data book of Iran, A preliminary survey of endemic, rare and endangered plant species in Iran 689–690.

447 Javidnia, K., Miri, R., Fahham, N., Mehregan, I., 2005. Composition of the essential oil of
448 dracocephalum kotschyi boiss. from Iran. J. Essent. Oil Res. 17, 481–482.
449 doi:10.1080/10412905.2005.9698970

450 Kadereit, J., Kubitzki, K., 2004. The families and genera of vascular plants VII. Flowering plants
451 dicotyledons: lamiales (except Acanthaceae including Avicenniaceae), Flowering Plants ·
452 Dicotyledons. Springer Berlin Heidelberg, Berlin, Heidelberg. doi:10.1007/978-3-642-18617-
453 2_11

454 Kiehlbauch, J.A., Hannett, G.E., Salfinger, M., Archinal, W., Monserrat, C., Carlyn, C., 2000. Use of
455 the National Committee for Clinical Laboratory Standards guidelines for disk diffusion
456 susceptibility testing in New York State Laboratories. J. Clin. Microbiol. 38, 3341–3348.
457 doi:10.1128/jcm.38.9.3341-3348.2000

458 Maksimović, Z., Stojanović, D., Šoštarić, I., Dajić, Z., Ristić, M., 2008. Composition and radical-
459 scavenging activity of Thymus glabrescens Willd. (Lamiaceae) essential oil. J. Sci. Food Agric.
460 88, 2036–2041. doi:10.1002/jsfa.3311

461 Martínez, A., Rojas, N., García, L., González, F., Domínguez, M., Catalán, A., 2014. In vitro activity
462 of terpenes against Candida albicans and ultrastructural alterations. Oral Surg. Oral Med. Oral
463 Pathol. Oral Radiol. 118, 553–559. doi:10.1016/j.oooo.2014.07.009

464 Mitić, Z.S., Jovanović, B., Jovanović, S., Mihajilov-Krstev, T., Stojanović-Radić, Z.Z., Cvetković,
465 V.J., Mitrović, T.L., Marin, P.D., Zlatković, B.K., Stojanović, G.S., 2018. Comparative study of
466 the essential oils of four Pinus species: Chemical composition, antimicrobial and insect larvicidal
467 activity. Ind. Crops Prod. 111, 55–62. doi:10.1016/j.indcrop.2017.10.004

468 Monsef-Esfahani, H.R., Karamkhani, F., Nickavar, B., Abdi, K., Faramarzi, M.A., 2007. The volatile
469 constituents of Dracocephalum kotschyi oils. Chem. Nat. Compd. 43, 40–43.
470 doi:10.1007/s10600-007-0027-z

471 Moradi, H., Ghavam, M. & Tavili, A. 2020. Study of antioxidant activity and some herbal compounds

of *Dracocephalum kotschyi* Boiss. in different ages of growth. *Biotech Reports*. **25**, e00408.

Morteza-Semnani, K., Saeedi, M., 2005. Essential oil composition of *Dracocephalum kotschyi* boiss. J. Essent. Oil-Bearing Plants 8, 192–195. doi:10.1080/0972060X.2005.10643443

Najafpour Navaei, M., Mirza, M., 2007. Comparative survay on the essential oil composition of cultivated and wild *Dracocephalum kotschyi*. Sci. J. Manag. Syst. 23, 128–133.

Nejad-Sadeghi, M., Taji, S., Goodarznia, I., 2015. Optimization of supercritical carbon dioxide extraction of essential oil from *Dracocephalum kotsch*yi Boiss: An endangered medicinal plant in Iran. Journal of chromatography. A. 1422, 73-81

Oussalah, M., Caillet, S., Saucier, L., Lacroix, M., 2007. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes. Food Control 18, 414–420. doi:10.1016/j.foodcont.2005.11.009

Raut, J.S., Karuppayil, S.M., 2014. A status review on the medicinal properties of essential oils. Ind. Crops Prod. doi:10.1016/j.indcrop.2014.05.055

Samadi, L., Larijani, K., Naghdi Badi, H., Mehrafarin, A., 2018. Qualitative and quantitative variations of the essential oils of *Dracocephalum kotschyi* Boiss. as affected by different drying methods. Journal of Food Processing and Preservation. e13816, 1-12.

Sani, T.A., Mohammadpour, E., Mohammadi, A., Memariani, T., Yazdi, M.V., Rezaee, R., Calina, D., Docea, A.O., Goumenou, M., Etemad, L., Shahsavand, S., 2017. Cytotoxic and apoptogenic properties of Dracocephalum Kotschyi aerial part different fractions on calu-6 and mehr-80 lung cancer cell lines. Farmacia 65, 189–199.

Sarikurkcu, C., Ozer, M.S., Calli, N., Popović-Djordjević, J., 2018. Essential oil composition and antioxidant activity of endemic Marrubium parviflorum subsp. oligodon. Ind. Crops Prod. 119, 209–213. doi:10.1016/j.indcrop.2018.04.023

496 Sartoratto, A., Machado, A.L.M., Delarmelina, C., Figueira, G.M., Duarte, M.C.T., Rehder, V.L.G.,
497 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in
498 Brazil. Brazilian J. Microbiol. 35, 275–280. doi:10.1590/S1517-83822004000300001

499 Shakib, P., Taherikalani, M., Ramazanzadeh, R., 2018. Chemical Composition, Genotoxicity and
500 Antimicrobial Activities of *Dracocephalum kotschyi* Boiss against OXA-48 Producing *Klebsiella*
501 *pneumoniae* Isolated from Major Hospitals of Kurdistan Province, Iran. Microbiol. Res. J. Int.
502 24, 1–8. doi:10.9734/mrji/2018/42064

503 Silva, A., Lopes, P., Azevedo, M., Molecules, D.C.-, 2012, U., 2012. Biological activities of a-pinene
504 and β-pinene enantiomers. Molecules 17, 6305–6316.

505 Singh, S.K., Vishnoi, R., Dhingra, G.K., Kishor, K., 2012. Antibacterial activity of leaf extracts of
506 some selected traditional medicinal plants of Uttarakhand, North East India. J. Appl. Nat. Sci. 4,
507 47–50. doi:10.31018/jans.v4i1.220.

508 Sodeifian, Gh., Sajadian, S.A., Nedasadat, A., 2015. Extraction of Dracocephalum Kotschyi Boiss
509 using supercritical carbon dioxide: Experimental and optimization. The Journal of Supercritical
510 Fluids. 107, 137-144.

511 Sonboli, A., Mirzania, F., Gholipour, A. 2019. Essential oil composition of *Dracocephalum kotschyi*
512 Boiss. from Iran. Nat Prod Res. 14, 2095-2098.

513 Srivastava, A.K., Singh, S., Marathe, R.A., 2002. Organic citrus: Soil fertility and plant nutrition. J.
514 Sustain. Agric. 19, 5–29. doi:10.1300/J064v19n03_03

515 Walker, L., Sirvent, T., Gibson, D., Vance, N., 2001. Regional differences in hypericin and
516 pseudohypericin concentrations and five morphological traits among Hypericum perforatum
517 plants in the northwestern United States. Can. J. Bot. 79, 1248–1255. doi:10.1139/b01-103

518 Xiang, H., Zhang, L., Yang, Z., Chen, F., Zheng, X., Liu, X., 2017. Chemical compositions,
519 antioxidative, antimicrobial, anti-inflammatory and antitumor activities of Curcuma aromatica
520 Salisb. essential oils. Ind. Crops Prod. 108, 6–16. doi:10.1016/j.indcrop.2017.05.058

Zarali, M., Hojjati, M., … S.D.-J. of F., 2016, U., 2016. EVALUATION OF CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF ECHINOPHORA CINEREA BOISS AND STACHYS LAVANDULIFOLIA VAHL ESSENTIAL OILS IN VITRO. Iran. J. FOOD Sci. Technol. 13, 1–12.

Zargoosh, Z., Ghavam, M., Bacchetta, G., Tavili, A., 2019. Effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata Boiss. Sci. Rep. 9. doi:10.1038/s41598-019-52605-8

Zobayed, S.M.A., Afreen, F., Kozai, T., 2005. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. Plant Physiol. Biochem. 43, 977–984. doi:10.1016/j.plaphy.2005.07.013