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Chemical analysis and anti-bacterial effect of essential oils from three different species of *Dracocephalum* in Iran

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Abstract
Dracocephalum is an herbaceous plant that has been recommended in the Persian traditional medicine for its amazing effects on the nervous system, the Heart, and the digestive system. Perylla aldehyde (PAH) is one of the main constituents of this genus. PAH has a lot of biological effects such as anti-depression, anticancer, antimicrobial and vasodilative effects. In this research, we compared the amount of the PAH in three species of *Dracocephalum* that grow in Iran to see which of them has the highest amount of PAH and the highest anti-bacterial effects. Our results indicated that *D. polychaetum* has the highest amount with 90.88% Of PAH in its essential oil also has the highest percentage of essential oil. *D. kotschyi* has the lowest MIC: 200 µg/ml against positive gram bacteria *S. epidermidis*. This is the first report of antibacterial activity of *D. multicauleae* essential oil.

Keywords: *Dracocephalum*, perylla aldehyde, essential oil, anti-bacterial, MIC

1. Introduction
Dracocephalum L. which is known as "Badarshaboo" in Persian language, is an annual, herbaceous, balm-scented and aromatic genus from Lamiaceae family [1, 2]. *Dracocephalum* consists of around 69 species distributed in the temperate regions of the Northern Hemisphere. In the flora of Iran, this genus is represented by 11 species, which are mainly distributed in the northern and central parts of the country, belonging to the Irano-Turanian phytogeographical region. Among 11 species of *Dracocephalum* which growing in Iran, 5 species are endemic [3]. According to World Health Organization (WHO) Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality. So finding a new anti-biotic is very important and critical. Medicinal plant always are a rich source of new medicine. Antibacterial activity has been reported from *Dracocephalum* species several times. Sonboli *et al*. reported the bioassays exhibited that all of the Gram-positive and Gram-negative bacteria tested were highly inhibited in the presence of essential oils of *D. polychaetum* and *D. surmandinum* [4]. Citral and limonen-10-al isolated from *D. subcapitatum* were found to be the most effective compounds against epimastigotes of Trypanosoma cruzi [5]. In another study where essential oil of *D. kotschyi* has been evaluated, the results indicated that the most sensitive bacteria to essential oil was found to be *S. aureus* with the lowest minimum inhibitory concentration value of 2 mg/mL [6]. So, three species have been selected for this survey; *D. kotschyi* and *D. polychaetum* that are endemic to Iran, and *D. multicauleae* of which essential oil has not been studied yet.
Perilla aldehyde (PAH) is a major component of the essential oils that exist in some *Dracocephalum* species [7]. With regards to the importance of PAH and its biological effects, the aim of this study was to analyze the essential oils and compare the amount of PAH in three species of *Dracocephalum*, to identify the main constituent of the plants and so the anti-bacterial activity of them.

2. Material and Methods
2.1 Plant material
The aerial parts of *D. multicauleae*, *D. polychaetum* and *D. kotschyi* were collected from Ardebil, Kerman and Zanjan countryside, respectively, during flowering season from May to July 2015. The plants were identified by Prof. Farideh Attar and the samples were kept at the herbarium of School of Science, University of Tehran (Tehran, Iran) with the voucher

specimens of 45869 TUH, 45866 TUH, and 45879 TUH, respectively.

2.2 Preparation essential oils (EOs)

The whole aerial part of each plant were grounded and 50 gr of each powder is subjected to a Clevenger apparatus. The EOs were obtained using hydro-distillation during 4 hours. The volatile oils were collected in n-pentane, concentrated, dehydrated with anhydrous sodium sulfate and stored in the sealed vials at low temperature.

2.3 Essential oil Analysis

The chemical analysis of the EOs were performed with GC/MS which was carried out by an HP 6890 Network GC System (Agilent Technology), equipped with a capillary column HP-5MS (DB-5) 30 m × 0.25 mm; film thickness, 0.25 µm; temperature program, 50-300 °C at a rate of 3 °C/ min. Helium (99.999 %) was used as carrier gas at a flow rate of 1.5 ml/min. The oils were analyzed by GC/MS using a Hewlett Packard 5973 mass selective detector connected to an HP 6890 gas chromatograph. The component percentages of the identified compounds were calculated from the GC peak areas. The identification of the components was based on direct comparison of their mass spectra with those of Wiley and NBS Libraries [8] and those described by Adams [9].

2.4 Anti-bacterial Activity

Broth micro dilution is one of the most basic antimicrobial susceptibility testing methods. The procedure involves preparing two-fold dilutions of the antimicrobial agent in a liquid growth medium dispensed in 96-well micro dilution plate. Then, each well is inoculated with a microbial inoculum prepared in the same medium after dilution of standardized microbial suspension adjusted to 0.5 McFarland scale. After well-mixing, the inoculated 96-well microdilution plate is incubated under suitable conditions depending upon the test microorganism [10]. The MIC values of the essential oils against four bacterial strains including two Gram-positive (S. *aureus* ATCC 6538p, *S. epidermidis* ATCC 12228) and two Gram-negative (E. coli ATCC 8739, *P. aeruginosa* ATCC 9027) bacteria were obtained using the conventional agar dilution procedure with ciprofloxacin as the standard according to the described method by Baron *et al.* [11]. Two-fold serial dilutions of samples and reference drug were prepared in Mueller-Hinton agar. Drugs (4 mg) were dissolved in DMSO (1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with Mueller-Hinton agar was performed to obtain the final concentrations of 0.001, 0.01, 0.05, 0.1, 0.5, 1, 3, 5 and 10 mg mL^1. The plates were inoculated with 1.5×10^8 CFU of microorganisms; including a control plate (containing 100 µL DMSO without any antibacterial agent) and incubated at 37 C^o for 18 h. The MIC was determined as the lowest concentration of the agent that completely inhibits visible growth of the microorganisms.

3. Results and Discussion

The essential oils from three *Dracocephalum* species were obtained using hydro-distillation. The physical properties of the volatile oils were summarized in table 1. Identified volatile constituents in the essential oils of the samples respectively have been shown in tables 2-4 in continue.

Table 1: Physical properties of Essential oils of three species of *Dracocephalum.*

	Dry weight of Plant (gr)	Color	Weight (mg)	Volume (ml)	Yield of oil (W/V)%
D. kotschyi	50	Yellow	225	0.5	0. 45%
D. multicauleae	50	Yellow	325	0.5	0. 65%
D. polychaetum	50	Yellow	350	0.8	0.7%

Table 2: Chemical composition of the essential oil from *D. kotschyi*

Number	Compound	Ki	RT (min)	Percentage of the Constituent
1	Limonene	1029	4.873	46.7%
2	Perilla aldehyde	1272	9.221	52.05%
3	α- terpinen-7-ol	1285	9.512	0.1%
4	Limonene-10-ol	1290	9.268	9.188%
5	Terpinen 4-ol-acetate	1300	9.73	0.02%
6	Trans- carvyl acetate	1342	10.295	0.41%
7	Neo- iso dihydrocarveol actate	1359	10.337	0.13%
8	Alpha-copaene	1377	13.337	0.26%

Ki: Kovats index, Rt: Retention Time

Table 3: Chemical composition of the essential oil from *D. multicaulea*

Number	Compound	Ki	RT (min)	Percentage of the Constituent
1	Alpha- pinene	939	2.99	2.73%
2	Limonene	1029	4.899	23.83%
3	Perilla aldehyde	1272	9.227	73.44%

Ki: Kovats index, Rt: Retention Time

Table 4: Chemical composition of the essential oil *D. polychaetum*

Number	Compound	Ki	RT (min)	Percentage of the Constituent
1	Limonene	1029	4.884	6.33%
2	Perilla aldehyde	1272	9.268	90.88%
3	Terpinen–7-al	1291	11.333	0.25%
4	Trans carvyl acetate	1342	11.494	1.52%
5	Cis-carvyl acetate	1368	11.676	0.57%
6	Exo Arbozol	1454	14.581	0.49%

Ki: Kovats index, Rt: Retention Time

As shown in tables 2-4, analysis of the essential oil resulted in identification 8 compounds for *D. kotschyi,* 6 compounds for *D. polychaetum* and just 3 compounds for *D. multicauleae* representing 100% of the total oil, while limonene and perilla aldehyde are the most compounds in all genus. In the previous investigation on the essential oil composition of *D. polychaetum,* perilla aldehyde (69.6 %) and limonene (16.5%) has been reported [12], in *D. multiculeae* Perilla aldehyde (71.5%) and limonene (28.1%) [13]. But, in *D. kotschyi*, other study report different monoterpenes such as a Citral (29.3%) and (E)-caryophyllene (21.5%) [14]. Limonene, Verbenone, α-Terpineol, Perillyl alcohol and Caryophyllene [15] was found to be the major constituents. Also Ashrafi and his coworker report the Geranil and Geranial (12.08%), Geraniol acetate (10.27%), α-pinene (10.34%), Limonene (6.95%), Geraniol (9.55%) as main constituents [16]. Trypinene-7-aldehyde and trans-carvyl acetate are existed in both *D. kotschyi* and *D. polychaetum*, although they do not exist in *D. multicauleae*.

PAH is known to be biologically active and it exhibits antimicrobial, anticancer, and vasodilatory effects [17, 18, 19]. In addition, PAH can activate the Transient Receptor Potential A1 (TRPA1) channel, which might be related to the anti-inflammatory properties of this compound [20]. Furthermore, both the oral and inhalation administration of PAH exhibit an anti-depression activity.

This is the first report of *D. multicauleae* essential oil and its antibacterial activity investigation. *In vitro* antibacterial activity of samples showed satisfied result which are summarized in table 4. *D. kotschyi* with MIC 200 µg/ml showed strongest antimicrobial activity against *S. epidermidis* and the *D. polychaetum* with MIC 5000 µg/ml showed the weakest result against *Ps. aeruginosa*. Sonboli and coworker report the MIC 0.3 mg/ml for *D. polychaetum* against *S. epidermidis* 2.4 mg/ml against *Ps. aeruginosa* [21]. Other researcher who investigate antibacterial of *D. sabcapitatum* report its essential oil exhibited potent activity against *S. aureus* and *E. coli* [22]. Previous studies on *D. kotschyi* show strong antibacterial activity against wide range of different bacterial with the MIC 80.0 to 460.0 µg/ml [23].

Table 5: MIC of essential oil of three different species of *Dracocephalum,* µg/ml

samples	*D. kotschyi*	*D. multicauleae*	*D. polychaetum*	*ciprofloxacin*
Staphylococcus epidermidis	200	500	1800	0.195
Staphylococcus aureus	200	840	1400	0.195
Escherichia coli	500	920	2200	0.012
Pseudomonas aeruginosa	900	1270	5000	0.195

4. Conclusion

It seems that, the efficiency of essential oils and percentages of the main constituents are really different between three species. In *D. kotschyi* 52%, and in *D. multicaule* 73.44% of the oil is perilla aldehyde but in *D. polychaetum* 90.88% of the oil is Perilla aldehyde. *D. polychaetum* has the highest efficiency of essential oil among the three species also has the highest amount of PAH among them. These properties make it a very important anticancer, anti-depression and anti-inflammatory agent among *Dracocephalum* species. But antibacterial activity in *D. polychaetum* is less than the other two. Maybe it's due to its smaller percentage of limonene or its high percentage of Perylla aldehyde.

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