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A comparative study of chemical compounds and antibacterial activity of medicinal plants of *Dracocephalum Lindbergii Rech.f* and *Dracocephalum subcapitatum* (kuntze) Lipsky growing in North Khorasan province, Iran

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Abstract

Background: A number of aromatic medicinal plants used for treating infection disease have been mentioned in different phytotherapy manuals due to their availability, fewer side effects, and reduced toxicity. *Dracocephalum L* is one of the most important genuses of Lamiaceae.The aim of our study was to evaluate the chemical compositions of *Dracocephalum Lindbergii* Rech.f and *Dracocephalum subcapitatum* (O.kuntze) Lipsky and there is antimicrobial activites.

Methods: Two species of *Dracocephalum* (*Dracocephalum Lindbergii* Rech.f and *Dracocephalum subcapitatum (*O.kuntze) Lipsky *)* were collected at North Khorasan province.Extracting the essential oil of the aerial parts of the plant was done through the method of hydrodistillation using Clevenger, and the identification of the essential oils components were carried out through GC-MS. The antibacterial activity was performed against three human pathogenic bacteria including: *Escherichia Coli, Staphylococcus aureus, Bacillus atrophaeuse,* using disk diffusion method.

Results: Forty-two components were identified in the essential oil of the species *Deracocephalum Lindbergii* Rech.f., representing 90.69% of the total oil. The highest percentage of the components belongs to β-ocimene with 17.58%. Thirty components were identified in the plant *Deracocephalum subcapitatum* (O.kuntze) Lipsky, representing 87.24% of the total oil, in which the highest percentage belongs to β-ocimene with 24.45%. The antibacterial effects showed that the bacteria of *Bacillus atrophaeus* and *Staphylococcus aureus* with the diameter inhibition zones of 29 and 33 mm were the most sensitive. In addition, the essential oil of *D.subcapitatum* (O.kuntze) with a diameter of 33 mm inhibition zone had the highest antibacterial activity against *Staphylococcus aureus* bacteria.

Conclusion: Based on the results of this study,the chemical variations of the volatile oils of two *Dracocephalum* species might be correlated with geographical regions and enviromental conditions play a significant role in biosynthesis of the components of the oil. Also,The results of the antibacterial examination of the essential oil s of the studied species can lead to the discovery of new antibacterial agents.

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Introduction

Dracocephalum Lindbergii Rech.f and *Dracocephalum subcapitatum* (O.kuntze) Lipsky *are* aromatic plants belonging to Lamiaceae family. *Dracocephalum L* is one of the most important genuses of Lamiaceae, which includes 186 species. Among these, 11 species are in Iran, and 8 are endemic in Iran (1). The essential oil of the herbaceous plant species is medicinal and possesses high antioxidant activity as well as antibacterial and antiseptic properties (2). The Austrian botanist, Karl Heinz Rechinger, in 1982, when he was working on Flora Iranica, identified 18 species of *Dracocephalum L*. in Iran, but currently only 11 species of the plant have been harvested in Iran (3). The *Dracocephalum L* is unique in terms of medicinal properties. In traditional medicine, this plant is used as an analgesic and anti-inflammatory agent, and boiling it can lead to the elimination of rheumatic pains and healing of the wounds (4). Investigating the essential oils of aerial part of *Deracocephalum Multicaule* Montbr & Auch collected from the valley on Haraz roads indicate that monotropic compounds are present (48.6%) (5). The most important compound in the essential oil of *D.Moldavica L* is the non-aromatic monotropic compounds, which include Geranial, Neral, Geraniol and Geranyl acetate (6).

In the study undertaken on the chemical compounds of the essential oil of *D.Kotschyi Boiss*, the most recognized compounds are citral (29.3%), beta-caryophyllene (21.5%), terpinyl acetate (12.2%) and myrcene (7.1%). *D.Heterophyllum* has been reported to have anti-cough and antiseptic effects (7). Compounds such as Limonene, α-pinene, Geranial, Nerol, Myrtenol, β-Caryophyllene are identified in the essential oils of other *Dracocephalum* species, such as *D.Multicaule* Montbr & Auch (5,8), *D. Moldavica L* (9), *D. Kotschyi Boissv* (10,11), *D.heterophyllum Benth* (12), and *D.surmandium* (13).

A study conducted on the essential oil of *Dracocephalum Heterophyllum* showed that antiasthmatic effect was the main feature of this plant (14). Dracocephalum is a source of flavonoids and terpenoids such as Luteolin and Apigenin, which is used in the treatment of chronic obstructive bronchitis (15). Xanthomicrol is one of the methoxylated flavones that is extracted from the leaves of the *Dracocephalum Kotschyi Boiss*, which is considered as cancer chemopreventive agent (16). Methanolic extract of *Dracocephalum multicaule* shows anticholinesterase, antioxidant and neuroprotective effects (17).

Dracocephalum is one of the native medicinal plants of North Khorasan province in Iran. Due to its significant distribution, *D. Lindbergiji* Rech.f and *D.Subcapitatum* (Kuntze) Lipsky are considered to be dominant in the second region (Mount Missinou and Mount Akhordag) and the people of North Khorasan province use this plant in traditional and indigenous medicine. We conducted this study due to the paucity of comparative research studies on the essential oils of *D. Lindbergiji* Rech.f and *D.Subcapitatum* (Kuntze) Lipsky, and in order to investigate the antibacterial activity as well as determining and comparing the essential oil composition of these two plant species.

Materials and Methods

Plant Material

The aerial parts of plant samples of *Dracocephalum subcapitatum* (kuntze) Lipsky were collected at the flowering stage from mountainous altitudes in Gifan region, North Khorasan province (37°, 54', 57″ N and 57°, 37', 29″ E), where the plant was grown and the plant samples were collected from a height between 2000 and 2600 meters above the sea level. Also, the aerial parts of plant samples of *Dracocephalum Lindbergii* Rech.f., which is located in Iran only in North Khorasan province, were collected from mountains of Akhoardagh (37°,34́,48″ N and 57°,13́,56″) between 1600 and 1850 meters above the sea level in 2017. The samples of the plants were identified by the Plant Sciences Institute of Ferdowsi University of Mashhad (*D.Lindbergii* Rech.f:

Voucher sp. no.: 43966 and *D.subcapitatum* (O.kuntze) Lipsky: Voucher sp. no.: 45065)

Isolation of the Essential Oils

The collected plants were dried in shade for a week. The plants' materials (100 g) were cut into small pieces and the essential oil was obtained by hydrodistillation with a Clevenger-type apparatus until there was no significant increase in the volume of the oil collected (2h). The yield of the oil was 0.8% (w/w) for *Dracocephalum Lindbergii* Rech.f., and 1.2% (w/w) for *Dracocephalum subcapitatum* (kuntze) Lipsky. After each extraction process, the essential oil was digested with dry sodium sulfate and then the extracted essential oil was stored in dark containers in the refrigerator at 4°C.

GC-MS analysis

Essential oils of these plants were delivered to the Research Center for Natural Products and Medicinal Plants in Bojnourd after identification. Isolation and measurement of essential components were performed by gas chromatography coupled with a mass spectrometer belonging to Shimadzu Company. GC-cromatogram of essential oil of *D.Lindbergii* Rech.f., and *D.subcapitatum* (kuntze) Lipsky shown in Figure1 and Figure2 respectively. The device specifications are as follows:

The Shimadzu- Qp2010SE gas chromatography is equipped with RTx-5MS (column length 30 m, internal diameter of 0.25 mm, static layer thickness of 0.25 µm). The oven temperature was programmed at 60 to 290°C at a rate of 10°C/min; and remained at this temperature for 13 minutes. Helium gas was used as carrier gas at a flow rate of 0.9 mm/min and ionization energy in mass was 70 eV; mass range was 40 to 300 amu and scan time was 1s.

Identification of Components

To detect the structure of the components in the essential oil, the Retention indices (RI) of compounds were determined by comparing the retention times of a series of n-alkanes with linear interpolation. Identification of the essential oil components was made by comparison of their mass spectra on both columns with those stored in NIST05 and Wiley275 libraries or with mass spectra from literature. Identification of each component was confirmed by the comparison of its retention index either with those of authentic compounds or with data in the literature (18).

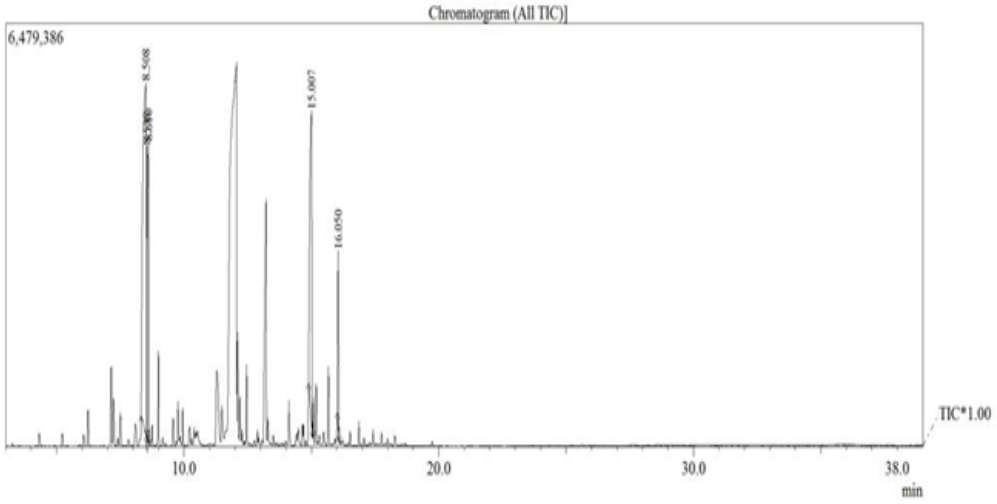

Figure 1.GC-cromatogram of essential oil of D. Lindbergii.Rech.f

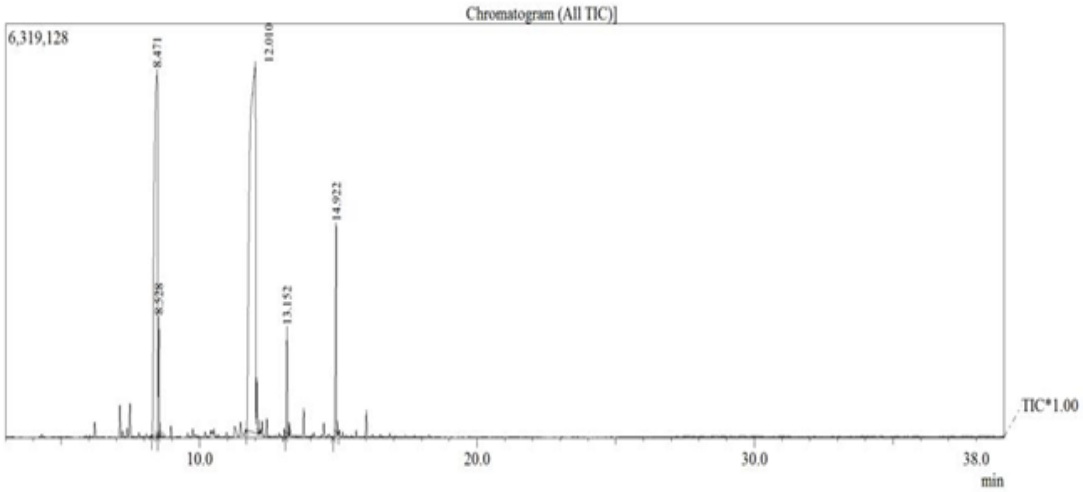

Figure 2. GC-cromatogram of essential oil of D. subcapitatum (O.kuntze) Lipsky

Antimicrobial activity

The microorganisms studied in the antimicrobial activity of the essential oil of the two species of *Dracocephalun* plant including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus atrophus* were provided from the Iranian Research Organization for Science and Technology (Table1). In order to investigate the anti-microbial effects of essential oils of the two plant species, bacterial strains were grown on nutrient agar at 37 °C. Two to three colonies from each culture were added to the sterilized physiology serum and the turbidity was adjusted to 0.5 McFarland (approxiately $1.5*10^8$ CFU/ml). Prepared bacterial suspension was inoculated onto Muller Hinton Agar. Then, sterile paper discs (diameter 6 mm) containing 2 µl of essential oil dissolved in 20 µl of dimethyl sulfoxide was placed on cultured media. Bacterial cultures were incubated at 37 ° C for 24 hours. The diameter of the inhibition zone was measured in millimeters (19). Disc containing dimethyl sulfoxide and antibiotic disk gentamicin (10 µg dose) were placed as control on culture. To ensure the results obtained for the essential oils of the species studied, the above experiments were repeated three times for each strain (Figure 3). In the dilution in the well (Micro broth dilution), the minimum inhibitory concentration of the antimicrobial agent (MIC) and the minimum bactericidal concentration of antimicrobial agent (MBC) were determined. MBC is the minimum concentration capable of killing 99.9% of the microorganisms. For this purpose, in this double-dilution series, the essential oils of the studied species were prepared in 10% dimethyl sulfoxide with a concentration of 0.05-0.8 µl / ml (20). Then 100 µl of each dilution was added to each well from a 96-well plate. The prepared standard (equivalent to 0.5 McFarland) was diluted in the previous step, 100µl of microbial suspension was added to each well and incubated at 37 ° C for 24 hours.The lowest concentrations without visible growth at the binocular microscope were recorded as MIC.

Table1. Species of microbial strains

name of microorganism	Type of microorganism	Abbreviated code
Escherichia Coli	G^-	*ATCC1533*
Staphylococcus aureuse	G^+	*ATCC1110*
Bacillus atrophaeuse	G^+	*ATCC1023*

Figure 3. Formation of no-growth holes around wells

Results

The yields of essential oils of *D.Lindbergii*Rech.f and *D.Subcapitatum* (O.kuntze) Lipsky were 0.8% and 1.2%, respectively. The yield was calculated based on dry weight. After transfusion to the GC-MS, the components of each essential oil were identified. Finally 42 comonents were identified from the essential oils of *D. Lindbergis* Rech.f and in the other species were *D.Subcapitatum*, 30 compounds in total were identified, which is given in Table 2. The major component of the essential oils of *D. Lindbergis* Rech.f and *D.Subcapitatum* was beta-Ocimene (17.58% and 24.45% respectively), beta-Ocimene is a linear monocarboxylic hydrocarbon mono-purple with a molecular formula ($C_{10}H_{16}$).

Table 2. Chemical compositions of the essential oils of D.subcapitatum and D.Lindbergii.

compounds	RI[a]	*D.Lindbergii* %	*D.subcapitatum* %
α- thujene	931	0.32	0.22
α- Pinene	939	0.8	0.85
Camphene	953	0.15	-
Sabinene	979	2.61	1.63
β- Pinene	985	0.18	-
β- Myrcene	994	1.39	2.31
Delta-3-Carene	1007	-	0.34
α- Terpinene	1021	0.81	0.27
β- Ocimene	1063	17.58	24.45
γ- Terpinene	1071	0.41	0.49
Terpinolene	1091	0.9	0.28
Linalool	1097	0.51	0.24
Octen-1-ol, acetate	1104	0.47	0.41
1- Octen-3-yl, acetate	1108	0.15	-
Trans- P- Mentha-2,8-dien-1-ol	1114	0.44	0.22
2,4,6-Octatriene,2,6-dimethyl	1119	0.39	0.22
Trans- Limonene oxide	1128	0.85	1.3
3,9-Epoxy-p-mentha-1,8(10)-diene	1168	2.17	-
Terpinen-4-ol	1170	8.2	0.29
Cis- sabinene hydrate	1171	6.13	-
1(2H)-Naphthalenone,4,4,5,6,7,8-Hexahydro	1207	-	16.75
2(3H)- Naphthalenone,4,4a,5,6,7,8-Hexahydro	1210	6.34	3.39
Bicyclo[3.3.0]octan-2-one,6-methyl-7-methylene	1212	5.27	13.7
Anisole,m-Tridecyl	1218	-	4.83
α- Terpineol	1220	6.36	-
Cis- Carveol	1229	0.94	0.34
D- Carvone	1234	0.47	0.79
Geranial	1238	4.83	-
Santene	1244	-	0.38
p-Menth-1(7),8(10)-Diene-9-ol	1251	-	3.71
Perillyl Alcohol	1260	0.24	-
P-Menth-1-en-9-ol	1263	0.98	0.64
Myrtenyl Acetate	1332	0.17	-
Cis- Carvyl Acetate	1336	0.19	-
Ethyl dihydroCinnamate	1346	0.83	-
α- Terpinyl Acetate	1352	1.51	0.44
Geranyl Acetate	1366	0.59	-
α- Copaene	1400	1.15	0.63
Caryophyllene	1406	0.59	-
β- Bourbonene	1412	0.38	-
β- Elemene	1413	0.24	-
Limonen-10-yl-acetate	1416	9.19	5.65
α- Humulene	1489	0.84	0.35
Bicyclosesquiphellandrene	1493	0.31	-
Germacrene- D	1517	3.67	1.21
Delta- cadinol	1522	0.16	-
γ- Cadinene	1537	0.37	0.23
Total		90.08	86.56

a: Retention Indices on RTX-5MS

The weight percent of the major and common compounds of the two *Dracocephalum* species, as well as the percentage of each essential oil composition of the two species are shown in Figures 4 and 5, respectively.

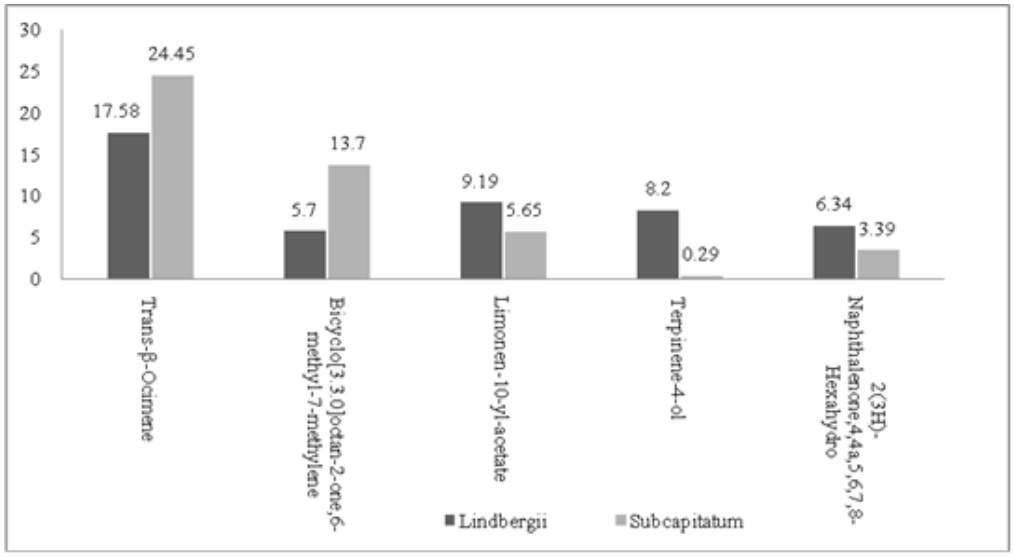

Figure 4. Comparison of the weight percentage of the major and common compounds between the two species

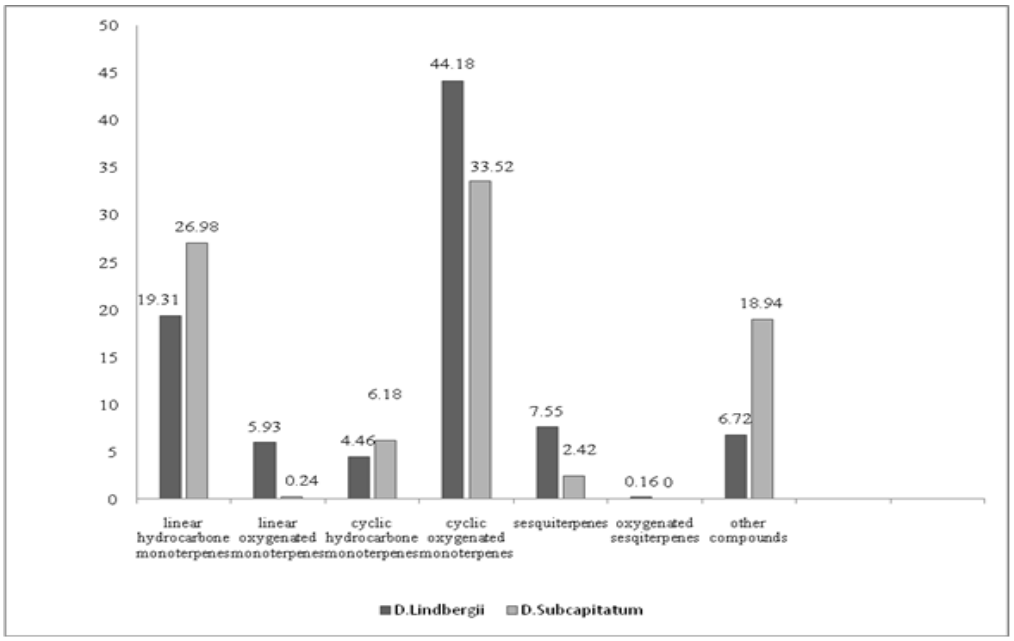

Figure 5. Comparison between the types of essential oil composition of the two species

The diameter of inhibition zones of essential oils of *D.Lindbergii* Rech.f and *D.Subcapitatum* (O.kuntze) Lipsky were assayed by disc diffusion method are illustrated in Table 3. The diameter of inhibition zone for these oils against tested bacteria was mostly 19 to 33 mm. The antibacterial effects showed that at the pure sample *Bacillus atrophaeus* and *Staphylococcus aureus* bacteria with the diameter inhibition zones of 29 and 33 mm were the most sensitive. additionly, the essential oil of *D.subcapitatum* (O.kuntze) with a diameter of

33 mm inhibition zone had the highest antibacterial activity against *Staphylococcus aureus* bacteria.

The effect of essential oils on 3 bacterial species showed that at the lowest concentration of essential oil (0.05 µl / ml), *Bacillus atrophus* bacteria was susceptible to the essential oil of *D.Lindbergii*Rech.f, and it has the highest diameter of the inhibition zone (21 mm) .in addition,*staphylococcus aureus* bacteria was also sensitive to essential oil of *D.Subcapitatum* (O.kuntze) Lipsky and it has the highest diameter of the inhibition zone (25 mm) . (Table 3).

Table3. The diameter of inhibition zones of D.Lindbergii (Rech.f.) and D.Subcapitatum (O.kuntze) Lipsky) in millimeters

Plan speciest	Type of bacteria	Positive control	Pure sample	concentration				
				0.8	0.4	0.2	0.1	0.05
DracocephalumLindbergiiRech.f.	*Escherichia Coli*	25	23	21	19	18	16	14
	Staphylococcus aureus	23	20	17	15	14	13	11
	Bacillus atrophaeus	32	29	27	26	25	23	21
DracocephalumSubcapitatum (O.kuntze)Lipsky	*Escherichia Coli*	21	19	18	18	17	15	12
	Staphylococcus aureus	36	33	31	29	28	27	25
	Bacillus atrophaeus	27	24	22	21	19	17	15

Discussion

The yield of the colorless essential oil obtained by hydrodistillation of dried aerial parts of *D.subcapitatum* and *D.Lindbergii* from north Khorasan province were 1.2% and 0.8%, respectively. The obtained yield of essential oil of the studied species are higher than other species such as *D. ruyschiana* (0.4%), *D. molduvica* (0.01-0.17%), and *D. thymiflorum* (0.04%)(10).

The essential oil compositions and the relative percentages are listed in Table 2. a total of 42 and 30 component of the essential oils of *D.Lindbergii* and *D.subcapitatum* were identified, representing 90.08% and 86.56% of the oil, respectively. The most important components identified from the essential oil of *D.Lindbergii* were Trans-β-Ocimene with 17.58% and Limonen-10-yl-acetate with 9.19% and the major compounds identified from *D.subcapitatum* were Trans- β-Ocimene with 24.45% and 1(2H)-Naphthalenone,4,4,5,6,7,8-Hexahydro with 16.75%.

As shown in Figure 5, the contribution of different compounds in the essential oils of *D.lindbergii* and *D.subcapitatum* were cyclic monoterpene hydrocarbons 6.18%, 4.46%; linear hydrocarbon monoterpenes 19.36%, 26.98%; linear oxygenated monoterpens 5.93%, 0.24%; cyclic oxygenated monoterpenes 44.18%, 33.52%; sesquiterpenes 7.55%, 2.42%; oxygenated sesquiterpens 0.16%, 0% and other compounds 6.72%, 18.94%, respectively.

Conclusion

The results of the identification of the compounds in the essential oils of the *D.lindbergii* and *D.subcapitatum* indicate that most of the compounds of the two species are common. compounds such as α-Pinene, Sabinene, β-Myrcene,β-Ocimene, Linalool and Germacrene- D are present in the essential oils of both plant species, although the percentage of these compounds is different, which indicates the effects of climate and other environmental conditions, topography and plant species variation on the compounds in the essential oil of the studied plants. The results of the antibacterial examination of the essential oil s of the studied species can lead to the discovery of new antibacterial agents.

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