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Short Communication

Chemical Composition and Antibacterial Activity of the Essential Oil of *Zosimia absinthifolia* Growing in East Azarbaijan (Iran)

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ABSTRACT

Background: *Z. absinthifolia*, with local name of "Zarak-e-Kuhi" has been consumed as a spice in some regions of Iran.
Methods: In this study, the essential oil obtained from the aerial parts of *Zosimia absinthifolia* (Apiaceae) (ZaeM) was analyzed by GC-MS. Also, the chemical profile of the oil was compared with the same species collected from different localities. Moreover, the essential oil was investigated for its antimicrobial activity by disc diffusion method.
Results: Analysis of ZaeM by GC-MS resulted nineteen components consisting 98.22% of the total oil. The essential oil was predominantly made up of esters (68.48%) and alcohols (26.04%). The most abundant components of the oil were octyl acetate (47.29%), n-octanol (25.79%), octyl butyrate (10.15%) and octanoic acid octyl ester (7.9%). The relative amounts of the main compounds of *Z. absinthifolia* essential oils growing in different places were comparable with each other. Both qualitative and quantitative variability of chemical composition of *Z. absinthifolia* essential oils were possibly related to the individual genotypes or geographical origin. Disc diffusion method was employed for the determination of antimicrobial activity of ZaeM. The results showed that the essential oil just had inhibitory activity against *B. subtilis*, a gram positive strain. The inhibitory activity could be attributed to the long chain alcohol, octanol (25.79%), which had previously shown antimicrobial activity.
Conclusion: As a conclusion, ZaeM could have antimicrobial potentials on *B. subtilis*. Further investigations are needed to isolate and identify antimicrobial compounds from this volatile oil.

Introduction

The family Apiaceae (Umbelliferae) consists of 150 genera and 3000 species largely exist in temperate regions.[1] The genus *Zosimia* includes two species in Iran: *Zosimia radiance* and *Zosimia absinthifolia*.[2] The seeds of *Z. absinthifolia*, with local name of "Zarak-e-Kuhi" have been consumed as spice in some regions of Iran.[3] The reported phyto constituents from methanolic extract of roots and fruits of *Z. absinthifolia*, were lactones of coumarin group, zosimin and deltoin. Moreover, two flavonoids as well as one chalcone have been identified from the methanolic extract of aerial parts of this species.[4] In another study, three flavonoids were separated from the methanolic extract of seeds of *Z. absinthifolia*.[3] Furthermore, GC and GC/MS analysis of the n- hexan extract from the seeds of this herb resulted n-octylacetate, octylpropanoate, decane, angelicin, umbelliferone as the major chemical compounds. Since Apiaceae (Umbelliferae) was selected as the source of essential oil containing herbs, so the volatile oil of the genus *Zosimia* would be a good candidate to investigate both in terms of the composition and biological activities. In the previous manuscript the essential oil composition of *Z. absinthifolia* collected from different geographical locations has been reported.[5-7] Even though the chemical composition of *Z. absinthifolia* essential oil (Zae) has been previously reported, in this case, due to the effect of harvesting time and season, geographical location, altitude and climate on the yield and

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composition of volatile oils,[8-9] this study aimed to determine firstly, the chemical composition of Zae growing in Mishoodaghi Mount in East-Azarbaijan Province of Iran (Zae M); secondly, to compare the main phytoconstituents of Zae M with Zae growing in the different regions with the different conditions. To the best of our knowledge there was no report on antibacterial activity of this herb so this paper will focus on the determination of antibacterial activity of ZaeM by disc diffusion method as well as the discussion of the connection between antibacterial activity and Zae phytoconstituents.

Materials and Methods

Plant Material

Aerial parts of *Zosimia absinthifolia* (Vent.) Link were collected from a place near Marand (Misho mountain) at E: 45° 47', N: 38° 19', altitude of 2036 in East-Azarbaijan Province, Iran in June 2014.Voucher specimen was deposited in the herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Essential oil Extraction

The fresh aerial parts of *Z. absintifolia* were shade-dried at the room temperature for 10 days. Then dried aerial parts of the herb (90 g) were subjected to hydrodistillation using a Clevenger-type apparatus) for 4 h. Then the oil was dried over anhydrous sodium sulfate, measured and stored in a dark glass at 4 ºC for further studies.

GC-MS and GC-FID Analysis

The volatile oil was analyzed using a Shimadzu GCMS-QP5050A Gas Chromatography Mass Spectrometer (GC-MS) fitted with a fused silica DB-1 capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.3 ml/min. The oven temperature was kept at 50°C for 3 min, and programmed to rise to 270°C at a rate of 5°C/min and then kept constant for 4 minutes. The injector temperature was 250°C and split ratio was adjusted at 1:24. The Mass Spectral (MS) data obtained at the following conditions; ionization potential 70 eV; ion source temperature 200°C; quadrupole temperature 100ºC; solvent delay 3 minutes and EM voltage 3000 volts. Identification of the essential oil components was performed by the comparison of their mass spectra with the NIST 21, NIST 107 and WILEY229 libraries, the spectral data banks, which make computer matching available. The comparison of the fragmentation patterns of mass spectra of unknown compounds with the reported components was carried out as well. Kovats indices (KI) of the components were calculated using standard n-alkanes (C_8-C_{20}) injection, under the same chromatographic conditions.

For the quantitation (area %), the GC analyses were performed on a Shimadzu QP-5050A GC series apparatus fitted with a FID detector. The FID detector temperature was 300 ºC. To obtain the same elution order as GC-MS, the simultaneous auto-injection was performed duplicate on the same column, applying the same operational conditions. The relative percentage amounts of separated compounds were calculated from the FID chromatograms. Moreover, flame ionization detector (FID) which was operated in ionization potential mode at 70 eV and used the same program reported above, was applied for quantification purpose and calculating the relative area percentage (area %) without the use of correction factors.

Antimicrobial assay

Disc diffusion method was employed for the determination of antibacterial activity of Zae M. The examined organisms were included three gram negative bacteria, *Pseudomonas aeroghinosa* (ATCC 9027), *Salmonella paratyphi* (ATCC 4420) and *Proteus mirabilis* (ATCC 29906), two gram positive species including *Bacillus subtilis* (ATCC 9372) and *Staphylococcus aureus* (ATCC 6538) and a fungus, *Candida albicans* (ATCC 10231) which were purchased in lyophilized culture from Pasture institute, Tehran, Iran. The activated microorganisms were transferred to Muller Hinton Broth medium (Merck, Germany) and were incubated overnight at 37°C. The turbidity was corrected until standard of 0.5 McFarland's [108 colony forming units (CFUs) per ml] by adding the sterile distilled water. About 10 ml of the prepared inoculums (106CFUs per ml) was spread over the autoclaved Muller Hinton Agar Medium. Then the sterile discs of Whatman paper with 6 millimeters diameter, which were impregnated with 10 µl of the essential oil and dissolved in 10 cc of 50% aqueous DMSO,were placed on the surface of the media. The plates were incubated for 30 min in the refrigerator to allow the essential oil to diffuse.Then they were incubated at 37°C for 24 h. Finally, the inhibition zones around the sterile discs were measured. In order to compare the potency of the antimicrobial activity of the volatile oil, standard discs of Amikacin (the concentration) as a positive control were placed on the seeded plates.[10-11] All of the experiments were performed in triplicate. Mean Inhibition Zone Diameter (MIZD) as well as the SD were calculated as well. The plates that indicated significant antibacterial activities were further assayed for their Minimum Inhibitory Concentration (MIC) at the tested concentration. Serial two-fold dilutions of sample were prepared in the broth. Cultures containing only sterile nutrient broth with no influence on bacterial growth were used as controls. An equal volume of the adjusted inoculums were added to the each test tube. After the incubation at 37ºC for 24 h, the MIC was read. The MIC was

defined as the lowestconcentration of a fraction which was able to completely inhibit the growth of the each bacterial strain.[12]

Results

The essential oil obtained by the hydrodistillation of aerial parts of *Z. absinthifolia* yielded a lemon yellow colored volatile oil of 0.15% (v/w). Table 1 illustrated phyto constituents of the ZaeM listed in the order of their elution from DB1 column. The listed components were qualitatively and quantitatively analyzed by GC-Mass and GC-FID. Nineteen components consisting 98.22% of ZaeM were identified while 1.78% of the essential oil remained unidentified. Among identified phytochemicals, octyl acetate was the main compound (47.29%). Moreover, percentage of n-octanol (25.79%), octyl butyrate (10.15%) and octanoic acid octyl ester (7.9%) were more than other identified components. Apart from the major volatiles, only (E)-5-decenyl acetate (2.25%) and octanal (1.19%) exceeded a content of 1% of the total oil, while the remaining volatiles (n=13) were present in low amounts.

Table 1. Percentage of chemical components of Zae M.

No.	Compound	%Area	RI[a]	Molecular formula	Identification method
1	n-Hexanal	0.13	781	$C_6H_{12}O$	RI+MS
2	α-Pinene	0.53	928	$C_{10}H_{16}$	RI+MS
3	Camphene	0.1	940	$C_{10}H_{16}$	RI+MS
4	β-Pinene	0.06	967	$C_{10}H_{16}$	RI+MS
5	Octanal	1.19	978	$C_8H_{16}O$	RI+MS
6	1-Hexyl acetate	0.14	1003	$C_8H_{16}O_2$	RI+MS
7	1,8-Cineol	0.61	1018	$C_{10}H_{18}O$	RI+MS
8	(Z)-3-Octen-1-ol	0.25	1042	$C_8H_{16}O$	RI+MS
9	n-Octanol	25.79	1059	$C_8H_{18}O$	RI+MS
10	Nonanal	0.11	1081	$C_9H_{18}O$	RI+MS
11	(E)-5-decenyl acetate	2.25	1177	$C_{12}H_{22}O_2$	RI+MS
12	Octyl acetate	47.29	1199	$C_{10}H_{20}O_2$	RI+MS
13	Bornyl acetate	0.66	1268	$C_{12}H_{20}O_2$	RI+MS
14	n-octyl propionate	0.09	1283	$C_{11}H_{22}O_2$	RI+MS
15	Octyl butyrate	10.15	1372	$C_{12}H_{24}O_2$	RI+MS
16	β-bourbonene	0.37	1383	$C_{15}H_{24}$	RI+MS
17	β-caryophyllene	0.42	1417	$C_{15}H_{24}$	RI+MS
18	Caryophyllene oxide	0.18	1571	$C_{15}H_{24}O$	RI+MS
19	Octanoic acid, octyl ester	7.9	1761	$C_{16}H_{32}O_2$	RI+MS
	Total compounds	19			
	Esters	68.48			
	Alcohol	26.04			
	Aldehyde	1.43			
	Monoterpenes	1.30			
	sesquiterpenes	0.97			
	Total Identified	98.22			
	Not identified	1.78			
	Total identified	98.22			

[a]Compounds listed in order of elution from a DB-1 column, [b]Identification method: (RI= Retention indices, MS=Mass spectroscopy.

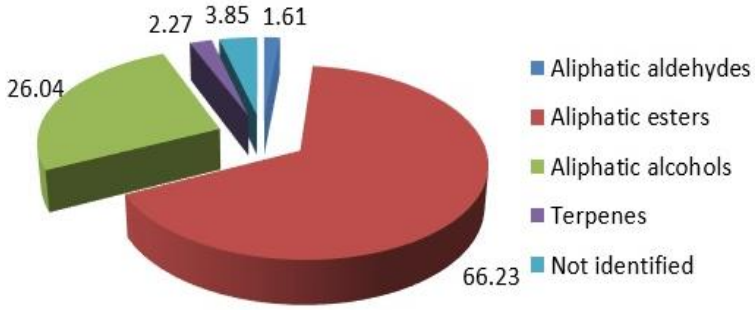

Figure 1. The identified chemical groups from the essential oils of the aerial parts of ZaeM.

Table 2. The percentage of the major compounds of different *Z. absintifolia* volatile oils growing in different areas.[3,5-7]

No	Compounds	ZaeM[1]	ZaeF[2]	ZaeL[3]	ZaeKh[4]
1	n-Hexanal	0.13	0.2	-	-
2	α-Pinene	0.53	1.1	5.3	-
3	Camphene	0.1	0.2	-	-
4	β-Pinene	0.06	0.1	-	-
5	Octanal	1.19	0.2	-	-
6	1,8-Cineol	0.61	-	0.2	0.3
7	n-Octanol	25.79	9.6	-	4.1
8	Octyl acetate	47.29	3.8	24.7	12.2
9	Bornyl acetate	0.66	1.5	-	-
10	Octyl butyrate	10.15	19.2	-	-
11	β-Bourbonene	0.37	-	3.3	0.4
12	β-Caryophyllene	0.42	13.9	22.2	14.6
13	Caryophyllene oxide	0.18	5.7	-	2.4

[1]Z. absintifolia essential oil gathered from Mishoodaghi mount in East-Azarbaijan Province of Iran.[2]Z. absintifolia essential oil gathered from northern mountains of Firozabad in Fars province of Iran. [3]Z. absintifoliaessential oil gathered fromLonbar in khalkhal, northwest of Iran. [4]Z. absintifolia essential oilgathered from north Khoramabad, Lurestan province of Iran.

As it could be seen in the Table 1 and Figure 1, phytochemicals of ZaeM were predominantly made up of firstly, esters (68.48%), secondly, alcohols (26.04%) and thirdly, Aldehydes (1.43%). Also the percentage of monoterpene and sesquiterpenes were 1.30% and 0.97 %, respectively. Table 2 exhibited the percentages of major constituents of ZaeM, *Z. absinthifolia* growing in northern mountains of Firozabad in Fars province (ZaeF), *Z. absinthifolia* growing in Lonbar of khalkhal, in northwest of Iran (ZaeL) and north Khoramabad, Lurestan province of Iran (ZaeKh) successively.

The results of the antimicrobial test demonstrated that the only susceptible strain to Zae M was *B. subtilis*, a gram positive bacterium, with MIZD±SD of 22.95±1.90 mm and MIC of 25 mg/ml. The MIZD±SD (mm) of Amikasin was 42.3±1.50 mm.

Discussion

There are several reports about qualitive and quantitative changes of chemical composition of the essential oils obtained from different species of *Z. absinthifolia* due to the effects of the harvesting time and season, the geographical location, the altitude, the climate and the other factors including the chemo type, the reproductive stage, the cutting height, the drying condition, which could extremely affect the yield and composition of volatile oils of the same species.[8,13] These findings could have important implications for collecting herbs from the best place and time to obtain the highest quality essential oils.

As it was illustrated in Table 2, the major constituents of Zae grown in different regions have shown significant differences. As it was evident in this table, the main constituents of the essential oils were not identical. The main similar chemical constituents were n-octanol, octyl acetate, octyl butyrate and β-caryophyllene, whereas ZaeM obtained the highest amount of n-octanol and octyl acetate among remaining ones. Likewise, ZaeF and ZaeKh contained the highest percentages of octyl butyrate and β-caryophyllene, respectively. So these findings further support the effect of the mentioned factors on the yield and chemical composition of the essential oils.

The plants of the Apiaceae family possess a range of compounds with many biological activities. Some of the previously reported pharmacological activities were their ability to induce apoptosis,[14] antibacterial,[15] hepatoprotective[16] and vaso-relaxant,[17] cyclooxygenase inhibitory effects and antitumorproperties.[18] In the recent years, there has been an increasing interest in screening herbs for their antibacterial activity due to development of new types of effective antimicrobial phyto compounds such as extracts of herbs and their essential oils, for using as the food preservatives or other goals.[19] Moreover, screening of the volatile oils as rich source of biologically active compounds have been carried out to indicate antibacterial activities of these natural products. So, it could be reasonable to expect a variety of phyto constituents in these oils with the antibacterial activity and antibiotic potential.[20] To the best of our knowledge, this was the first report on the antimicrobial activity of ZaeM. The results obtained from the *in vitro* antimicrobial test, showed that ZaeM indicated the inhibitory activity against *B. subtilis*, a gram positive strain. As seen in Table 1, octanol, as a long chain alcohol and a fundamental phyto constituent that includes almost a quarter of ZaeM (25.79%), has been reported to possess antimicrobial activity.[21]

Conclusion

As a conclusion, ZaeM could have antimicrobial potentials against *B. subtilis*. Further investigations are needed to isolate antimicrobial compounds from this volatile oil. Moreover, ZaeM worth to the further investigations for exploring further

biological activities as well as the isolation and identification of biologically active components.

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Conflict of interests

The authors claim that there is no conflict of interest.

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