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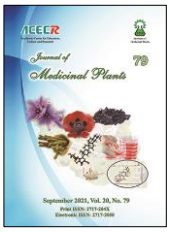

Research Article

Essential oil analysis and biological activities of the aerial parts of *Zygophyllum eichwaldii* C. A. Mey., a native plant from Iran

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ABSTRACT

Background: *Zygophyllum eichwaldii* C. A. Mey. is a medicinal plant from Zygophyllaceae family. This family consists of 27 genera and 285 species. **Objective:** The present study was performed to evaluate the chemical composition of the essential oil of *Z. eichwaldii* and evaluation of biological activities of its extracts. **Methods:** Essential oils extraction of *Z. eichwaldii* aerial parts was performed by three different procedures and its chemical composition were evaluated by GC and GC-MS analyses. Evaluation of the biological activities was carried out by spectrophotometric methods. **Results:** GC-MS analysis showed menthol, thymol and palmitic acid as major components of the plant essential oils. In biological activity evaluation, while methanol extract showed moderate to week potency in antioxidant assessments, the ethyl acetate fraction was strong in these tests (IC_{50} = 178.63 µg/ml in DPPH assay and 70.52 % inhibition in β-carotene/linoleic acid tests). This fraction also showed significant phenol, flavonoid and tannin contents (99.83, 118.29 and 188.05 µg/mg, respectively). Also, plant extracts exhibited considerable antimicrobial activities against most selected bacteria. **Conclusion:** Significant amount of phenol, flavonoid and tannin compounds in the ethyl acetate fraction and high antimicrobial activity against most bacterial strains, candidates this plant as a good case for further studies in this respect.

1. Introduction

Natural products, such as plants extract, either pure compounds or standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [1]. According to the World Health Organization (WHO), more than 80 % of the world's population relies on

Abbreviations: DPPH, 2,2-Diphenyl-1-PicrylHydrazyl; BHT, Butylated Hydroxyl Toluene; DMSO, Dimethyl Sulphoxide; HD, Hydrodistillation; SDE, Simultaneous Distillation Extraction; SFME, Solvent Free Microwave Extraction; GC, Gas Chromatography; GC/MS, Gas Chromatography/Mass Spectrometry; IROST, Iranian Research Organization for Science and Technology; NA, Nutrient Agar; SDA, Sabouraud Dextrose Agar; PDA, Potato Dextrose agar; BHI, Brain Heart Infusion; SDB, Sabouraud Dextrose Broth; MIC, Minimal Inhibition Concentration; MBC, Minimum Bactericidal Concentration; IC_{50}, Mean Inhibitory Concentration; SD, Standard Deviation; RI, Retention Indices

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traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. Many beneficial biological activities such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing were reported from the plants. In many cases, people claimed good benefit for certain natural or herbal products. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim. The first steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation [2].

The genus *Zygophyllum* belongs to the family Zygophyllaceae. According to some botanical sources, the number of genera belonging to this family is about 27 genera and 285 species, most of which are distributed in arid and semi-arid regions of the world. There are more than 9 species of herbaceous, semi-shrub and shrub plants of this genus in Iran.

Zygophyllum eichwaldii grows on sand dunes in relatively humid parts of the deserts. Geographical distribution of this plant is the central parts of the Iranian-Turanian zone (central deserts in Isfahan province and Khorasan Razavi province), Central Asia and Afghanistanat an altitude range of 780 to 1200 meters above the sea level. Different species of *Zygophyllum* are used as anti-diabetic, anti-spasmolytic, anti-eczema, antiseptic, anti-diarrheal and anti-inflammatory. *Z. eichwaldii* has antiseptic, anti-eczema and anti-diabetic activities [3].

In literature, there is no report on essential oil composition and biological activities of *Z. eichwaldii*. In the present study, essential oil chemical composition of *Z. eichwaldii* is reported. Meanwhile, extraction of aerial parts of this plant was prepared and evaluated for antioxidant and antimicrobial activity.

2. Materials and Methods

2.1. Plant material

The aerial parts (leaves, stems & flowers/inflorescences) of *Z. eichwaldii* were collected in September 2019 from sand dunes of northern Aran and Bidgol deserts, around Kashan (Isfahan province, Iran) at an altitude of ca. 980 m. The plant was authenticated and its voucher specimen was then deposited in the herbarium of Kashan Botanical Garden, Iran (Voucher No. HKBG: 2016).

2.2. Preparation of the extracts

2.2.1. Isolation of the essential oils

Three portions (150 g each) of the crushed plant material were individually subjected to three different essential oil extraction methods: usual hydrodistillation (HD) for 3.5 h using a Clevenger-type apparatus, simultaneous distillation extraction (SDE, a well-known distillation-extraction method for volatile materials extensively reviewed by Alain Chaintreau [4] with *n*-pentane as solvent for 2.5 h, and solvent free microwave extraction (SFME) in power 850 watts for 20 min. After decanting and drying over anhydrous sodium sulphate, yellow colored oils were recovered and stored at low temperature (4 ºC) under nitrogen atmosphere in amber vials and were used for analyses within a few days.

2.2.2. Extraction and fractionation

Dried powdered aerial parts of *Z. eichwaldii* (2 Kg) were subjected to extraction by maceration with enough volumes of methanol (7 L) for four times, to obtain total methanol extract (444.00 g). Five fractions were obtained from elution of total methanol extract by *n*-hexane (HE; 41.00 g), chloroform (CL; 18.50 g), ethyl acetate (EA; 7.25 g) and *n*-butanol (BU; 91.00 g), respectively and the residue was labeled as methanol extract residue (ME; 160.00 g). The obtained extracts were concentrated using a rotary evaporator under the reduced pressure at 40°C. The concentrated fractions were kept at refrigerator temperature for further investigations.

2.3. Chromatographic analysis

2.3.1. Gas chromatography (GC) analysis

Essential oil samples obtained from aerial parts of *Z. eichwaldii* were analyzed using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a FID detector using a HP-5MS 5 % phenylmethylsiloxane capillary column (60 m, 0.25 mm, 0.25 µm film thickness; Restek, Bellafonte, PA). Oven temperatures were programmed as follows: 60-246 ºC (3 ºC/min). Injector and detector temperatures were set at 220 ºC and 290 ºC, respectively. Ultra-high pure helium (flow rate: 1 ml/min), hydrogen (flow rate: 40 ml/min) and nitrogen (flow rate: 50 ml/min) were used as carrier, fuel and make up gases respectively and compressed air (flow rate: 450 ml/min) was used for combustion. Diluted samples (1/1000 in n-pentane, v/v) of 1.0 µl were injected manually in the splitless mode. Peak area percent of each compound relative to the area percent of the entire spectrum (100 %) were used for obtaining their quantitative data.

2.3.2. Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of the oils were carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (ionization energy: 70 eV), operating under the same conditions as described in 2.4.1., using a HP-5MS 5 % phenylmethylsiloxane capillary column (30 m, 0.25 mm, 0.25 µm film thickness; Restek, Bellafonte, PA). Retention indices were calculated for all components using a homologous series of *n*-alkanes injected in conditions equal to the sample one. Identification of components of essential oils were based on retention indices (RI) relative to *n*-alkanes and computer matching with the NIST14.L and W10N14.L libraries, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature [5].

2.4. Antioxidant activity

2.4.1. Radical scavenging assay

Radical-scavenging activities of the extracts were determined using a published 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity assay method with minor modifications [6].

Absorbance values were recorded on an ultraviolet and visible (UV–Vis) spectrometer at 517 nm using a blank containing the same concentration of DPPH radicals. Inhibition percentages (I %) of DPPH radicals were calculated using following equation:

$$I\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

2.4.2. β-Carotene/linoleic acid bleaching assay

The method described by Miraliakbari and Shahidi [7] was used for the evaluation of *β*-carotene/linoleic acid bleaching ability of the plant samples with slight modifications [8]. The absorbance values were measured at 470 nm onan ultraviolet and visible (UV–Vis) spectrometer. Antioxidant activities (inhibition percentages, I %) of the samples and BHT (positive control) were calculated using the equation:

$$I\ \% = (A_{\beta\text{-carotene after }2\ h}/A_{initial\ \beta\text{-carotene}}) \times 100$$

All tests were carried out in triplicate and inhibition percentages were reported as means ± SD of triplicates.

2.5. Quantitative estimation of total phenol, flavonoids and tannin contents

2.5.1. Estimation of total phenol content

Total phenol content of *Z. eichwaldii* extracts were calculated using published colorimeteric procedure involving Folin-Ciocalteu phenol reagent and gallic acid standard [9].

According to this test, total phenol compounds content of each extract, as gallic acid equivalent, were determined using its absorbance measured at 760 nm as input to the obtained standard curve and following equation.

$$\text{Absorbance} = 0.0012 \times \text{gallic acid (µg)} + 0.0033$$

All tests were carried out three times and obtained values as gallic acid equivalents were reported as mean ± SD of three determinations.

2.5.2. Estimation of total flavonoid content

The flavonoids content of the *Z. eichwaldii* extracts were determined by Aluminium chloride method using quercetin as reference compound [10]. The absorbances were measured against the blank at 415 nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of quercetin and their corresponding absorbance with the following equation:

$$\text{Absorbance} = 0.0079 \times \text{Quercetin (µg)} + 0.0206$$

Total flavonoid content of each extract, were determined using its absorbance measured at 415 nm as input to the obtained standard curve and equation and expressed as µg quercetin/mgdried extract. All tests were carried out three times and reported as mean ± SD of three determinations.

2.5.3. Estimation of total tannin content

The tannins were determined by Folin-Ciocalteu method and tannic acid as standard [10] Absorbance of standard solutions was measured against the blank at 725 nm with an UV/Visible spectrophotometer. A standard graph was plotted using various concentrations of tannic acid and their corresponding absorbance with the following equation:

$$\text{Absorbance} = 0.008 \times \text{tannic acid (µg)} + 0.0480$$

Total tannin content of each extract, was determined using its absorbance measured at 725 nm as input to the obtained standard curve and equation and expressed as µg tannic acid/mg dried extract. All tests were carried out three times and reported as mean ± SD of three determinations.

2.6. Antimicrobial activity

2.6.1. Microbial strains

The extracts and isolated compounds of *Z. eichwaldii* were individually tested against a panel of 12 and 6 microorganisms, respectively. Following microbial strains were provided by Iranian Research Organization for Science and Technology (IROST) and used in this research: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC

29737), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus epidermidis* (*CIP 81.55*), *Shigella dysenteriae* (PTCC 1188), *Streptococcus pyogenes* (ATCC 19615), *Salmonella paratyphi-A serotype* (ATCC 5702), *Candida albicans* (ATCC 10231) *Aspergillus brasiliensis* (ATCC 16404) and *Aspergillus niger* (ATCC 9029). Bacterial strains were cultured overnight at 37 ºC in nutrient agar (NA) and fungi were cultured overnight at 30 ºC in sabouraud dextrose agar (SDA).

2.6.2. Well diffusion assay

Determination of antimicrobial activity of the extracts and isolated compounds of *Z. eichwaldii* were accomplished by agar well diffusion method [11]. Gentamicin (10 µg/disc) and Rifampin (5 µg/disc) were used as positive controls for bacteria and Nystatin (100 IU) for fungi and yeast. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated three times [12].

2.6.3. Micro-well dilution assay

Minimal inhibition concentration (MIC) values of the extracts and isolated compounds of *Z. eichwaldii* were studied using micro-well dilution assay method [13]. Gentamicin and Rifampin for bacteria and Nystatin for yeast were used as standard drugs for positive control in conditions identical to tests materials. The MIC value was defined as the lowest concentration of the plant extracts required for inhibiting the growth of microorganisms. All tests were repeated three times.

2.6.4. MIC agar dilution assay

MIC values of the extracts for the fungi were evaluated based on the agar dilution method [14]. The MIC was defined as the lowest concentration of the compounds needed to inhibit the growth of microorganisms. Each test was repeated three times.

2.6.5. Minimum bactericidal concentration (MBC) assay

To determine the minimum concentration able to kill the bacteria the same microdilution assay described above was used [15]. The MBC was the lowest concentration able to effectively reduce the growth of microorganisms by 99.5 %. Each test was repeated three times.

3. Results

3.1. Chemical composition of the essential oils

The pale yellowish essential oils of dried aerial parts of *Z. eichwaldii* were obtained by hydrodistillation (HD), simultaneous distillation extraction (SDE) and solvent free microwave extraction (SFME) in the yields of 0.0122, 0.0159 and 0.0101 (% w/w on the basis of dried plant material), respectively. Essential oils were analyzed by GC/FID and GC/MS systems and the oils components were identified both quantitatively and qualitatively. Forty components were identified in the essential oils of the plant obtained from three different methods accounting for 96.52 %, 99.98 % and 99.99 % of the oils, respectively. Table 1 shows the percentage of volatile fraction compositions. Thirty two components (98.05 %) were identified in the oil obtained by HD with menthol (12.89 %), thymol (12.02 %), *β*-damascenone (8.21 %), hexadecanoic acid methyl ester (6.69 %) and carvone (4.71 %) as major components. The main constituents of the essential oil extracted using SDE apparatus were palmitic acid (28.36 %), (*Z*)-9,17-octadecadienal (21.22 %), linoleic acid (14.90 %), eicosane (11.28 %), 9,12-octadecadienoic acid methyl ester (9.45 %) and thymol (3.49 %) constituting 99.98 % of the oil.

Table 1. Chemical composition of the essential oils of *Z. eichwaldii*

NO	Compound[a]	Composition (%)			RI[b]	RI[c]	Identification method
		HD	SDE	SFME			
1	Linalool	3.15	-	2.14	1102	1103	d,f
2	Menthone	2.82	-	2.18	1163	1154	d,f
3	Neo-Menthol	2.14	-	-	1178	1165	d,f
4	Menthol	12.89	2.32	10.57	1186	1167	d,f
5	*α*-Terpineol	3.46	1.00	2.69	1203	1195	d,f
6	Citronellol	1.85	-	-	1229	1245	d,f
7	Carvone	4.71	1.01	2.64	1252	1246	d,f
8	Piperitone	0.98	-	1.38	1262	1268	d,f
9	Thymol	12.02	3.49	8.88	1294	1306	d,f
10	Carvacrol	1.39	-	-	1303	1312	d,f
11	(*E, E*)-2,4-Decadienal	1.59	-	-	1322	1326	d,f
12	(6*Z*, 8*E*) -(+)-2,5-Epoxy-6,8-megestigmadiene	1.26	-	-	1340	1312	d,f
13	Ascaridole	1.29	-	-	1351	1257	d,f
14	Geranyl acetate	1.53	-	-	1378	1378	d,f
15	*β*-Damascenone	8.21	1.94	4.27	1387	1383	d,f
16	Tetradecane	1.22	-	-	1400	1400	d,f
17	Dihydrodehydro-β-ionone	4.09	-	-	1417	1424	d,f
18	Butanoic acid, 2-methyl-, octyl ester	3.29	-	1.09	1433	1438	d,f
19	Geranyl acetone	3.03	-	-	1450	1453	d,f
20	*trans-β*-Ionone	1.32	-	-	1485	1487	d,f
21	Pentadecane	0.90	-	-	1500	1500	d,f
22	Dodecanoic acid, methyl ester	1.44	-	-	1523	1526	d,f
23	Caryophyllene oxide	2.46	-	-	1599	1582	d,f
24	Tetracosanoic acid, methyl ester	-	-	8.73	1599	1729	d,f
25	1,9-Tetradecadiene	-	1.12	-	1626	1411	d,f
26	Cyclopropaneoctanal, 2-octyl-	-	-	7.99	1672	2062	d,f
27	Pentadecanal	0.98	-	-	1717	1715	d,f
28	Tetradecanoic acid, methyl ester	1.12	-	-	1724	1722	d,f
29	Tetradecanoic acid, 12-methyl-, methyl ester	0.44	-	-	1795	1786	d,f
30	Eicosane	-	11.28	-	1802	2000	d,f
31	Hexahydrofarnesyl acetone	1.81	-	-	1843	1835	d,f
32	Linoleic acid	-	14.90	10.62	1843	2132	d,f
33	Farnesyl acetone	2.00	-	-	1805	1915	d,f
34	Hexadecanoic acid, methyl ester	6.69	1.65	6.17	1814	1926	d,f
35	Palmitic acid	2.71	28.36	10.88	1841	1959	d,f
36	2-Methyl-*Z,Z*-3,13-octadecadienol	-	2.24	3.46	1850	2104	d,f
37	Hexadecanoic acid, 14-methyl-, methyl ester	1.62	-	-	1866	1983	d,f
38	Oleic Acid	-	-	12.83	1866	2141	d,f
39	(*Z*)-9,17-Octadecadienal,	-	21.22	1.00	1895	2297	d,f
40	9,12-Octadecadienoic acid, methyl ester	2.11	9.45	2.47	1935	2088	d,f
	Total	96.52%	99.98%	99.99%			

[a] Compounds listed in order of elution from HP-5MS column.
[b] Relative retention indices to C_8–C_{24} n-alkanes on HP-5MS column.
[c] Literature retention indices.
[d] Retention index in HP-5MS column.
[e] GC/MS comparison with NIST08.L, W10N14.L and Adams (2001) libraries.

Finally, in the oil collected by SFME 18 components were identified, which made up 99.99 % of the total oil. The major constituents were oleic acid (12.83 %), palmitic acid (10.88 %), linoleic acid (10.62 %), menthol (10.57 %) and thymol (8.88 %).

3.2. Antioxidant activity

In vitro antioxidant tests are designed to mimic the oxidation–reduction reactions popularly occurring in the live biological systems and are used to estimate the antioxidant potentials of various chemical and biological samples. In this work, two classical antioxidant tests namely DPPH and *β*-carotene/linoleic acid tests were carried out.

In 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical test the capacity of the samples to donate hydrogen atom and/or electron to this blue/purple stable radical and converting it to yellow diphenyl picrylhydrazine molecule was measured [16]. This reaction is used for measuring the ability of the extracts or pure molecules (such as BHT) to scavenge free radicals. The results of this test are presented in Table 2. Compared to synthetic standard antioxidant BHT (IC_{50} = 19.20 ± 0.68 µg/ml), extracts and fractions of the plant exhibited moderate to weak radical-scavenging activities, but the free radical scavenging activity of the ethyl acetate fraction (IC_{50} = 178.63 ± 0.85 µg/ml) was superior to that of other extracts and methanol extract residue showed the weakest activity (IC_{50} = 1098.83 ± 1.18 µg/ml).

Potential ability of the antioxidants to delay lipid peroxidation by reacting with chain propagating peroxyl radicals faster than the reaction of these radicals with proteins or fatty acid side chains is usually evaluated by *β*-carotene/linoleic acid test [17]. In this test, the inhibition percentages of the extract and fractions of *Z. eichwaldii* on the oxidation of β-carotene in the presence of linoleic acid oxidation intermediates are listed in Table 2. The effectiveness of the extract ethyl acetate fraction (70.52 ± 0.99 %) was comparable to that of BHT (89.70 ± 1.05 %), but the inhibition values observed for other extracts were lower than that of BHT, and again methanol extract residue showed the weakest activity (14.64 ± 0.64 %).

Table 2. Antioxidant activities of aerial parts from *Z. eichwaldii*

NO.	Sample	DPPH assay (IC_{50}, µg/mL)	*β*-Carotene assay (% I)
1	Total methanol extract	460.81 ± 0.93	53.20 ± 0.53
2	Hexane fraction	548.15 ± 1.15	50.85 ± 0.42
3	Chloroform fraction	407.94 ± 1.06	59.33 ± 0.65
4	Ethyl acetate fraction	178.63 ± 0.85	70.52 ± 0.99
5	*n*-Butanol fraction	580.16 ± 0.89	61.58± 1.09
6	Methanol extract residue	1098.83 ± 1.18	14.64 ± 0.64
7	BHT	19.20 ± 0.68	89.70 ± 1.05

Values are mean ± SD (n = 3)

Table 3. Quantitative estimation of total phenol, flavonoids and tannin content of *Z. eichwaldii* aerial parts

NO.	Sample	Total phenol contents Gallic acid equivalent (µg/mg)	Total flavonoid content Quercetin equivalent (µg/mg)	Total tannin content Tannic acid equivalent (µg/mg)
1	Total methanol extract	68.33 ± 1.10	58.30 ± 1.03	63.32 ± 1.30
2	Hexane fraction	60.30 ± 1.06	62.13 ± 0.89	60.69 ± 1.11
3	Chloroform fraction	79.33 ± 0.65	78.26 ± 1.30	79.43 ± 1.14
4	Ethyl acetate fraction	99.83 ± 0.92	118.29 ± 0.68	188.05 ± 0.92
5	*n*-Butanol fraction	53.50 ± 0.98	68.48 ± 0.93	67.45 ± 1.13
6	Methanol extract residue	11.42 ± 0.82	12.01 ± 0.72	41.7 ± 1.08

Values are mean ± SD (n = 3)

3.3. Quantitative estimation of total phenol, flavonoids, and tannin

Total phenol content of the plant extracts were determined using a colorimetric assay method based on Folin-Ciocalteu reagent reduction. Results, expressed as gallic acid equivalents, and presented in Table 3. As expected, due to the major contribution of phenol compounds in antioxidant activity, ethyl acetate fraction showed the highest phenol content (99.83 ± 0.92 µg/mg) and methanol extract residue showed the lowest phenol content (11.42 ± 0.82 µg/mg). Other extracts and fractions of the plant showed moderate to weak amounts of phenol compounds content.

The content of total flavonoids in extractsand fractions were determined and reported (Table 3) asµg per mg of the dried extracts based on quercetin. The total flavonoid varied from 12.01 ± 0.72 to 118.29 ± 0.68µg/mg and the in the extracts. Maximum flavonoid content was found in the ethyl acetate fraction (118.29 ± 0.68µg/mg) of the plant. The content of tannin expressed as tannic acid equivalents, varied from 41.7 ± 1.08 to 188.05 ± 0.92 µg tannic acid equivalent/mg extract (Table 3). The ethyl acetate fraction showed the highest amount of tannin contents (188.05 ± 0.92 µg/mg).

3.4. Antimicrobial activity

Antimicrobial activity of *Z. eichwaldii* extracts against a panel of 12 microorganisms was examined and their potencies were assessed both qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC & MBC values. The results are given in Table 4. The maximum inhibition zones and MIC values for microbial strains sensitive to the extracts of the plant were in the range of 8-12 mm and 15.63-1000 µg/ml, respectively.

Total methanol extract showed great antimicrobial activities against *Candida albicans*, *Escherichia coli*, *pseudomonas aeruginosa* and *Streptococcus pyogenes* (MIC & MBC < 15.63 µg/ml), Also, ethyl acetate fraction showed considerable antimicrobial activities against *Candida albicans* and *pseudomonas aeruginosa* (MIC & MBC < 15.63 µg/ml).

On the other hand, methanol extract residue of the plant showed weak antimicrobial activities compared to other extracts in both MIC and MBC assay tests. The fungi strains tested *Aspergillus niger* and *Aspergillus brasiliensis* were weakly sensitive to all samples of the plant.

Table 4. Antimicrobial activities of the extracts of *Z. eichwaldii* aerial parts against tested microbial strains

Test Microorganism	Total methanol extract			Hexane fraction			Chloroform fraction			Ethyl acetate fraction		
	ZI [a]	MIC[b]	MBC[c] or MFC[d]	ZI	MIC	MBC or MFC	ZI	MIC	MBC or MFC	ZI	MIC	MBC or MFC
***A. brasiliensis* (ATCC 16404)**	—	—	1000	—	—	1000	—	—	1000	—	—	1000
***A. niger* (ATCC 9029)**	—	—	1000	—	—	1000	—	—	1000	—	—	1000
***B. subtilis* (ATCC 6633)**	—	31.25	62.50	—	31.25	250	—	125	500	—	31.25	500
***C. albicans* (ATCC 10231)**	—	< 15.63	< 15.63	—	< 15.63	< 15.63	—	62.50	62.50	—	< 15.63	< 15.63
***E. coli* (ATCC 25922)**	—	< 15.63	< 15.63	—	62.50	62.50	—	62.50	250	—	62.50	62.50
***K. pneumonia* (ATCC 10031)**	—	< 15.63	62.50	—	31.25	1000	—	62.50	1000	—	62.50	1000
***P. aeruginosa* (ATCC 27853)**	—	< 15.63	< 15.63	—	31.25	62.50	—	< 15.63	< 15.63	—	< 15.63	< 15.63
***S. paratyphi-A serotype* (ATCC 5702)**	—	125	> 1000	—	31.25	500	—	62.50	1000	—	62.50	500
***S. dysenteriae* (PTCC 1188)**	—	125	> 1000	—	31.25	1000	—	62.50	125	—	62.50	125
***S. aureus* (ATCC 29737)**	—	250	1000	—	250	1000	—	250	> 1000	—	500	1000
S. epidermidis (CIP 81.55)	9	125	500	11	62.50	500	—	125	250	8	250	500
***S. pyogenes* ATCC 19615**	—	< 15.63	< 15.63	—	31.25	31.25	—	250	250	12	62.50	62.50

A dash (-) indicate no antimicrobial activity.

[a.] ZI: Zone of inhibition in diameter (mm); [b.] MIC: Minimal inhibition concentrations (µg/ml)

[c] MBC: Minimum Bactericidal Concentrations (µg/ml); [d] MFC: Minimum Fungicidal Concentrations (µg/ml)

Table 4. Antimicrobial activities of the extracts of *Z. eichwaldii* aerial parts against tested microbial strains (Continue)

Test Microorganism	*n*-Butanol fraction			Methanol extract residue			Antibiotics					
							Rifampin		Gentamicin		Nystatin	
	ZI [a]	MIC[b]	MBC[c] or MFC[d]	ZI	MIC	MBC or MFC	ZI	MIC	ZI	MIC	ZI	MIC
***A. brasiliensis* (ATCC 16404)**	—	—	1000	—	—	1000	NA[e]	NA	NA	NA	30	31.2
***A. niger* (ATCC 9029)**	—	—	1000	—	—	1000	NA	NA	NA	NA	27	31.2
***B. subtilis* (ATCC 6633)**	—	62.50	62.50	—	62.50	> 1000	19	31.25	30	3.90	NA	NA
***C. albicans* (ATCC 10231)**	—	< 15.63	62.50	—	< 15.63	< 15.63	NA	NA	NA	NA	33	125
***E. coli* (ATCC 25922)**	—	62.50	62.50	—	125	125	11	3.90	20	3.90	NA	NA
***K. pneumonia* (ATCC 10031)**	—	62.50	1000	—	62.50	250	8	15.63	17	3.90	NA	NA
***P. aeruginosa* (ATCC 27853)**	—	< 15.63	62.50	—	< 15.63	250	-	62.50	22	3.90	NA	NA
***S. paratyphi-A serotype* (ATCC 5702)**	—	62.50	1000	—	62.50	1000	8	15.63	18	3.90	NA	NA
***S. dysenteriae* (PTCC 1188)**	—	62.50	1000	—	62.50	1000	9	15.63	17	3.90	NA	NA
***S. aureus* (ATCC 29737)**	—	500	1000	9	250	1000	21	31.25	27	1.95	NA	NA
S. epidermidis (CIP 81.55)	—	125	250	8	125	250	27	1.95	45	1.95	NA	NA
***S. pyogenes* ATCC 19615**	10	125	125	10	125	125	21	0.975	32	0.975	NA	NA

A dash (-) indicate no antimicrobial activity.

[a.] ZI: Zone of inhibition in diameter (mm); [b.] MIC: Minimal inhibition concentrations (µg/ml)

[c] MBC: Minimum Bactericidal Concentrations (µg/ml); [d] MFC: Minimum Fungicidal Concentrations (µg/ml)

[e] NA: Not Applicable

4. Discussion

Chemical compositions of the essential oils of *Z. eichwaldii* extracted by three different methods HD, SDE and SFME were presented in Table 1. The overall yields of essential oil extraction by all of these methods were low recording a low essential oil production potential for the plant. The plant essential oils mainly consist of alcoholic, ketonic and peroxide monoterpene hydrocarbons and fatty acids and their esters. Due to aqueous media used in the HD and SFME methods, identified components are mainly classical terpene essential oils components leaving behind water insoluble fatty acid of the plant unextracted. On the other hand, using n-pentane as an organic media in the SDE method, led to the extraction of fatty acids of the plant and appearance of them as the major constituents of the oil obtained by this method.

One hundred components have been reported in the essential oil of *Zygophyllum album* aerial parts and (E)-β-Damascenone (11.8 %) was reported as the major component [18]. Kchaou et al [19], reported 19 components in the essential oil obtained from the fresh leaves of *Z. album* and 2,6-Di (tert-butyl) phenol, delta decalactone, cocolactone and carvacrol were detected as major constituents. A study of the sources revealed that there is no report on essential oil composition of *Z. eichwaldii* and the results in this work are reported for the first time.

According to the results of antioxidant activity tests, antioxidant capacity of the ethyl acetate fraction of the plant in DPPH test showed the highest antioxidant activity compared to other extracts may be a consequence of its high phenol compounds content which was reflected in its Folin–Ciocalteu test result. The ethyl acetate fraction of *Z. eichwaldii* was also considerably potent in blocking of the oxidation of β-carotene by the active peroxyl radicals resulted from the oxidation of linoleic acid molecules in the presence of O_2 and an activity comparable to that of BHT was recorded for it (Table 2), which may be a consequence of the presence of molecules with easy hydrogen donating parts such as allylic and/ or benzylic functional groups. Other extracts and fractions of the plant were moderately active in this test and showed inhibition percentage of about two third of that of BHT.

Antioxidant capacity may be associated with high phenol content. Structurally, phenols comprise of anaromatic ring bearing one or more hydroxyl substituents. The antioxidant activity of this type of molecule is due totheir ability to scavenge free radicals, donate hydrogen atoms or electrons or chelate metal cations.

There are few reports on the antioxidant activity of the *Zygophyllum* genus plant species [20] such as *Z. simplex* [21], *Z. Album* [22], *Z. cocceniem* [23], and *Z. cornutum* [24]. But, according to our best knowledge, literature has no report on the antioxidant activity of the *Z. eichwaldii* and this is the first one.

Natural antioxidants such as phenols, flavonoids and tannins are increasingly attention attracting because they are natural disease preventing, health promoting and anti-ageing substances. Phenol are the most abundant widely distributed secondary metabolites of the plants, with about 8000 structures currently identified. Plant phenolics include phenolic acids, flavonoids, tannins, stilbenes and lignans, are a class of antioxidant agents acting as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. Several pharmacological activities have been associated with phenolic compounds. These include anti-ulcer, anticancer, antioxidant, anti-inflammatory, antimicrobial and anticholinesterase [25]. Flavonoids are a group of polyphenolic

compounds with known properties including free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight capable of forming complexes with proteins [26]. Based on the antioxidant activity results (Table 2) and as shown in Table 3, the ethyl acetate fraction of the plant showed the highest amount of total phenol, flavonoid and tannin contents. Literature review shows the presence of different hydroxyl containing and phenolic compounds such as polyphenols, tannins, phenolic acids and flavonoids, in the plants of the genus *Zygophyllum* [20-22, 27]. But, literature has no report on the estimation of total phenol, flavonoids, and tannin of *Z. eichwaldii* and this is the first one.

As can be seen from the antimicrobial test results, total methanol extract and ethyl acetate fraction of the plant showed stronger and broader range of antimicrobial activity than the other samples, compared with positive control drugs Rifampin, Gentamicin and Nystatin. Also, the weakest antimicrobial activity was observed in methanol extract residue of the plant in both MIC and MBC assay tests.

Although, this is the first report on antimicrobial activity of *Z. eichwaldii,* the only report in literature on antimicrobial activity of a plant extract from the genus *Zygophyllum* and shows considerable antimicrobial activity for leaves aqueous extract of this plant [28].

5. Conclusions

This report is on the essential oil composition and biological activities of the plant extracts of *Z. eichwaldii*. The plant was detected as a poor source of essential oil quantitatively, but the monoterpene contents of its essential oil are mainly oxygenated and commercially valuable and candidates the plant as a potential source of aromatic raw material for health, hygiene and cosmetic industries. Ethyl acetate fraction showed both the highest antioxidant activities and phenolic, flavonoid and tannin contents. High antimicrobial activity against most bacterial strains, candidates this plant as a good case for further studies.

Author contributions

All authors contributed toward data analysis, drafting, and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of interest

The authors declare that there is no conflict of interest.

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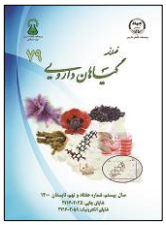

مقاله تحقیقاتی

بررسی ترکیبات شیمیایی اسانس و ارزیابی خواص بیولوژیکی سرشاخه‌های هوایی قیچ کاشانی (*Zygophyllum eichwaldii* C. A. Mey.)، گیاهی بومی ایران

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اطلاعات مقاله

گل‌واژگان:
قیچ کاشانی
خانواده اسفند
اسانس‌ها
کروماتوگرافی گازی
متصل به
طیف‌سنجی جرمی
آنتی‌اکسیدانی
ضدمیکروبی

چکیده

مقدمه: گیاه قیچ کاشانی *Zygophyllum eichwaldii* C. A. Mey. یک گیاه دارویی متعلق به خانواده Zygophyllaceae (اسفند) است. این خانواده مشتمل بر ۲۷ جنس و ۲۸۵ گونه است. **هدف:** مطالعه حاضر برای بررسی ترکیبات شیمیایی اسانس گیاه قیچ کاشانی و ارزیابی فعالیت‌های بیولوژیکی عصاره‌های آن می‌باشد. **روش بررسی:** استخراج اسانس از سر شاخه هوایی گیاه قیچ کاشانی توسط سه روش مختلف انجام و ترکیبات شیمیایی اسانس‌های حاصل با روش کروماتوگرافی گازی و کروماتوگرافی گازی متصل به طیف‌سنجی جرمی شناسایی شد. ارزیابی فعالیت‌های بیولوژیکی با استفاده از روش‌های اسپکتروفتومتری انجام شد. **نتایج:** آنالیز کروماتوگرافی گازی متصل به طیف‌سنجی جرمی نشان داد که منتول، تیمول و پالمیتیک اسید، از اجزای اصلی اسانس‌های استخراج شده از گیاه هستند. در بخش فعالیت‌های بیولوژیکی، در حالی که عصاره متانولی در ارزیابی‌های آنتی‌اکسیدانی قدرت متوسط تا ضعیف را نشان داد، بخش اتیل استاتی در این آنالیزها قوی بود (IC_{50} برابر با ۱۷۸/۶۳ میکروگرم بر میلی لیتر در سنجش DPPH و ۷۰/۵۲ درصد مهار در آزمون بتا-کاروتن/ اسید لینولئیک). این بخش اتیل استاتی همچنین محتوای قابل توجه فنلی، فلاونوئیدی و تانینی را نشان داد (به ترتیب برابر با ۹۹/۸۳، ۱۱۸/۲۹ و ۱۸۸/۰۵ میکروگرم بر میلی‌گرم). همچنین، عصاره‌های گیاه فعالیت‌های ضد میکروبی قابل توجهی را در برابر بیشتر باکتری‌های انتخاب شده نشان دادند. **نتیجه‌گیری:** مقدار قابل توجهی از ترکیبات فنولی، فلاونوئیدی و تانینی در بخش اتیل استات و فعالیت ضد میکروبی بالا در برابر بیشتر سویه‌های باکتریایی، این گیاه را به عنوان یک مورد مناسب برای مطالعات بیشتر در این زمینه معرفی می‌کند.

مخففها: DPPH، ۲،۲- دی فنیل-۱- پیکریل هیدرازیل؛ BHT، بوتیل هیدروکسی تولوئن؛ DMSO، دی متیل سولفوکسید؛ HD، تقطیر با آب؛ SDE، تقطیر همزمان با استخراج؛ SFME، استخراج بدون حلال با ماکروویو؛ GC، کروماتوگرافی گازی؛ GC/MS، کروماتوگرافی گازی متصل به طیف‌سنجی جرمی؛ IROST، سازمان پژوهش‌های علمی و صنعتی ایران؛ NA، نوترینت آگار؛ SDA سابرو دکستروز آگار؛ PDA، پوتیتو دکستروز آگار، BHI، برین هارت اینفیوژن؛ SDB، سابرو دکستروز براث؛ MIC، حداقل غلظت مهارکنندگی رشد؛ MBC، حداقل غلظت کشندگی باکتریایی؛ IC_{50}، غلظت مهارکنندگی میانگین؛ SD انحراف معیار؛ RI، اندیس بازداری

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