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Chemical composition and bioactivities of the volatile oil of the seeds of *Eryngium bungei* Boiss.

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The volatile oil of the seeds of *Eryngium bungei* Boiss. (family Apiaceae), was obtained by hydrodistillation and analyzed by the GC-MS and the GC-FID. The analyses revealed at least 69 compounds representing 94 % of the total oil. The results showed that the oil was dominated by chrysanthenyl acetate (20.0 %), spathulenol (17.2 %), *endo*-isofenchol (10.8 %) and α-pinene (5.1 %). The free-radical-scavenging activity of the oil was evaluated by DPPH assay and RC_{50} value was calculated as 7.5 µg/mL. The antifungal and phytotoxic activities of the oil were tested against *Sclerotinia sclerotiorum*, and some common weeds. The MIC value for anti sclerotinia activity of the seed oil was evaluated as 12.5 µg/mL. The IC_{50} values were determined as 1.32-2.1 µg/mL for inhibitory effects of the oil on seed germination of different weeds. This is the first report on chemical composition and bioactivities of the volatile oils of the seeds of *Eryngium bungei.*

Keywords: Allelopathy, Antifungal activity, Chrysanthenyl acetate, *Eryngium bungei*, Phytotoxicity.
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Introduction

The genus *Eryngium* L. of Apiaceae family comprises 250 annual or perennial species distributed all over the world. It is regarded as one of the largest genera of the Apiaceae with well known pharmaceutical and biological properties[1-6]. *Eryngium bungei* Bioss., commonly known as "*Zool Khorasani*" and "*Chichagh*", is the most common species of the genus distributed from Iran to Afghanistan and Central Asia[7]. It has been used in Iranian folk medicine from ancient time[8]. The plant is well known as an appetizer, diuretic and stimulant agent[9-11]. It also exhibits cytotoxic, anti-inflammatory, antibacterial, antifungal, antimalarial, antioxidant, and antihyperglycemic properties[3]. The plant leaves are used as a vegetable, and food flavouring agent, as well[3-8]. As part of the on-going studies on the volatile oils from the Iranian flora[12-15],the authors report the composition of the volatile oils from the seeds of *E. bungei* and its bioactivities, for the very first time.

Materials and Methods

Plant material

The seeds of *Eryngium bungei* Boiss. were collected during August 2012 from Oojan, (Birjand, east of Iran), at 59° 17' east longitude and 32° 56' north latitude and 1875 m altitude. The plant was identified in Department of Biology, University of Mohaghegh, Ardabili. A voucher specimen (No. 1391-3) was deposited in the herbarium of the Faculty of Basic Sciences, University of Mohaghegh, Ardabili.

Essential oil hydrodistillation

The ground dried seeds (100 g) of *E. bungei* were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours to yield 0.25 % of a yellowish oil. The extraction process was repeated several times to obtain enough amounts for biological assays. The oils were dried over anhydrous sodium sulphate and stored at 4 °C in the dark until tested and analyzed[16].

GC-MS and GC-FID conditions

The volatile oil was analyzed by the GC-MS using a Hewlett Packard GC-MS (GC-7890A & MS-5975C) equipped with a HP-5 fused silica capillary column (30 m×0.25 mm, film thickness 0.25 µm), which was programmed as follows: 60 °C (3 minutes), then 60-230 at 5 °C/min and finally held isothermal at 230 °C for 10 minutes. The carrier gas was helium (99.999 %) at a flow rate of 1 mL/ min. Diluted sample (1/20, v/v, in *n*-hexane) of 1.0 µL was injected manually in a splitless mode. The relative percentage of the volatile oil constituents was expressed as percentages by peak area normalization. Linear retention indices then were calculated by injecting series of *n*-hydrocarbons (C_6-C_{24}) after the oil at the same temperature and conditions.

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For quantitation (area %), the GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a HP-5 fused silica column (30 m×0.25 mm, film thickness 0.25 µm). Nitrogen was used as the carrier gas at the constant flow of 1 mL/min, the split ratio was same as it was for the GC-MS. The oven temperature was raised from 60 to 230 ºC at a rate of 5 ºC/min and held for 10 minutes. The injector and detector (FID) temperatures were kept at 230 and 280 ºC, respectively. Semi-quantitative data was obtained from FID area percentages without the use of correction factors**.** To obtain the same elution order as with GC-MS, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of the essential oil components

Identification of compounds was performed based on direct comparison of the Kovats indices and MS data with those for standard compounds, and computer matching with the NIST NBS54K Library and Wiley Library[17,18]. Calculations were performed by using retention times of *n*-alkanes (C8-C20), which were injected after the oil at the same temperature and conditions.

DPPH assay

The free-radical-scavenging property of the volatile oil of *E. bungei* was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described in the literature[19-21]. DPPH was purchased from Fluka Chemie AG, Bucks. The essential oil was dissolved in $CHCl_3$ to obtain the stock concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 5×10^{-1}, 2.5×10^{-1}, 1.25×10^{-1}, 6.25×10^{-2}, 3.13×10^{-2} and 1.56×10^{-2} mg/mL. Diluted solutions (5 mL each) were mixed with DPPH (5 mL; 0.08 mg/mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control ascorbic acid. The percentage of DPPH radical scavenging was estimated, using the following equation:

$$I\ (\%) = 100[(Ac - As)/Ac]$$

where, Ac and As are the absorbance of the negative control and the samples, respectively. RC_{50} values (the half maximal reducing concentration) were then calculated, using the fitted regression line equation in Excel software, and were expressed in µg/mL.

Fungitoxic assay

The fungitoxic assay was conducted in Completely Randomized Design (CRD) with three replications to evaluate the efficacy of the volatile oil as an antifungal agent against *Sclerotinia sclerotiorum* (KACC 41065), using the Poisoned Food Technique[22]. Four concentrations of the volatile oil (1, 10, 100 and 1000 µg/mL) were prepared in combination with the molten potato dextrose agar (PDA) medium[23] using Tween-20 (0.01 % v/v) Sterile Petri dishes (90 mm diameter) containing 15 mL of PDA were used. The positive and negative controls were amphotericin B (10 µg/mL) and distilled water, respectively. In order to eliminate the possible effect of Tween-20, the same amount of Tween-20 (0.01 % v/v) was used to prepare the solutions. The plates were inoculated with 6 mm plugs of 7-day-old cultures, and their border was sealed with a plastic film[24] and incubated at 25 °C for 3-7 days until the mycelia growth in the negative control plates reaches the edges of the plates[25]. Growth inhibition of the fungal strain was calculated as the percentage of inhibition of radial growth relative to the negative control, using the equation:

$$I_{MG}\ (\%) = 100[(Dc - Dt)/Dc]$$

Where I_{MG} (%) is inhibition of mycelial growth, Dc and Dt are average diameters of fungal mycelia growth in the negative control and treatment groups, respectively.

The minimum inhibitory concentration (MIC), defined as the lowest concentrations that inhibited the visible growth of fungal mycelia after incubation (growth was indicated as turbidity), of the volatile oil and amphotericin B against *S. sclerotiorum* was determined by two-fold dilution method. Six dilutions (6.25, 12.5, 25, 50, 100 and 200 µg/mL) of the test samples were prepared separately, using potato dextrose broth (PDB) and Tween-20 (0.01 % v/v). A control medium was used without oil and amphotericin B, but with 0.01 % v/v Tween-20. The media were inoculated with10 µL fungal spore suspension and incubated at 25-30 °C for 48 h.

Phytotoxicity assay

Phytotoxicity assays were conducted with four replications to examine the effects of the volatile oil

on seed germination, and radicle and epicotyl growth of Lettuce (*Lactuca sativa*), Amaranth (*Amaranthus retroflexus*), Branyard grass (*Echinochloa crus-gali*), Wild mustard (*Sinapis arvensis*), Purslane (*Portulaca oleracea*) and Nightshade (*Solanum nigrum*). All plant seeds were surface sterilized with sodium hypochloride (1 %) for 10 min and rinsed with sterile distilled water. Three concentrations of the volatile oil (1, 10 and 100 µg/mL) were prepared by dilution in distilled water, using Tween-20 (0.01 % v/v). A total of 25 seeds were treated with 5 mL of each concentration of the volatile oil, in sterile Petri dishes (90 mm diameter) lined with one sterile filter paper (Whatman, number 2). A control test was performed with distilled water with 0.01 % v/v Tween-20. The seeds were placed uniformly to avoid competition during germination. The dish border was sealed with a plastic film[24]. Petri dishes were incubated at 25 °C with 12 h of photoperiod. The length of radicle and epicotyl and germination of all seeds were recorded on the 7th day. Seedlings with radicle protruding at least 1 mm through the seed coat were counted as germinated. The phytotoxic potential of the essential oil was calculated using the equation:

$$I_{GS}\ (\%) = 100[1- (GS_{Sample}/GS_{Control})]$$

Where I_{GS} (%) is inhibition of seeds germination, GS_{Sample} is the number of germinated seeds on the petri dishes where the volatile oil was applied, $GS_{ControL}$ is the number of germinated seeds on the petri dishes where the essential oil was not applied. To calculate the inhibition percentages of radicle and epicotyl growth, the above equation was modified where seed germination (GS) is replaced by radicle growth (RG) and epicotyl growth (EG), respectively; as follows:

$$I_{RG}\ (\%) = 100[1- (RG_{Sample}/RG_{Control})] \text{ and } I_{EG}\ (\%) = 100[1- (EG_{Sample}/EG_{Control})]$$

The half maximal inhibitory concentration (IC_{50}) values which correspond to the concentrations of the oil that gave 50 % phytotoxic effect[25], were calculated using the fitted regression line equation in Excel software and were expressed in µg/mL.

Statistical analysis

Statistical analysis of the data was performed using IBM SPSS Statistics 20 software, and the related means were compared by Duncan test at $p \leq 0.01$. Graphs were plotted using Excel 2013 software, the data were fitted with linear regression at the intercept of zero. RC_{50} and IC_{50} values were then estimated using the fitted regression line equation.

Results and Discussion

The GC-MS and the GC-FID analyses of the volatile oil of the seeds of *E. bungei* identified 69 compounds representing 94 % of the oil. The oil was dominated by chrysanthenyl acetate (20.0 %), spathulenol (17.2 %), *endo*-isofenchol (10.8 %) and α-pinene (5.1 %) as the characteristic constituents of the oil (Table 1). Oxygenated monoterpenes (38.9 %), oxygenated sesquiterpenes (28.5 %), monoterpenes (6.9 %), sesquiterpenes (6 %), alkanes (5.7 %) and

Table 1 — GC-MS and GC-FID data of the components of the volatile oils of the seeds of *Eryngium bungei* Boiss.

S.No.	Component	Retention time (min)	Real area (%)	Calculated Kovats indices (KI)
1	n-Octane	3.33	3.2	800
2	(E)- 2-hexenal	4.26	0.1	857
3	n- Hexanol	4.57	0.1	861
4	Heptanal	5.35	0.6	885
5	α-Pinene	6.26	5.1	936
6	Benzaldehyde	6.75	0.2	978
7	Verbenene	6.80	1.0	976
8	β-Pinene	7.37	0.1	987
9	1-Hexyn-3-ol	7.63	0.1	997
10	2-Pentyl-furan	7.76	0.2	1000
11	n-Decane	8.03	1.0	1001
12	n- Octanal	8.18	3.1	1023
13	Limonene	8.86	0.5	1031
14	1,8-Cineole	8.92	0.1	1033
15	(E)-β-Ocimene	9.11	0.2	1047

(*Contd*.)

Table 1 — GC-MS and GC-FID data of the components of the volatile oils of the seeds of *Eryngium bungei* Boiss. (*Contd*.)

S.No.	Component	Retention time (min)	Real area (%)	Calculated Kovats indices (KI)
16	2-Octenal	9.68	0.1	1055
17	n-Octanol	10.06	0.1	1075
18	2-Nonanone	10.70	0.5	1092
19	(E)-2,6-dimethyl-2,4,6-Octatriene	10.84	0.1	1099
20	α-Terpinolene	10.98	0.2	1102
21	n-Nonanal	11.08	0.4	1104
22	*trans*-Chrysanthenol	11.44	0.2	1149
23	Verbenol	12.89	3.8	1193
24	β-Citral	12.31	0.2	1230
25	(E)-2-Decenal	12.72	0.2	1252
26	Chrysanthenyl Acetate	16.11	20.0	1262
27	Di-ethyl ether	16.42	0.1	1301
28	Carvacrol	16.61	3.7	1314
29	3-Pyridinol	17.06	0.3	1327
30	2,4-Decadienal	17.16	0.1	1332
31	Camphene	17.74	0.5	1356
32	*p*-Menthane	17.94	0.1	1369
33	α-Cubebene	18.06	0.1	1376
34	α-Copaene	18.76	0.3	1390
35	2-Butanone	18.94	0.2	1391
36	β-Elemene	19.18	0.2	1392
37	n-Tetradecane	19.29	0.1	1400
38	α-Gurjunene	19.67	0.4	1415
39	β-Funebrene	19.78	0.1	1428
40	*trans*-Caryophyllene	19.92	0.3	1437
41	Germacrene D	20.15	0.1	1462
42	*AR*-Curcumene	21.47	0.5	1493
43	Bicyclogermacrene	21.80	1.9	1500
44	Cuparene	22.00	0.2	1510
45	*endo*-Isofenchol	22.46	10.8	1513
46	Δ-Cadinene	22.50	1.9	1514
47	α-Muurolene	22.86	0.1	1516
48	α-Calacorene	22.98	0.1	1517
49	Elemol	23.14	0.8	1527
50	Palustrol	23.61	0.5	1565
51	Spathulenol	24.19	17.2	1640
52	ν-Costol	24.22	0.6	1643
53	Viridiflorol	24.34	0.8	1649
54	Ledol	24.62	3.6	1657
55	Aromadendren Epoxide	24.67	0.1	1662
56	Hinesol	25.07	0.4	1688
57	Isospathulenol	25.53	2.3	1695
58	Benzeneacetic acid	25.43	1.0	1696
59	Caryophyllenol	25.90	0.6	1709
60	α-Bisabolol	26.23	1.4	1718
61	Camphen	26.62	0.2	1726
62	Aromadendrene oxide	26.92	0.2	1728
63	Isoaromadendrene epoxide	27.04	0.1	1730
64	m-Cymene	27.14	0.2	1733
65	Mintsulfide	27.41	0.1	1750
66	Benzylbenzoate	27.86	0.1	1762
67	Eicosane	28.49	0.1	1809
68	Methyl palmitate	31.03	0.1	1928
69	9-Octadecenoic acid	34.34	0.1	2124
Total			94.0 %	

aldehydes (4.6 %) were the predominant classes of components present in the oil. A comparison of our results with those of the previous reports revealed that the volatile oil profile of the plant seeds was significantly different from the other plant parts. Whereas, the volatile oil of the plant aerial parts was dominated by borneol (44.4 %), isobornyl formate (14.7 %), isoborneol (9.2 %), 1,8-cineol (9.1 %) and camphor (7.9 %)[5], the seeds oil was enriched by chrysanthenyl acetate (20.0 %) and spathulenol (17.2 %). On the other hand, other study reported curmin alcohol (55.3 %) a terpinolene (14.6) as the major components in the plant aerial parts oil[6] It is consistent with the fact different habitats may affect plant metabolic pathways to produce various phytochemicals.

Previous studies on the volatile oils of a few other *Eryngium* species, e.g., *E. billardieri*, *E. caeruleum* and *E. caucasicum*, revealed monoterpenes and sesquiterpenes as two major classes of components, and the dominant constituents of the volatile oils of the inflorescences and seeds of *Eryngium* species were mainly chrysanthenyl esters[26-28].

The DPPH assay results showed that the *E. bungei* seed oil possessed significant free-radical-scavenging activity. The related RC_{50} value was calculated as 7.5 µg/mL, which was comparable to that of the positive control ascorbic acid (6.37 µg/mL). This finding is quite comparable with those of some previous reports[28].

The volatile oil of the of *E. bungei* seeds exhibited notable antifungal activity against *S. sclerotiorum* mycelia growth also. Mycelia growth was almost completely inhibited at the concentrations higher than 10 µg/mL, while the growth reached to the plates edges on the third day of an experiment in the negative control (Fig. 1). The MIC values for the oil and amphotericin B were recorded as 12.5 and 25 µg/mL, respectively. So, it was revealed that the antifungal effect of the oil appeared to be stronger than that of the positive control amphotericin B. In a previous study the antibacterial property of the extract of *E. bungei* was shown against *Streptococcus aureus, S. mutans, S. pyogens* and *S. sanguis*, as well[29].

In the phytotoxicity assay, the oil displayed intense phytotoxicity on Lettuce (*Lactuca sativa*), Amaranth (*Amaranthus retroflexus*), Branyard grass (*Echinochloa crus-gali*), Wild mustard (*Sinapis arvensis*), Purslane (*Portulaca oleracea*) and Nightshade (*Solanum nigrum* (Fig. 2). The IC_{50} values of the oil are shown in Table 2.

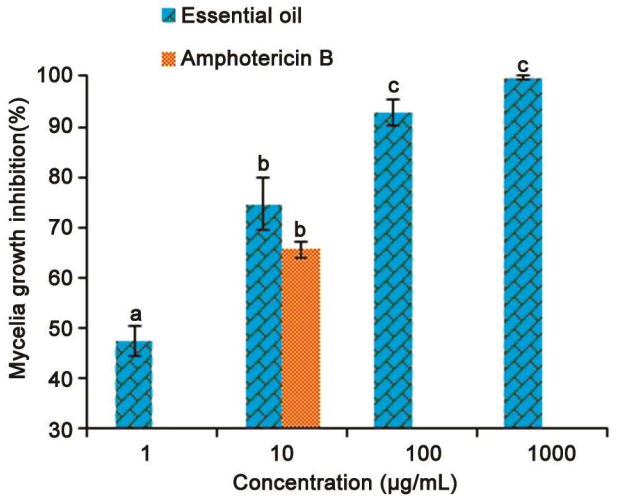

Fig. 1 — Inhibitory effects (%) of the volatile oil of the seeds of *E. bungei* and amphotericin B against *Sclerotinia sclerotiorum* mycelia growth. Mean values followed by the same letter are not significantly different at the 0.05 level according to the Duncan test.

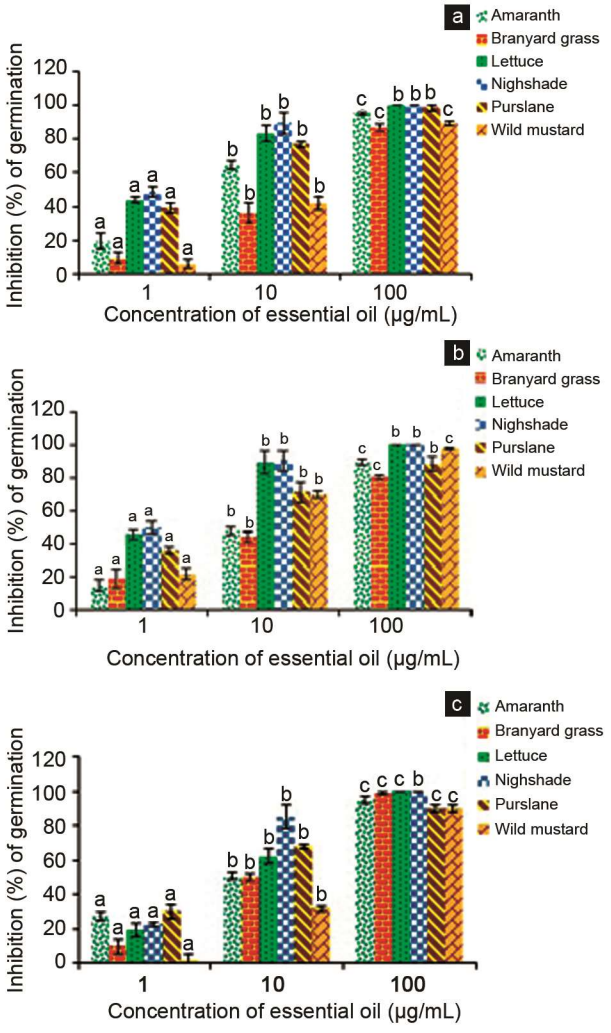

Fig. 2 — Inhibitory effect (%) of the essential oil on seed germination (a) radicle growth (b) and epicotyl growth (c), relative to the control. Mean values followed by the same letter are not significantly different at the 0.05 level according to the Duncan test.

Table 2 — IC_{50} values (µg/mL) of the volatile oil of the seeds of *E. bungei* on seed germination, and radicle and epicotyl growth of various plants

Plants	IC_{50} values (µg/mL)		
	Seed germination	Radicle growth	Epicotyl growth
Amaranth (*Amaranthus retroflexus*)	1.61±0.08	1.85±0.13	1.69±0.13
Branyard grass (*Echinochloa crus-gali*)	2.05±0.11	2.02±0.12	1.73±0.15
Lettuce (*Lactuca sativa*)	1.37±0.09	1.36±0.10	1.58±0.13
Nightshade (*Solanum nigrum*)	1.32±0.07	1.32±0.08	1.42±0.10
Purslane (*Portulaca oleracea*)	1.43±0.10	1.58±0.09	1.61±0.11
Wild mustard (*Sinapis arvensis*)	1.96	1.54	2.1

Previous studies on the genus *Eryngium* extracts or isolates demonstrated various bioactivities, e.g., antioxidant, antibacterial and antifungal properties[3]. Phytotoxic activities of some species of the Apiaceae plants are already known. Oxygenated compounds present in the plant volatile/essential oils are generally implicated for various bioactivities of the oils. It is worthy of note that the oxygenated classes of compounds (oxygenated monoterpenes, oxygenated sesquiterpenes and aldehydes) found in *E. bungei*, have been approximately accounted for 72.0 % of total oil. Therefore, the bioactivities of the volatile oil of the seeds of *E. bungei*, as observed in the present study, could reasonably be attributed to the presence of high amounts of the oxygenated terpenes.

Conclusion

In conclusion, the results indicated that the essential oil of seeds of *E. bungei* has a potential to combat *S. sclerotiorum*, a plant pathogen fungus causing root rot in wild plants and crops. It also indicates a considerable phytotoxic activity causing the plant to stunt the growth of surrounding weeds or other competing plants.

Allelochemicals from plants can be utilized for the purpose of managing weeds, pathogens, disease, insects and nematodes. It may be replaced with synthetic herbicides or pesticides that their use is claimed to negatively affect the environment and actually they do not represent an appropriate tool for the control of some resistance organisms.

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