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Essential Oil Profile in Different Parts of *Echinophora cinerea* (Boiss.)

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ABSTRACT

Echinophora cinerea is grown in the mountains of Iran and has aromatic and medicinal properties. The secondary metabolites of this plant are used as sources of natural antioxidants and anticancer agents. This study aimed to investigate the chemical compounds of the essential oils in different parts of *Echinophora cinereal*. The *E. cinerea* plants were collected in July, in the Zagros mountains, Iran. The chemical compositions of different essential oils were detected using gas chromatography coupled with mass spectrometry. The highest essential oil content belonged to the flower and root of *E. cinerea* (0.90 and 0.85%, respectively). The essential oil content of the flower and root was similar, and the amount of essential oil in the leaf and stem was also the same (P>0.05). The total components of essential oil in the root, stem, leaf, and flower of *E. cinerea* were 96.23, 97.28, 89.95, and 97%, which included 44, 36, 27, and 38 components, respectively. The major important components of root essential oil included α-phellandrene (25.86%), p-cymene (18.17%), γ-terpinene (11.87%), (-)-Spathulenol (5.58%), and α-pinene (5.17%). The greatest important components of the stem essential oil included p-cymene (35.25%), α-phellandrene (23.17%), and α-pinene (10.66%). The most important components of leaf essential oil include α-phellandrene (16.5%), 6-Octen-1-ol, 3,7-dimethyl-, (R) (14.02%), linalool (11.87%), p-cymene (7.8%), carvacrol (6.48%), β-phellandrene (5.87%), and sabinol (5.77%). The major important components of flower essential oil include α-phellandrene (27.31%), p-cymene (9.86%), β-phellandrene (6.84%), 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl- (6.78%), linalool (5.75%), α-pinene (5.26%), sabinol (5.06%), and 6-Octen-1-ol, 3,7-dimethyl-, (R) (4.92%). In general, it can be concluded that the different parts of *E. cinerea* differ in terms of volatile components, and the amount of each compound can be considered for exploiting this plant for industrial and pharmaceutical purposes.

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1. Introduction

Echinophora cinerea (*E. cinerea*), which grows in the mountains of Zagros and Alborz, has aromatic and medicinal properties (Hosseini *et al.*, 2017). In traditional medicine, it is used as a stomach stimulant and the metabolites in its extract are anti-cancer (Gholamhosseinpour *et al.*, 2023; Mahdavi *et al.*, 2022). The essential oil and extract of its aerial parts contain monoterpenoids, including α-phellandrene, α-pinene, β-phellandrene, p-cymene, sesquiterpenoids, and phenolic components along with flavonoids (Sajjadi and Ghannadi, 2002).

The chemical composition of essential oils strongly depends on different factors, including the type of species, climatic conditions, geographical area, harvesting time, storage conditions and production method (Koljančić *et al.*, 2023). In the study conducted by Jahantab et al. (2022), the essential oils of different populations of *E. cinerea* at the full bloom stage were extracted. The study revealed that the major chemical components of essential oils were α-phellandrene (23.7-36.2%), α-pinene (9.5-24.8%), β-myrcene (3.3-17.5%), linalool (1.8-6.7%), β-phellandrene (3.4-5.5%), methyl eugenol (1.1-15.1%), citronellol (0.5-6.4%), and verbenone (1.9-4.9%). Several studies have analyzed the essential components present in the aerial parts of *E. cinerea* cultivated in the Khorramabad region. These studies have identified α-phellandrene

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(24.08-40.64%), p-cymene (10.75-16.32%), limonene (16.28%), carvacrol (3.79-9.12%), α-pinene (5.18-9.75%), Z-b-ocimene (17.28%), and β-myrcene (2.65%) as the key components of the essential oils (Hashemi *et al*., 2009; Zarali *et al*., 2016; Rashidipour *et al*., 2020). Previous studies demonstrated that the chemical composition of medicinal plants essential oils was dependent on various factors such as plant species, and environmental conditions such as altitude and climate (Khalil *et al*., 2020; Zengin *et al*., 2022). Existing literature has indicated the presence of valuable volatile oils in the aerial parts of *E. cinerea*. However, no previous reports have explored the specific quantity of essential oil found in different parts of the plant. Consequently, this study was conducted to investigate the allocation of plant carbon toward the biosynthesis of essential oils in various parts of *E. cinerea*.

2. Materials and methods

Qeysari protected area, Zagros Mountains, Iran, located at an altitude of 2587 m asl (35°65'38" N latitude, 43°77'28" E longitude), was the study site during 2019. In July 2019, flowering plants were harvested (Fig. 1). Then, the plant was identified as *E. cinerea* Hedge et Lamond (Boiss.) by the Agricultural Education and Natural Resources Research Center of Chaharmahal and Bakhtiari with herbarium code D-7062.

Figure 1. Photographs of *E. cinerea* plants showing the differences in aerial parts at full flowering stage (July 2019).

Different parts of *E. cinerea*, including root, stem, leaf, and flower, were separated and dried in the shade with proper ventilation. After peeling, the roots were cut into small pieces and dried. The stems were also cut into small pieces and dried. The different dried parts were stored in a refrigerator at a temperature of 4-5 °C until the time of essential oil extraction. The dried parts of the plant were completely crushed by an electric mill. For extracting essential oils, a Clevenger apparatus with a temperature of 250 to 280 °C was used. For each sample, 400 g of a specific plant part was used, and the essential oil was extracted by hydrodistillation, using a Clevenger-type glass system attached to a cooling system for maintaining the condensation water for 3 h. The collected essential oil was dehydrated with sodium sulfate to the amount of 15 g, and then the percentage of essential oil in each organ was calculated.

GC–MS analysis was conducted with SCION SQ W/436 model SSL-T21 coupled with SCION-MS. The components were separated by a capillary column with a diameter of 25 *M*m and a cover of DB-5 ms and an injector (injection site) split / without split (slit / without a slot) with a glass liner diameter of 1 mm. Pure helium gas with an ionization voltage of 70 eV was used. The temperature of the injection site and interface was 280°C and 260°C, respectively, and the mass value was between 35 and 450 m/z. The same oven temperature program as mentioned for GC was used. Essential oil components were calculated by calculating the inhibition index under the same temperature program conditions for n-alkane (C8-C20) and essential oil with a DB-5 chromatography column. The identification of essential oil components was done by comparing their mass spectrum with the mass spectrum available in internal reference libraries (NIST11).

The data of essential oils content in this study were statistically analyzed using a completely randomized design with ten replications, and the least significant difference (LSD) was used to compare means at $p \leq 0.05$. The SAS 9.1 software was used for the data analysis.

3. Results and discussion

The essential oil content of different parts of *E. cinerea* is presented in Fig. 2. The highest essential oil content belonged to the flower and root of *E. cinerea* (0.90 and 0.85%, respectively). The essential oil content of the flower and root was similar (0.90 and 0.85%, respectively, $P>0.05$), and the amount of essential oil in the leaf and stem was also the same (0.42 and 0.40%, respectively, $P>0.05$) (Fig. 2).

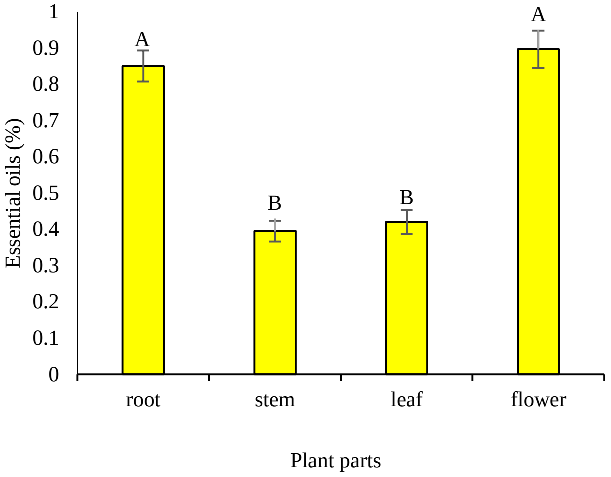

Figure 2. The essential oil content of different parts in *E. cinerea*. Means with similar letters are not significantly different ($P<0.05$) based on the LSD test.

The essential oil content and composition were analyzed in the root, stem, leaf, and flower of *E. cinerea*. The results showed that the total components of essential oil were 96.23% in the root, 97.28% in the stem, 89.95% in the leaf, and 97% in the flower. These essential oils comprised a total of 44, 36, 27, and 38 components, respectively, as detailed in Tables 1-4 and Fig. 3A-D.

Table 1. Chemical compositions of essential oil in *E. cinerea* root

No.	Compounds	RI	Root
1	Hexanal	800	0.21
2	1-Hexanol	865	0.06
3	Heptanal	901	0.27
4	α- Thujene	911	0.31
5	α-Pinene	937	5.17
6	Camphene	953	0.21
7	Sabinene	977	0.02
8	β-Pinene	980	0.01
9	β- Myrcene	991	1.42
10	α-Phellandrene	1010	25.86
11	p-Cymene	1026	18.17
12	γ-Terpinene	1056	11.87
13	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	1083	2.49
14	Linalool	1098	4.01
15	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans	1135	0.78
16	Verbenol	1145	0.01
17	Bicyclo[3.1.1]heptan-3-ol, 2,6,6-trimethyl-, (1α,2β,3α,5α)-	1162	3.08
18	Sabinol	1179	2.94
19	Terpinen-4-ol	1180	0.65
20	2,6-Dimethyl-3,5,7-octatriene-2-ol, ,E,E-	1187	0.23
21	Benzenemethanol,.alpha.,.alpha.,4-trimethyl-	1188	0.85
22	L-.alpha.-Terpineol	1192	0.79
23	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	1208	0.53
24	6-Octen-1-ol, 3,7-dimethyl-, (R)	1220	2.26
25	Isobornyl formate	1227	0.34
26	2-Decenal, (Z)-	1254	0.61
27	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-	1256	0.13
28	Thymol	1287	0.38
29	Carvacrol	1299	2.69
30	(1S,2S,3R,5S)-(+)-Pinanediol	1313	0.91
31	Dihydrocarvyl acetate	1344	0.34
32	.gamma.-Elemene	1425	0.31
33	Germacrene D	1480	0.26
34	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1483	0.15
35	Butanoic acid, 3-methyl-, 1-ethenyl-1,5-dimethyl-4- hexenyl ester	1484	0.08
36	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)- 4-(1-methylethylidene	1492	0.46
37	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl -3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	1549	0.11
38	(-)-Spathulenol	1572	5.58
39	Globulol	1583	0.06
40	Carotol	1597	0.32
41	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	1618	0.32
42	.alpha.-Cadinol	1645	0.19
43	.gamma.-Dodecalactone	1673	0.36
44	Ledene oxide-(II)	1682	0.43
Total	Essential oils (%)		96.23

RI: denotes Retention indices on the DB-5 column.

In particular, the essential oils obtained from the leaf and flower contained components with a retention index greater than 937. However, the root essential oil exhibited components with a retention index of less than 937, including hexanal, heptanal, and α-thujene, as indicated in Tables 1, 3, and 4.

Table 2. Chemical compositions of essential oil in *E. cinerea* stem

No.	Compounds	RI	Stem
1	Hexanal	800	0.03
2	o-Xylene	880	0.09
3	α-Pinene	937	10.66
4	Camphene	953	0.14
5	β-Pinene	980	0.02
6	β- Myrcene	991	2.60
7	α-Phellandrene	1010	23.17
8	p-Cymene	1026	35.25
9	Benzeneacetaldehyde	1044	0.01
10	γ-Terpinene	1056	1.3
11	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	1083	0.39
12	Linalool	1098	4.21
13	2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	1112	0.03
14	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans	1135	0.70
15	Verbenol	1145	0.71
16	Bicyclo[3.1.1]heptan-3-ol, 2,6,6-trimethyl-, (1α,2β,3α,5α)-	1162	0.50
17	Sabinol	1179	2.74
18	Terpinen-4-ol	1180	0.52
19	Benzenemethanol,.alpha.,.alpha.,4-trimethyl-	1188	1.47
20	L-.alpha.-Terpineol	1192	0.74
21	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	1208	0.55
22	6-Octen-1-ol, 3,7-dimethyl-, (R)	1220	3.63
23	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-	1256	0.2
24	Thymol	1287	0.92
25	Carvacrol	1299	3.03
26	(1S,2S,3R,5S)-(+)-Pinanediol	1313	0.37
27	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	1398	0.35
28	Methyleugenol	1401	0.28
29	cis-.beta.-Farnesene	1446	0.04
30	(-)-Spathulenol	1572	1.05
31	Caryophyllene oxide	1581	0.47
32	Carotol	1597	0.09
33	.alpha.-Cadinol	1645	0.27
34	.gamma.-Dodecalactone	1673	0.47
35	Ledene oxide-(II)	1682	0.20
36	Benzyl Benzoate	1760	0.08
Total	Essential oils (%)		97.28

RI: denotes Retention indices on the DB-5 column

Compounds including hexan-1-ol, heptanal, α-thujene, bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-, 2,6-dimethyl-3,5,7-octatriene-2-ol, E,E-, isobornyl formate, 2-decenal, (Z)-, and globulol with 2.36% of total components were observed only in the roots (Table 1; Fig. 3A). However, o-xylene, benzeneacetaldehyde, 2H-pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-, 2-cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z), cis-beta.-farnesene, caryophyllene oxide and benzyl benzoate with 1.07% of total components were observed only in the stem (Table 2; Fig. 3B). Compounds including 2-ethylhexanoic acid, pinocarvone, neric acid with 1.73% were detected only in leaves (Table 3; Fig. 3C). 1.84% of flower essential oil components included 2-furanmethanol, 5-ethenyltetrahydro-α, α,5-trimethyl-, cis-, p-mentha-1,5-dien-8-ol and 2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl- which were exclusively found in this organ (Table 4; Fig. 3D). Nine compounds were observed in the roots that were not detected in the aerial parts, and 12 components were observed in the aerial parts that were not detected in the roots (Tables 1-4; Fig. 3A-D).

Table 3. Chemical compositions of essential oil in *E. cinerea* leaf

No.	Compounds	RI	Leaf
1	α-Pinene	937	1.04
2	β- Myrcene	991	1.47
3	α-Phellandrene	1010	16.50
4	p-Cymene	1026	7.80
5	β-Phellandrene	1031	5.87
6	γ-Terpinene	1056	0.37
7	Linalool	1098	11.87
8	2-ethylhexanoic acid	1116	0.71
9	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans	1135	0.90
10	Verbenol	1145	2.92
11	Pinocarvone	1164	0.53
12	Sabinol	1179	5.77
13	Terpinen-4-ol	1180	1.25
14	Benzenemethanol,.alpha.,.alpha.,4-trimethyl-	1188	1.76
15	L-.alpha.-Terpineol	1192	2.65
16	6-Octen-1-ol, 3,7-dimethyl-, (R)	1220	14.02
17	Thymol	1287	2.67
18	Carvacrol	1299	6.48
19	(1S,2S,3R,5S)-(+)-Pinanediol	1313	2.89
20	Neric acid	1347	0.49
21	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	1391	2.55
22	Methyleugenol	1401	0.42
23	(-)-Spathulenol	1572	1.07
24	Caryophyllene oxide	1578	1.18
25	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl- [1R-(1R*,3E,7E,11R*)]-	1600	0.52
26	.alpha.-Cadinol	1645	1.07
27	.gamma.-Dodecalactone	1673	1.12
Total	Essential oils (%)		95.89

RI: denotes Retention indices on the DB-5 column

The root essential oil of *E. cinerea* exhibited significant amounts of α-phellandrene (25.86%), p-cymene (18.17%), γ-terpinene (11.87%), (-)-spathulenol (5.58%), and α-pinene (5.17%), making them the major important components in the oil (Table 1; Fig. 3A).

Table 4. Chemical composition of essential oil of *E. cinerea* flower

No.	Compounds	RI	Flower
1	α-Pinene	937	5.26
2	Camphene	953	0.13
3	β- Myrcene	991	3.94
4	α-Phellandrene	1010	27.31
5	p-Cymene	1026	9.86
6	β-Phellandrene	1031	6.84
7	γ-Terpinene	1056	1.10
8	2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-	1065	0.32
9	Linalool	1098	5.75
10	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans	1135	1.07
11	Verbenol	1145	1.04
12	p-Mentha-1,5-dien-8-ol	1170	1.36
13	Sabinol	1179	5.06
14	Terpinen-4-ol	1180	0.88
15	Benzenemethanol,.alpha.,.alpha.,4-trimethyl-	1188	1.19
16	L-.alpha.-Terpineol	1192	1.73
17	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	1208	0.89
18	6-Octen-1-ol, 3,7-dimethyl-, (R)	1220	4.92
19	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-	1256	0.53
20	Thymol	1287	0.62
21	Carvacrol	1299	3.11
22	(1S,2S,3R,5S)-(+)-Pinanediol	1313	0.20
23	Dihydrocarvyl acetate	1344	0.09
24	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	1391	0.57
25	.gamma.-Elemene	1425	0.23
26	Germacrene D	1480	0.50
27	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	1483	0.22
28	Butanoic acid, 3-methyl-, 1-ethenyl-1,5-dimethyl-4- hexenyl ester	1484	0.15
29	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl -3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	1549	0.31
30	(-)-Spathulenol	1572	2.35
31	Caryophyllene oxide	1578	0.37
32	Carotol	1597	0.51
33	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl- [1R-(1R*,3E,7E,11R*)]-	1600	0.27
34	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	1618	6.78
35	.alpha.-Cadinol	1645	0.31
36	.gamma.-Dodecalactone	1673	0.52
37	Ledene oxide-(II)	1682	0.55
38	α-Sinensal	1686	0.16
Total	Essential oils (%)		97

RI: denotes Retention indices on the DB-5 column

In the stem essential oil, the major constituents were p-cymene (35.25%), α-phellandrene (23.17%), and α-pinene (10.66%) (Table 2; Fig. 3B). The leaf essential oil contained α-phellandrene (16.5%), 6-Octen-1-ol, 3,7-dimethyl-, (R) (14.02%), linalool (11.87%), p-cymene (7.8%), carvacrol (6.48%), β-phellandrene (5.87%), and sabinol (5.77%) as the major important components (Table 3; Fig. 3C), and in the flower essential oil, the key components were α-phellandrene (27.31%), p-cymene (9.86%), β-phellandrene (6.84%), 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl- (6.78%), linalool (5.75%), α-pinene (5.26%), sabinol (5.06%), and 6-Octen-1-ol, 3,7-dimethyl-, (R) (4.92%) (Table 4; Fig. 3D). These compositions highlight the significant components present in the essential oils of different parts of *E. cinerea*, including the root, stem, leaf, and flower. The changes of some major components in different parts of the plant show that the content of α-phellandrene, α-pinene, p-cymene, carvacrol, linalool and 6-Octen-1-ol, 3,7-dimethyl-, (R) in the leaf is different from other parts. Also, unlike the aerial parts, a significant amount of γ-terpinene (11.87%) was observed in the roots (Tables 1-4).

The essential oils extracted from different species of medicinal plants have influenced a great deal of scientific attention due to their capability to act as a source of natural agents to enhance the shelf life and safety of foods and natural bio-active compounds (Khan *et al*., 2023). In the present study, the essential oil content obtained from the aerial parts is higher than that previously reported in different regions of Kohghiluyeh and Boyer Ahmad province, at altitudes of 2800 to 3000 m (Sajjadi and Qanadi, 2002; Jahantab *et al*., 2017). However, the essential oil of flower and root was approximately similar to the essential oil of aerial parts of *E. cinerea* collected from the Fars region (0.8%) (Ahmadi *et al*., 2001). The difference in the amount of essential oil in our study samples with the results of other studies can be attributed to environmental factors, geographical origin, topography, and phenological growth stages of the plant at the time of collection.

The essential oil compounds obtained from *E. cinerea* were similar to the essential compounds of *Thymus vulgaris* and *Achillea millefolium* in terms of some compounds such as p-cymene, α-pinene, carvacrol and linalool (Shahdadi *et al*., 2022). The chemical composition of essential oils strongly depends on different factors, including the type of species, climatic conditions, geographical area, harvesting time, storage conditions and production method (Koljančić *et al*., 2023). Thus, the unfavorable conditions of Chaharmahal and Bakhtiari' highlands, like the semi-arid conditions in Jiroft, can stimulate the biosynthetic pathway of secondary metabolites and create relatively similar compounds in the *E. cinerea* essential oil by creating environmental stresses such as high solar radiation. However, the difference between *E. cinerea* and the studied species by Shahdadi et al. (2022), and the complete mismatch of environmental conditions in these places have prevented the complete similarity of the compounds. Previous studies demonstrated that the chemical composition of essential oils was dependent on various factors such as plant species (Zengin *et al.*, 2022), environmental conditions such as altitude, climate (Khalil *et al.*, 2020), and natural habitat of plant species (Flamini *et al.*, 2004).

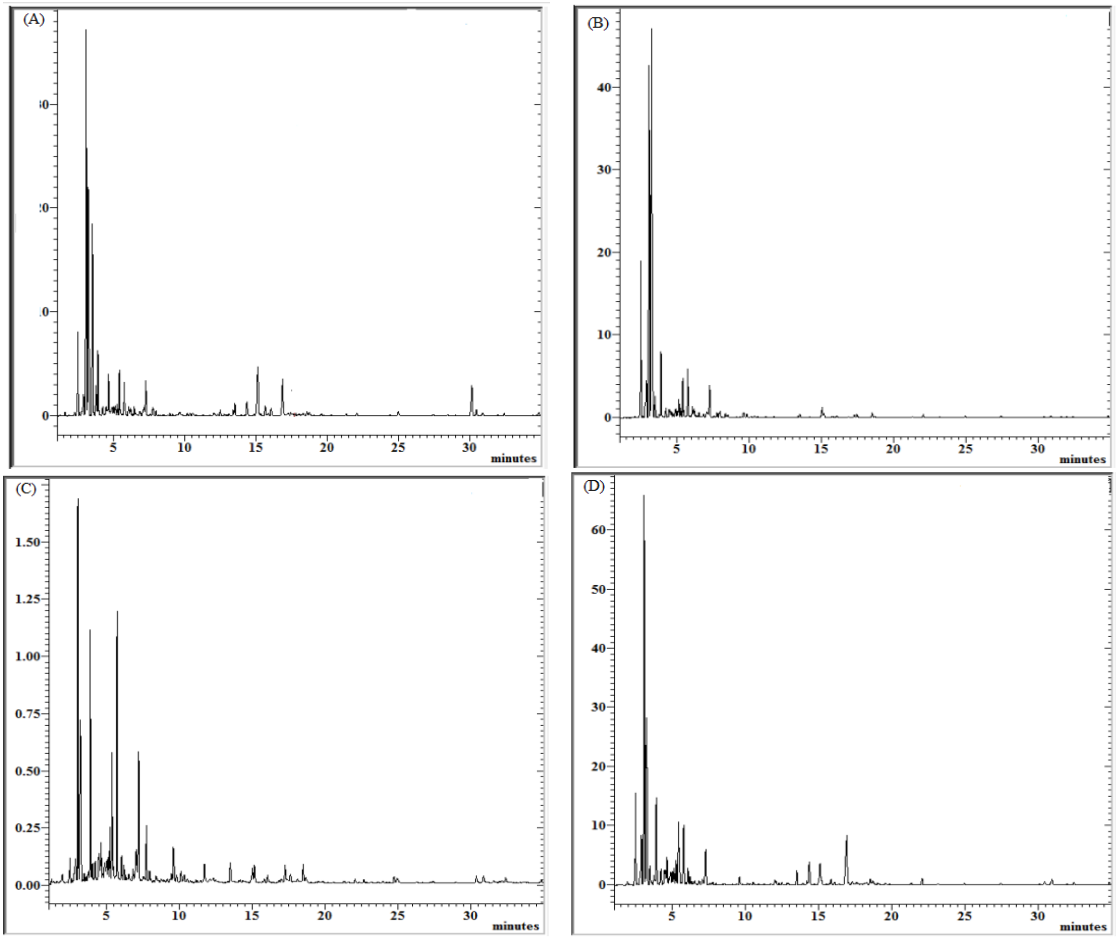

Figure 3. GC-MS profile of essential oil from root (A), stem (B), leaf (C), and flower (D) of *E. cinerea*.

In the present study, the maximum content of some essential oils such as p-cymene (18.17%), γ-terpinene (11.87%), and linalool (11.87%) was much higher than in other studies (Zarali *et al.*, 2016; Rashidipour *et al.*, 2020; Jahantab *et al.*, 2022). However, a study conducted by Hashemi et al. (2009) using hydrodistillation methods reported a significant presence of p-cymene at 34.43% in this plant. This differs from the previous composition mentioned. The key attributes of essential oils and active substances are their hydrophobic property, which leads to the change and destruction of the cell membrane structure, and their greater permeability (Shahdadi *et al.*, 2023). Therefore, *E. cinerea* essential oil can be effective in controlling pathogens and pests by increasing the leakage of ions and essential components of the cell. The important point is that it has been determined in the field observations that livestock avoid eating this plant. This finding can be considered a valuable ecological indicator to follow the inhibitory effects of *E. cinerea* essential oil on microorganisms and weeds in future studies. Furthermore, the present study also revealed the identification of various components such as sabinol and (-)-spathulenol, which were reported for the first time in *E. cinerea.* Notably, the presence of spathulenol in different parts of the plant, particularly in the roots and flowers, suggests the potential use of this compound for reducing blood pressure. It is worth noting that this specific compound has not been documented in other studies conducted on *E. cinerea* thus far.

4. Conclusion

These findings highlight the novel discoveries and potential therapeutic applications associated with the unique composition of *E. cinerea*, particularly regarding the presence of spathulenol in various plant parts. In addition, the possible effects of growth inhibition by the essential oil of this plant are considered as an approach to producing a biocide. Our results indicated that the stem, leaf, flower, and root of *E. cinerea* have volatile components that are different in type or concentration. Therefore, we concluded that not only the above-ground parts but also the roots of *E. cinerea* can be used for pharmaceutical purposes. This finding can be used to exploit this plant for industrial and pharmaceutical products.

Conflict of interests

All authors have to declare their conflicts of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

Sina Fallah: Conceptualized and designed the study, Project administration, Supervision, Writing-original draft, Writing-review & editing, Funding acquisition. Ali Nasiri: Conceptualized and designed the study, Investigation, Collected the data and analysis. Amir Sadeghpour: Conceptualized and designed the study, Writing-review & editing, Project advisory committee. Hossien Barani-Beiranvand: Writing-review & editing, Project advisory committee.

Informed consent

The authors declare not to use any patients in this research.

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