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Phytochemical Survey and Antioxidant Activity of Natural Population of *Dorema aucheri* Boiss. from Dalahoo

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ABSTRACT

Dorema aucheri Boiss. (Bilhar) is an important perennial herb belonging to the Apiaceae family. The bioactive compounds found in *D. aucheri* have been documented in traditional medicine to have liver-health effects, as well as stimulant, anti-spasmodic, and expectorant properties. Considering the extinction risk of this valuable species, the present study was conducted. Roots, stems, leaves, and flowers of *D. aucheri* were collected from Dalahoo County (46º 17′ 57″ N, 34º 16′ 50″ E), Kermanshah Province, Iran. Their volatile components and compositions were investigated with gas chromatography techniques. The essential oil (EO) yield of *D. aucheri* was 0.45, 0.2, 0.35 and 0.4 w/w % (relative to dry weight of plant) for roots, stems, leaves and flowers, respectively. The main compounds identified in the oil of root were thymol (15.9%), β-Caryophyllene (11.3%), and β-bisabolene (8.1%). The highest amounts of compounds in stem were β-Caryophyllene (16.4%), thymol (15.3%), β-bisabolene (6.3%), and Caryophyllene oxide (5.1%). The main constituents of leaf oil were heptacosane (17.3%), thymol (14.2%), β-Caryophyllene (10.0%), β-bisabolene (7.0%), and Caryophyllene oxide (5.1%). The major constituents of the flower oil were β-Caryophyllene (20.2%), thymol (12.4%), heptacosane (7.3%), and β-bisabolene (4.1%). A methanolic extract was also prepared from all samples and their total tannin content, saponin, antioxidant activity, including DPPH and FRAP, total phenolic and flavonoid contents were evaluated. The results obtained showed there was a significant difference among all extracts of *D. aucheri* in terms of the AA, tannin content, saponin, total phenol content and total flavonoid content ($p \leq 0.01$). The flower sample showed the maximum antioxidant activity with an IC_{50} of 148.1 µg ml^{-1} and FRAP of 23.3 mg Fe^{2+} g^{-1} DW. It is suggested to expand the cultivation of this valuable plant in pastures to compensate for the excessive harvesting of the plant.

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1. Introduction

Medicinal herbs are abundant sources of diverse chemical compounds that exhibit beneficial therapeutic properties. These compounds found in medicinal herbs can be utilized to treat or provide relief for various health issues and conditions (Nazir *et al.*, 2021; Sliwinska, 2018). The medicinal herbs industry encompasses the cultivation, harvesting, processing, and distribution of medicinal plants and their derived products (Hosseini *et al.*, 2018). Many chemical compounds derived from plants have demonstrated significant pharmacological activities and are often utilized as the basis for synthesizing pharmaceutical compounds (Hassan *et al.*, 2019). While the production of secondary metabolites in medicinal herbs is primarily controlled by genetic factors, the actual concentrations and accumulation levels of these compounds are significantly influenced by environmental conditions (Pant *et al.*, 2021). *Dorema aucheri* Boiss. is a perennial herb that grows in arid or semi-arid regions (Fig. 1). It can reach a height of up to 1.5 meters and has small and yellow flowers and leaves are pinnately compound with linear segments (Claßen-Bockhoff and Ajani, 2025). *D. aucheri*, is one of the endemic plant species with economic and ecological values, growing in the central Zagros Mountains of the

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southwest of Iran. Increasing anthropogenic pressures, including deforestation, re-forestation, intensification of agriculture, and drainage of wetlands, have already had a great impact on the growth, survival and distribution of native species in Iran, especially the rare and endemic species (Mirinejad and Ardakani, 2014). *D. aucheri* can be cultivated and grows in cold areas (precipitation mostly snow).

Figure 1. Developmental stages of *D. aucheri* plant. Letters indicate different growth phases: (a) seedling, (b) vegetative stage, (c) flowering stage, and (d) mature plant (Askari, 2022).

To provide this plant for public use (medicinal plant as well as vegetable crop), we demonstrated that it can be cultivated (Mirinejad and Ardakani, 2014). The essential oils (EOs) of *D. aucheri* from the natural habitats in various regions across Iran have been reported. Studies have identified the main chemical constituents of the essential oils, which include β-caryophyllene (7.2 – 35.7%), thymol (23.45 – 29.64%), β-gurjunene (2.6 – 5.8%), carvacrol (1.3 – 2.7%) and cuparene (2.0 – 3.0%) (Akbarian *et al.*, 2016). The main constituents of the leaf oils were α-pinene, limonene, β-pinene, germacrene D, and spathulenol (Arabjafari *et al.*, 2023). The root oils contained pathulenol, caryophyllene oxide, and germacrene D as the main components (Akbarian *et al.*, 2016). Studies on the volatile compounds from the aerial parts (stems) are more limited. However, the stem oil also contains significant monoterpenes like α-pinene and limonene (Akbarian *et al.*, 2016). The main compounds of the flower oil of *D. aucheri*, collected from Birjand, were sesquiterpenes like β-caryophyllene and germacrene D (Arabjafari *et al.*, 2023). The quality and quantity of essential oils produced by medicinal herbs are highly dependent on the prevailing climate conditions (Moghaddam and Farhadi, 2015).

Essential oils extracted from *D. aucheri* have been shown to possess multiple beneficial biological properties, including a protective effect on the liver, as well as stimulant, antispasmodic, and expectorant activities (Akbarian *et al.*, 2016). In another study, the results of flow cytometry showed that the essential oil significantly increased the apoptosis in SW48 cell line compared with the vincristine. It also increased the apoptosis in SW1116 cells compared with vincristine, but this difference is not significant. In fact, the essential oil of *D. aucheri* showed significant cytotoxic effects against SW48 and SW1116 cancerous cell lines (Hossiniyan *et al.*, 2021). The non-volatile compounds are mainly made up of phenolic and flavonoid compounds that are developmentally regulated during

the plant's life and in response to different conditions (Masoudi and Kakävand, 2017). Phenolic and flavonoid compounds are recognized for their ability to prevent the generation of free radicals, thereby mitigating oxidative stress (Chaudhary *et al.*, 2023). Numerous clinical studies have demonstrated the significant value of phenolic and flavonoid compounds. Not only do they possess potent antioxidant properties, but they also exhibit diverse antimicrobial activities, including antiviral, antifungal, and antibacterial effects (Sim *et al.*, 2019). There are reports of other bioactive compounds present in extracts of the *D. aucheri* plant. Several coumarin compounds have been isolated from *D. aucheri*, such as umbelliferone and scopoletin (Das *et al.*, 2023), and various flavonoids and phenolic acids (Mianabadi *et al.*, 2015). The production and biological activity of phytochemicals are influenced by ecological factors such as climate, soil, light availability, and the presence of other organisms (Prinsloo and Nogemane, 2018). This study aimed to identify the essential oil variation in different organs (root, stem, leaf and flower) of *Daucus aucheri* plants found in the Dalahoo region of Iran by GC-MS. The study also evaluated the total tannin content, saponin content, antioxidant activity including DPPH and FRAP assays, total phenol content and total flavonoid content of the root, stem, leaf and flower of *D. aucheri*.

2. Materials and methods

2.1. Plant materials

Fresh roots, stems, leaves and flowers of *D. aucheri* were collected during the flowering stage (April 2019), from Dalahoo county (46º 17′ 57″ N, 34º 16′ 50″ E), Kermanshah Province, Iran and then air-dried under shade conditions and room temperature.

2.2. Habitat characteristics

Site attributes such as altitude, longitude, and latitude were determined using GPS (Table 1). The various climatic factors, including mean annual rainfall, average annual temperature and relative humidity, were determined using the nearest meteorological stations (Table 1).

Concurrently, soil samples were collected from the rhizosphere, the zone surrounding the roots of plants, at the study site. The important properties of the soil, including soil texture analyzed using the hydrometer method, were examined (Taubner *et al.*, 2009). Soil acidity (pH) and Electrical Conductivity (EC) were evaluated from the saturated soil paste extract. Soil organic carbon with titration method (Gelman *et al.*, 2012), soil nitrogen by Kjeldahl method (Chakraborty *et al.*, 2019), Phosphorus by Olsen method (Sims, 2000), Potassium by Flame photometer (Picard *et al.*, 2025) and microelements by Atomic method (Moron and Cozzolino, 2003) were measured (Table 2).

Table 1. Geographical coordinates and collection site information for *D. aucheri* population in 2019

Sampling location	Province	Average rainfall (mm)	Average annual temperature (ºC)	Relative humidity (%)	Latitude	Longitude	Altitude (m a.s.l.)*
Dalahoo	Kermanshah	527	13.7	50	46º14′07″	34º16′50″	1553

*Meters above sea level

Table 2. Physical and chemical properties of soil (depth of 0-30 cm) of collection site

Parameter	Measurement method	-
Clay (%)	Hydrometer method	12
Silt (%)	Hydrometer method	20
Sand (%)	Hydrometer method	68
Texture	-	Sandy loam
EC (ds m^{-1})	Conductometer	1.86
pH	pH meter	7.48
OC (%)	Titration method	0.51
Nitrogen (%)	Kajeldal	0.05
Phosphorus (ppm)	Olsen method	7.8
Potassium (ppm)	Flame photometer	126.8
Fe (ppm)	Atomic	5.04
Zn (ppm)	Atomic	0.76
Cu (ppm)	Atomic	1.28
Mn (ppm)	Atomic	3.8

EC: Electrical conductivity

2.3. Isolation and analysis of essential oils

About 50 grams of air-dried roots, stems, leaves and flowers of *D. aucheri* were chopped off separately in a fine manner and individually immersed in 500 mL of distilled water. For each essential oil sample, hydrodistillation using a Clevenger apparatus was conducted for 3 hours. The essential oils were separated from the water and dried over anhydrous sodium sulfate and stored at 4°C for further analysis. The EO yields were calculated based on the dry weight of the plant material. Analysis using GC was performed using Agilent Technologies 7890B (Santa Clara, CA, USA) with a flame ionization detector. The instrument was equipped with an HP-5 fused silica column (length 30

m, inner diameter 0.32 mm and film thickness 0.25 µm) and helium was used as the carrier gas at a flow rate of 1.1 mL minute^{-1}. Each essential oil (1 µl) was injected into the Thermoquest-Finnigan gas chromatograph, coupled with a trace mass spectrometer with the same parameter for fused silica column (except for the inner diameter of 0.25 mm), oven temperature, injector temperature, carrier gas and flow rate. The ionization voltage was 70 eV and the ion source and interface temperatures were set at 200 and 250°C, respectively. The identification of essential oil compounds was based on the mass spectrum of each respective compound that was then compared with the internal Wiley 7.0 and Adams mass spectral libraries. Further identification was based on comparison of peak retention indices by using a homologous series (C8 to C24) recorded under the same operating conditions and published data were also used as a reference (Adams, 2007).

2.4. Preparation of different extracts

For this study, roots, stems, leaves, and flowers of plant *D. aucheri* were extracted. The extraction was done by sonicating 10 g of the dried plant material for 15 minutes at 30°C in 50 mL of methanol, using an ultrasonic device operating at 120 Hz frequency. The obtained extracts were filtered by Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40°C under vacuum. After the extracts were completely dried, they were stored at 4°C until further analysis was conducted.

2.5. Determination of total tannins

The tannin contents of samples were specified by the method of Luthar and Kreft (1999). Briefly, 400 µL of the solution was mixed with 3 ml of vanillin reagent (vanillin 4% in methanol) and 1.5 mL of concentrated hydrochloric acid and kept for 20 minutes at 28°C. The mixture was shaken well, kept at 28°C for 30 min and absorbance was measured at 520 nm. Tannic acid was used to draw the standard curve. Tannic acid was employed to construct the standard calibration curve. The total tannin contents (TTC) of methanolic extracts are presented in milligrams of tannic acid equivalent per gram of dry plant weight (mg TA g^{-1} DW).

2.6. Determination of saponin

The experimental procedure commenced with the pulverization of the plant samples into a fine powder. This powdered material, weighing 5 grams, was combined with 50 milliliters of a 20% aqueous ethanol solution within a flask. Over 90 minutes, the contents were periodically agitated to facilitate extraction. Following this heating and mixing regimen, the extract was filtered. The solid residue retained on the filter was subjected to an additional extraction using 50 milliliters of 20% ethanol. The primary and secondary extracts were then pooled together. The combined extract was concentrated by evaporation at 90 degrees Celsius, reducing the volume to approximately 40 milliliters. This concentrated extract was transferred to a separatory funnel, where 40 milliliters of n-hexane were added. The biphasic mixture was vigorously shaken to facilitate the partitioning of the components. This liquid-liquid extraction with n-hexane was repeated until the aqueous layer exhibited a clear appearance. The saponins were then selectively extracted from the aqueous phase using 60 milliliters of normal butanol. The combined butanol extracts were washed with a 5% sodium chloride solution before being evaporated to dryness in a pre-weighed container (Ezeabara *et al.*, 2014). The dried saponin extract was then placed in an oven at 60 degrees Celsius to complete the drying process. After cooling in a desiccator, the extract was reweighed. Saponin content was estimated by difference and calculated as a percentage of the original sample using Equation 1.

$$(1) \qquad Saponin\ (\%) = \frac{W_2 - W_1}{Weight\ of\ sample} \times 100$$

In this equation, W_1 is weight of evaporating dish and W_2 is weight of evaporating dish + sample.

2.7. Determination of total phenolic compounds

The total phenolic contents of the *D. aucheri* extracts were obtained by the Folin–Ciocalteu method (Slinkard and Singleton, 1977). A calibration curve was provided with a series of methanolic Gallic acid solutions at different concentrations. For the sample extracts, 20 µL of each extract (at a concentration of 0.05 g ml^{-1}) was mixed with the Folin–Ciocalteu reagent and sodium carbonate, and the absorbance was measured at 765 nm. Gallic acid was used as the standard, and the total phenolic content was expressed as mg of Gallic acid equivalents per gram of dry weight of extract (mg GAE g^{-1} DW ext).

2.8. Determination of total flavonoid

The total flavonoid content of the *D. aucheri* extracts was estimated using the colorimetric method described by Ordonez et al. (2006). The extracts were prepared at a concentration of 0.5 g ml^{-1} in DMSO. An equal volume of the extract and 2% aluminum chloride solution (in methanol) was mixed in a test tube. After 10 minutes, the absorbance of the mixture was measured at 420 nm using a spectrophotometer. This was done in triplicate for each extract. A calibration curve was prepared using a series of methanolic quercetin solutions at different concentrations. The flavonoid content was expressed as mg of quercetin equivalents per gram of dry weight of extract (mg QE g^{-1} DW Ext).

2.9. Antioxidant capacity

2.9.1. DPPH method

The DPPH (2,2-diphenyl-2 picrylhydrazyl hydrate) radical scavenging activity was assessed based on the method described by Bozin et al. (2007). The absorbance of the samples was measured at 517 nm using an ELISA reader (Epoch, BioTek instrument). The antioxidant capacity was calculated using Equation 2.

$$(2) \quad Inhibition\ (\%) = \left[\frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}}\right] \times 100$$

Where Abs_{blank} is the absorbance of the blank and Abs_{sample} is the absorbance of the sample extract or the positive control BHT. The IC_{50} value, which is the concentration required to inhibit 50% of the DPPH radical, was determined from the equation relating inhibition percentage to sample concentration. The results were used to compare the antioxidant activity of the *D. aucheri* extracts.

2.9.2. FRAP method

The FRAP assay was performed following the method described by Tomasina et al. (2012). For the assay, 50 µL of the extracts were mixed with 3 mL of fresh FRAP reagent. The FRAP reagent was prepared by mixing 0.3 M acetate buffer (pH 3.6), 0.01 M TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 M HCl, and 0.02 M $FeCl_3 \cdot 6H_2O$ in a 10:1:1 (v/v/v) ratio. The absorbance was then measured at 593 nm using an ELISA reader with the control serving as the reference. The test was performed in triplicate, and the results were calculated using a standard curve of $FeSO_4$. The reducing power was expressed as mg Fe^{2+} equivalents per g of dry weight (mg Fe^{2+} g^{-1} DW).

2.10. Statistical analysis

All the data were analyzed using a randomized complete block design (RCBD) with three replications. The statistical analysis was performed using the SAS Statistical Package Program version 9.0. The PROC UNIVARIATE within SAS was used to test the assumptions of the ANOVA, and the residuals were confirmed to be normally distributed. The mean values were compared using the least significant difference (LSD) at the 5% probability level.

3. Results and discussion

3.1. Essential oil composition

The hydrodistillation of *D. aucheri* oil gave 0.45, 0.2, 0.35 and 0.4 w/w % (relative to dry weight of plant) yield for roots, stems, leaves and flowers, respectively. Generally, 32 compounds containing 92.0% were identified by GC/MS analysis in the root oil of *D. aucheri* (Table 3). The main constituents identified in the oil of root were thymol (15.9%), β-Caryophyllene (11.3%), and β-bisabolene (8.1%) (Table 3). Thirty-four compounds comprising 97.3% of the stem oil were identified in the stem oil and accumulating at the highest amounts were thymol (15.3%), β-Caryophyllene (16.4%), β-bisabolene (6.3%), and Caryophyllene oxide (5.1%) (Table 3).

Approximately 29 phytochemicals, comprising 92.0% of the leaf oil of *D. aucheri*, were detected via GC-MS (Table 3). The major components of leaf oil were heptacosane (17.3%), thymol (14.2%), β-Caryophyllene (10.0%), β-bisabolene (7.0%) and Caryophyllene oxide (5.1%) (Table 3). A total of thirty compounds containing 93.5% were detected in the flower oil (Table 3). For this oil, thymol (12.4%), β-Caryophyllene (20.2%), β-bisabolene (4.1%), and heptacosane (7.3%) were the important constituents. The chemical compositions of oils of *D. aucheri* consisted mainly of sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Fig. 2).

3.2. Total tannin content (TTC)

The extracts from the organs of *D. aucheri* about the tested TTC content were significantly different (Table 4). The maximum total tannin content was found in the

samples that were designated as flower and root, with 1.1 and 1.05 mg TA g^{-1} DW (Fig. 3), whereas the lowest value of total tannin in the samples designated as leaf and the TTC value was recorded as 0.35 mg TA g^{-1} DW. This is the first report about tannin contents in different organs of *D. aucheri*.

Table 3. Chemical composition (%) of root, stem, leaf and flower essential oils of *D. aucheri*.

No	RT	Compounds	Root (%)	Stem (%)	Leaf (%)	Flower (%)	RI*
1	8.6	Myrcene	0.5 + 0.04		1.0 ± 0.09	0.2 + 0.01	988
2	9.5	*p*-Cymene	0.3 + 0.02		1.1 ± 0.18	0.1 + 0.01	1024
3	9.8	β-Ocimene	2.5 + 0.18	1.2 ± 0.04	1.1 ± 0.04		1032
4	10.1	(Z)-Sabinene hydrate		0.5 ± 0.01	0.3 ± 0.04		1065
5	11.5	iso-Pentyl isovalerate	1.1 + 0.10	0.7 ± 0.02		0.4 + 0.02	1103
6	11.8	(E)-2-Nonenal	2.1 + 0.14			1.8 + 0.14	1150
7	12.9	*trans*-Pinocamphone		0.9 ± 0.08	1.7 ± 0.12		1158
8	13.9	*n*-Dodecane	0.3 + 0.20		0.6 ± 0.05	2.4 + 0.21	1200
9	14.5	*endo*-Fenchyl acetate	0.1 + 11	2.3 ± 0.9		4.2 + 0.31	1218
10	14.8	Thymol, methyl ether		2.9 ± 0.08		3.2 + 0.31	1232
11	15.1	Carvacrol, methyl ether	5.3 + 0.21		2.4 ± 0.11		1241
12	16.9	Thymol	15.9 + 0.21	15.3 ± 0.41	14.2 ± 0.17	12.4 + 0.45	1289
13	17.7	a-Cubebene		0.7 ± 0.04	1.6 ± 0.11	1.1 + 0.10	
14	17.8	2-Undecanol		0.8 ± 0.03		0.2 + 0.18	
15	19.2	α-Elemene			4.8 ± 0.15	4.8 + 0.32	1389
16	19.6	β -Caryophyllene	11.3 + 0.12	16.4 ± 0. 4	10 ± 0.14	20.2 + 0.5	1408
17	20.4	*E*-Caryophyllene	0.8 + 0.07		0.8 ± 0.07		1417
18	20.6	Dehydroaromadendrane	2.8 + 0.18	0.7 ± 0.05	2.6 ± 0.14	0.4 + 0.03	1460
19	20.7	*ar*-Curcumene	1.0 + 0.11	0.4 ± 0.06			1475
20	21	γ-muurolene	0.5 + 0.04		0.5 ± 0.08	1.3 + 0.11	1478
21	21.4	(*Z*)-Farnesene		0.7 ± 0.01	0.7 ± 0.07		1481
22	21.6	Germacrene D		0.1 ± 0.01	1.2 ± 0.21	0.4 + 0.03	1484
23	21.8	β-Selinene		2.9 ± 0.9		1.8 + 0.10	1489
24	21.9	2-Pentadecanol	5.3 + 0.24	4.1 ± 0.1	0.7 ± 0.12	6.1 + 0.36	-
25	22.0	(E)- β –Ionone		2.3 ± 0.13		0.7 + 0.07	
26	22.2	Bicyclogermacrene	1.8 + 0.21		4.3 ± 0.17		1502
27	22.4	β-Bisabolene	8.1 + 0.27	6.3 ± 0.31	7.0 ± 0.19	4.1 + 0.36	1505
28	22.7	(*Z*)-α-Bisabolene		4.5 ± 0.14		4.2 + 0.32	1506
29	22.8	Cuparene		0.4 ± 0.04	4.6 ± 0.12	1.5 + 0.12	
30	23.2	Caryophyllene oxide	0.9 + 0.08	5.1 ± 0.12			1567
31	23.4	Tridecanol		1.0 ± 0.02		0.2 + 0.01	1570
32	23.6	*ar*-dihydro Turmerone			2.1 ± 0.14		1595
33	23.9	Junenol		0.3 ± 0.04	0.7 ± 0.08	0.8 + 0.06	1618
34	24.1	γ-Eudesmol	0.4 + 0.03				1630
35	24.2	α-Muurolol		5.2 ± 0.46	1.5± 0.14	3.4 + 0.26	1644
36	24.3	Cubenol	2.3 + 0.19	0.4 ± 0.02	0.9± 0.06	0.9 + 0.08	1645
37	24.7	(6*Z*)-Pentadecen-2-one	3.1 + 0.27	3.3 ± 0.05			1667
38	25.2	Pentadecanal	1.2 + 0.18				1682
39	25.3	(2*Z*,6*Z*)-Farnesal			0.2 ± 0.03	3.0 + 0.21	1684
40	25.7	(2*Z*,6*Z*)-Farnesol		0.7 ± 0.03	2.2 ± 0.21	3.7 + 0.21	1698
41	26	(2*E*,6*E*)-Farnesol	2.3 + 0.21	2.7 ± 0.09			1742
42	26.5	*n*-Hexadecanol	4.0 + 0.34		4.4 ± 0.21		1874
43	26.9	Hexadecanoic acid	0.5 + 0.04			0.5 + 0.04	1959
44	27.2	*n*-Eicosane	1.3 + 0.12	1.2 ± 0.07	1.5 ± 0.14		2000
45	28.2	*n*-Octadecanol	0.5 + 0.04				2077
46	28.4	*n*-Heneicosane	1.4 + 0.11	0.3 ± 0.04		1.3 + 0.12	2100
47	28.5	(*E*)-Phytol acetate		1.2 ± 0.04		1.2 ± 0.03	
48	29	*n*-Tricosane	1.9 + 0.011	1.5 ± 0.09			2300
49	31.9	*n*-Pentacosane	0.7 + 0.06	2.1 ± 0.05			2500
50	32	Hexacosane	4.8 + 0.32				2600
51	41.6	Heptacosane	5.1 + 0.30	6.1 ± 0.14	17.3 ± 0.14	7.3 + 0.3	2700
		Monoterpene hydrocarbons	6.6	2.6	5.2	0.3	
		Oxygenated monoterpene	21.5	18.2	16.6	15.6	
		Sesquiterpene hydrocarbons	26.3	40.3	35.5	39.6	
		oxygenated sesquiterpenes	6.1	14.4	7.6	11.8	
		Diterpenes	-	1.2	-	1.2	
		Total compounds	92.0	97.3	92.0	94.7	

*RI: retention indices according to the normal alkanes between C8-C24. The bold typeface means the compounds have the highest value.

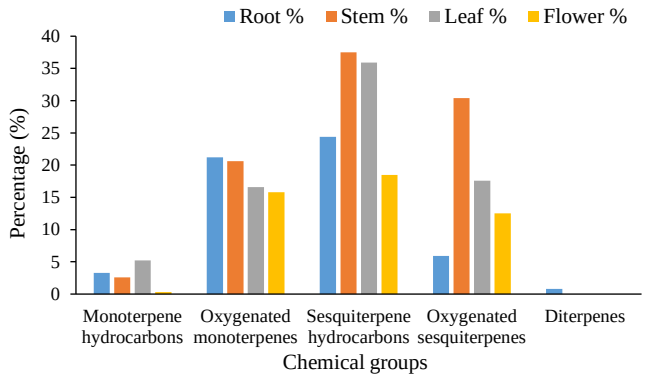

Figure 2. Chemical groups of the essential oil's compositions from organs of *D. aucheri*.

Table 4. Analysis of variance (mean squares) for the effects of plant parts on total tannin content (TTC), saponins, total phenol content (TPC), total flavonoid content (TFC), DPPH and FRAP in *D. aucheri*.

SoV	df	TTC	Saponins	TPC	TFC	DPPH	FRAP
Block	2	0.005	0.001	6.21	5.49	18.9	7.0
Treat	3	0.376*	0.00006*	57.1**	11.16*	13813.4**	90.97**
Error	6	0.11	0.0003	3.67	2.27	23.46	8.88
CV (%)	-	24.4	5.9	14.3	18.5	3.3	18.3

**, * and ns significant at 1%, 5% level of probability and non-significant, respectively.

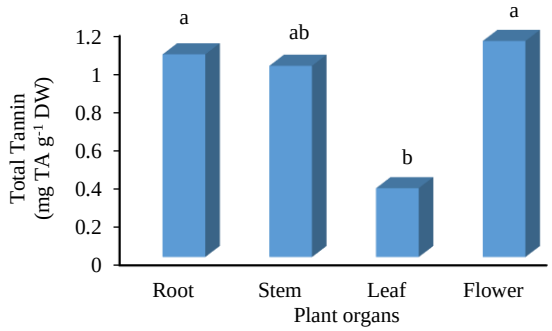

Figure 3. Mean comparison of TTC (total tannin content; mg TA g^{-1} DW) of *D. aucheri* methanolic extracts. Different letters indicate statistical significance based on least significant difference (LSD) test (p<0.05).

3.3. Saponins

The analysis of variance (ANOVA) showed a significant difference (p≤0.05) in the saponin content between the extracts from different plant organs of the *D. aucheri* populations. As shown in Table 4, the plant organ with the highest saponin content was the root extract, which contained 0.10% saponin (Fig. 4). Similarly to the tannin content, this is the first report about saponins being variable in different organs of *D. aucheri*.

3.4. Antioxidant activity: DPPH and FRAP

The antioxidant capacity of the samples was estimated by DPPH and FRAP methods. Significant differences were noted between the extracts from the organs of *D. aucheri* in antioxidant activity (p≤0.01) (Table 4). The results of DPPH assay are shown in Fig. 5. In DPPH assay, the maximum value of antioxidant capacity (lowest IC_{50}) was discovered in the flower extract with an IC_{50} of 148.1 µg ml^{-1} compared to BHT (46.3 µg ml^{-1}), a synthetic industrial antioxidant, respectively. The lowest activity (IC_{50} 300.0 µg ml^{-1}) was associated with the stem sample. However, for the FRAP activity, a sample of flowers was higher than all the other samples and the amount of antioxidant activity was 23.3 mg Fe^{2+} g^{-1} DW (Fig. 6).

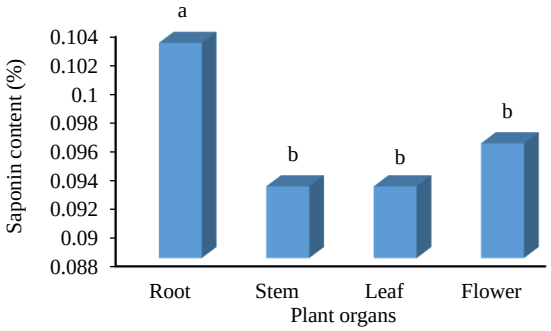

Figure 4. Mean comparison of saponin content (%) of all *D. aucheri* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test (p<0.05).

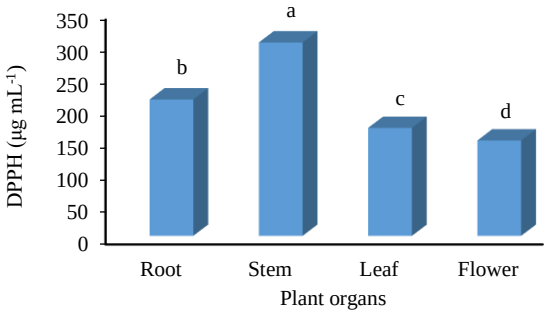

Figure 5. Mean comparison of DPPH (antioxidant activity by DPPH assay; IC_{50}) of all *D. aucheri* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test (p<0.05).

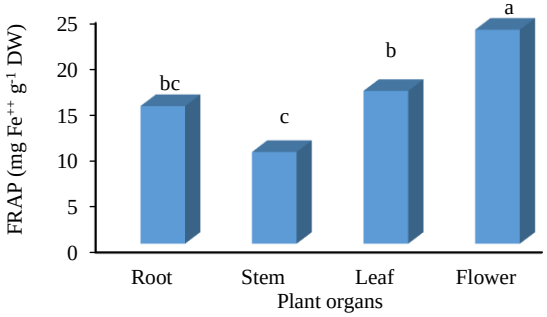

Figure 6. Mean comparison of FRAP (antioxidant activity by FRAP assay; mg Fe^{++} g^{-1} DW) of all *D. aucheri* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test (p<0.05).

3.5. Total phenolic and flavonoid contents

The results indicated a significant difference ($p \leq 0.01$) in the total phenolic and flavonoid content across all extracts of *D. aucheri* (Table 4). The root and flower extracts had the highest total phenolic content, with 17.2 mg GAE g^{-1} DW and 16.6 mg GAE g^{-1} DW respectively, while the leaf extract had a much lower phenolic content of 8.3 mg GAE g^{-1} DW (Fig. 7). Additionally, the root and stem extracts contained the highest flavonoid content, at 9.9 mg QE g^{-1} DW and 9.6 mg QE g^{-1} DW respectively (Fig. 8). These findings indicate significant variations in the phytochemical composition across the different plant organs of *D. aucheri*.

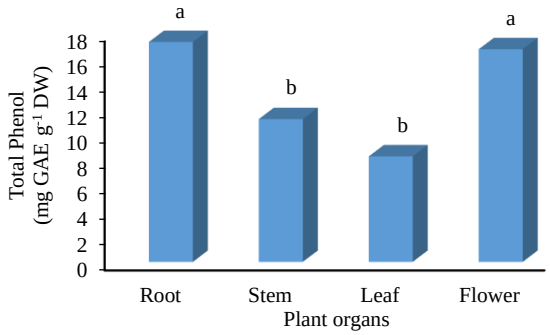

Figure 7. Mean comparison of TPC (total phenolic content; mg GAE g^{-1} DW) of methanolic extract of *D. aucheri*. Different letters indicate statistical significance based on least significant difference (LSD) test ($p<0.05$).

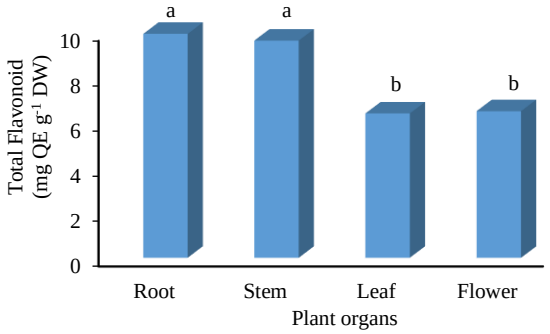

Figure 8. Mean comparison of TFC (total flavonoid content; mg QE g^{-1} DW) of methanolic extract of *D. aucheri*. Different letters indicate statistical significance based on least significant difference (LSD) test ($p<0.05$).

3.6. Correlation between TTC, saponin, TPC, TFC and AA

The results showed that there were significant positive correlations between several phytochemical parameters and the antioxidant activity measured by DPPH and FRAP assays in the *D. aucheri* extracts (Table 5). Specifically, the total phenolic content, saponin, and total tannin content all exhibited significant positive correlations with both DPPH and FRAP antioxidant activity, with correlation coefficients ranging from 0.30 to 0.35. However, the total flavonoid content (TFC) did not show a significant correlation with the overall antioxidant capacity. These findings indicate that the phenolic compounds, saponins, and tannins are likely the primary phytochemical contributors to the potent antioxidant properties demonstrated by the *D. aucheri* plant extracts.

Table 5. Correlation between six main traits on studied *D. aucheri* samples: TTC, total tannin content; TPC, total phenolic content; TFC, total flavonoid content; DPPH, antioxidant activity by DPPH assay; FRAP, antioxidant activity by FRAP assay.

	Tannin	Saponin	TPC	TFC	DPPH	FRAP
Tannin	1					
Saponin	0.05^{ns}	1				
TPC	0.04^{ns}	-0.18^{ns}	1			
TFC	0.10^{ns}	0.19^{ns}	0.22^{ns}	1		
DPPH	0.33^{**}	0.30^{*}	0.31^{*}	0.08^{ns}	1	
FRAP	0.32^{**}	0.35^{**}	0.35^{**}	0.02^{ns}	0.89^{**}	1

**, * and ^{ns} significant at 1%, 5% level of probability and non-significant, respectively.

The yield of oil of *D. aucheri* organs averaged 0.3-0.4 w/w %. Akbarian et al. (2016) reported the EO content of leaves of *D. aucheri* from several areas was 0.67 and 2.6 w/w%, respectively. There are several previous studies that indicate that essential oil content can vary among different plant organs such as the Apiaceae family, including *Oliveria decumbens*, *Trachyspermum ammi* and *Heracleum persicum* (Hazrati *et al*., 2020). In comparison, (Akbarian *et al*., 2016) reported the essential oil content of stem and fruit of *D. aucheri* was 0.28 to 0.68 % v/w %. Also, the EO yields of the aerial part and root of *D. ammoniacum* were 0.2 and 0.3 v/w %, respectively (Delnavazi *et al*., 2014).

Thymol, as a main compound in some samples, is a food additive and it is used to improve the digestive secretion as it better aids the functioning of digestive system, thereby assisting with feeding (Alagawany *et al*., 2021). However, in a separate study, Arabjafari et al. (2023) reported the major components in leaf oil to be β-caryophyllene. β-bisabolene is the common major component in roots, stems and flower oil of *D. aucheri* is used in personal care products and flavoring agent in beverages (Barton and Chickos, 2020). It has a balsamic aroma and food additive approval (Barton and Chickos, 2020). It has also been shown to exhibit cytotoxicity in breast cancer cell lines, in vitro and in

vivo (Yeo *et al*., 2016). In comparison, the chemical composition analysis of the volatile components from *Dorema Glabrum* revealed that the main compound in the root was δ-cadinene (12.77%), followed by β-bisabolene (7.48%), α-fenchyl acetate (6.32%), and copaene (5.68%) (Asnaashari *et al*., 2011). Otherwise, Yousefzadi et al. (2011) found that (Z)-ocimenone (22.3%), (E)-ocimenone (18.1%) and β-cyclocitral (9.9%) were the main constituents in the fruit oil of *D. ammoniacum*. Hosseini et al. (2014) indicated that 2-pentadecanone (19.1%), β-eudesmol (17.2%), germacrene D (5.8%), α-eudesmol (5.8%) and spathulenol (5.0%) were the main constituents in the EO of seeds. In general, the essential oils of *D. aucheri* have been reported to be primarily composed of sesquiterpene hydrocarbons. Specifically, these sesquiterpene hydrocarbons account for 65.5-87.5 % of the essential oil composition in the stems, and 59.4-84.3 % in the leaves of *D. aucheri* (Arabjafari *et al.*, 2023). The change in the essential oil components may also be impacted by the maturity and growth stage of the individual plants sampled (Hazrati *et al.*, 2020). The differences in essential oil composition between *Dorema* species can be attributed to factors like genetic diversity, climate, soil, location/timing of sampling, and environmental stresses. The essential oil profiles of medicinal plants can vary significantly based on these geological and environmental conditions (Mutlu-Ingok *et al.*, 2020; Norani *et al.*, 2023).

Since the effects of factors like climate, soil, and growing location can vary significantly, it is necessary to carefully evaluate the specific role each of these plays in influencing the growth, development, and essential oil profiles of medicinal plants (Aboukhalid *et al.*, 2017). Climatic characteristics, topography, and soil fertility have a great effect on the growth, quantity, and quality of essential oils in medicinal plants (Hassiotis *et al.*, 2014). Identifying the beneficial environmental factors that positively influence the amount and effectiveness of the essential oil components is of great importance (Ahmed and Tavaszi-Sarosi, 2019).

Tannins are an important class of phytochemicals that can be categorized as either hydrolyzable or condensed tannins, depending on their chemical structure. The process of tannin synthesis within plants is relatively short and is more closely tied to the metabolism of carbohydrates compared to other secondary metabolites. Environmental factors such as light stress and shading, atmospheric changes, temperature fluctuations (between day and night), exogenous plant hormones, pathogen infections (bacterial and fungal), solar radiation, and nutrient deficiencies (in nitrogen, water, and phosphorus) can all potentially influence the biosynthesis of tannins (Qaderi *et al.*, 2023). In contrast to triterpenoid saponins, steroid saponins are common in medicinal herbs or are exploited for their health-promoting characteristic (Singh and Kaur, 2018). Saponins are a diverse group of plant-derived compounds that have been extensively studied for their pharmacological activities. These components have been reported to possess a vast range of therapeutic applications, including anti-inflammatory, antioxidant, antimicrobial, antiviral, and anticancer properties (Sparg *et al.*, 2004). Beyond their general medicinal properties, saponins have also been specifically associated with the tonic and stimulating activities observed in various medicinal herbs and plants. This is because saponins can interact with and modulate numerous physiological and biochemical processes within the human body (Ezeabara *et al*., 2014).

Previous studies of the hydroalcoholic extracts from *D. aitchisonii* aerial parts and ethanolic extracts of *D. aucheri* aerial parts confirmed that these species had a weak antioxidant activity based on the DPPH assay, as IC_{50} values of 488 and 200 μg mL^{-1}, respectively, were recorded (Nabavi *et al.*, 2012). In case of *D. aucheri*, n-hexane fraction showed more inhibition (68.44%) of DPPH (Khan *et al*., 2014). As a radical scavenging investigation (DPPH) on several species of the Apiaceae (Falcaria vulgaris, *Smyrniopsis munzurdagensis*, *Smyrnium cordifolium*, and *Actinolema macrolema*), Zengin et al. (2019) reported that the highest antioxidant activity was observed in *Smyrnium cordifolium* methanolic extract with 59.2 mg TE g^{-1} extract and *Smyrniopsis munzurdagensis* extract showed low antioxidant activity with 2.29 mg TE g^{-1}. The difference in antioxidant capacity observed among samples from various populations can indeed be attributed to differences in their polyphenolic compound composition. These differences in the polyphenolic composition of samples from different populations are often responsible for the observed variations in their overall antioxidant capacity (Rostaei *et al.*, 2018).

In this paper, the phenolic and flavonoid contents in different organs of *D. aucheri* are recorded for the first time. Mianabadi et al. (2015) reported that total phenolics of stem in *D. aucheri* were 22.72 mg GAE g^{-1} dW, and the maximum value of flavonoid (1.95 mg QE g^{-1} dW) was observed in the flower. Additionally, the main inhibition was reported in the leaf (48.52%) and flower (54.24%) of *D. aucheri*. Also, the total phenols ranged from 52 ± 7 to 67 ± 2 mg g^{-1} in *D. aucheri* extracts (Khan *et al*., 2014). Furthermore, the ferric reducing antioxidant power (FRAP) was also found to be maximum in the stem and flower. Nazir et al. (2021) reported that the total phenolic contents and flavonoids of *D. ammoniacum* aerial parts (methanolic extraction) from Pakistan were 68.2 mg GAE g^{-1} and 66.97 mg QE g^{-1}. Phenolic and flavonoid compounds are highly effective at neutralizing various oxidizing molecules, including singlet oxygen and different types of free radicals. Free radicals have been implicated in the development of several diseases (Owen *et al*., 2003). The polyphenol compounds in plant extracts can differ, depending on several influencing factors. These factors play major roles in the plant's defense mechanisms against threats such as predators, auto-oxidative effects, induced oxidative stress, and extreme weather conditions (Tuladhar *et al*., 2021). The natural habitats of Iran are regarded as valuable resources for medicinal plants production in nature (Jamshidi-Kia *et al*., 2017). Regarding the remembrance of native medicinal plants or plants well-adapted to definite ecological zones of Iran, it is possible to domesticate and cultivate these plants on a large scale to enable commercial-scale production. This could help harness the benefits of these valuable native plant resources (Sharafzadeh and Alizadeh, 2012). Furthermore, environmental factors can improve the growth and phytochemical properties of medicinal plants. When the environmental conditions are optimized to produce desirable metabolites, the plants will yield higher quantities of these beneficial compounds (Biondi *et al*., 2021). Therefore, by carefully selecting the environmental factors and suitable plant varieties, it is possible to achieve maximum production of the desired secondary metabolites in medicinal plants (Yang *et al*., 2018). The results of the study showed a positive and significant correlation between the TTC (0.33 and 0.32), saponin (0.30 and 0.35) and TPC (0.31 and 0.35) with antioxidant activity (DPPH and FRAP).

Generally, there is a significant relation between total tannin contents (Medda *et al*., 2021), saponin (Puente-Garza *et al*., 2017) and total phenolic contents (Zhang *et al*., 2023) with antioxidant activities of plants such as *Myrtus communis* L., *Agave salmiana*, *H. officinalis*, *P. oleracea*, and *O. vulgare*, which showed antioxidant activity with comparable phenolic content (Nile *et al*., 2017). Antioxidant capacity displays the existence of different and invaluable components, inclusive of phenols and flavonoids and identification of various bioactive resources may be a path to the discovery of traditional medicines and remedies for many critical diseases (Basgedik *et al*., 2014).

4. Conclusion

The essential oil yield of *D. aucheri* is relatively low; however, its chemical composition varies notably among different plant organs, with sesquiterpene hydrocarbons and oxygenated sesquiterpenes being predominant. This study provides novel insights into the phytochemical profiles, antioxidant activities, and bioactive compound contents—such as phenolics, flavonoids, tannins, and saponins—across roots, stems, leaves, and flowers of *D. aucheri*. Significant variations in antioxidant capacity were observed, with the flower extracts exhibiting the highest activity. Positive correlations between phenolic content, saponins, tannins, and antioxidant activity suggest these compounds play key roles in the plant's bioactivity. Given the ecological importance and medicinal potential of *D. aucheri*, expanding its cultivation is recommended to sustainably meet demand and reduce pressure from wild harvesting. These findings contribute valuable data for future pharmacological applications and conservation efforts involving this endemic species.

Abbreviation

AA= antioxidant activity, DPPH= 2,2-diphenyl-2 picrylhydrazyl hydrate, EC= Electrical Conductivity, EO= essential oil, FRAP= ferric reducing antioxidant power, LSD= least significant difference, pH= Soil acidity, RSC= radical scavenging capacity, TFC: Total flavonoid contents, TPC= Total phenolic contents.

Conflict of interests

The authors have to declare their conflicts of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

The authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

The first author [M.N.]: performance of the research project and writing the article. The second author [O.K.]: cooperation in the implementation of the research project.

Informed consent

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

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