



Eco-phytochemical Properties of Ten *Dorema ammoniacum* Populations in Different Climates of Iran

Mohamad Norani, Mohammad-Taghi Ebadi, Mahdi Ayyari*

Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

*Corresponding author's email: m.ayyari@modares.ac.ir

Article history:

Received: 18 November 2023,
Received in revised form: 26 March 2024,
Accepted: 23 November 2024

Article type:

Research paper

Keywords:

Antioxidant activity,
 β -bisabolene,
D. ammoniacum
Eco-phytochemical,
Volatile compounds

ABSTRACT

This study evaluated the contents and composition of the essential oil (EO) derived from *Dorema ammoniacum*, a perennial plant belonging to the Apiaceae family. Roots and flowers of *D. ammoniacum* were collected from ten different regions in Iran. The EO yield ranged from 0.2% to 0.5% in roots and 0.2% to 0.46% in flowers (w/v). The primary components of the oil were β -bisabolene (3.4–14.9%), δ -elemene (0.3–14.5%), heptacosane (1.2–27.3%), and n-dodecane (0.1–46.6%). Additionally, methanolic extracts were prepared from all samples, and various parameters were assessed, including total tannin content, saponin content, antioxidant activity (via DPPH and FRAP assays), total phenolic content (TPC), and total flavonoid content. Significant variations were observed among the extracts in antioxidant activity, tannin content, saponin content, TPC, and total flavonoid content. Principal component analysis (PCA) based on ecological and phytochemical characteristics classified the root samples into three groups. Group 3 demonstrated the highest levels of antioxidant activity, TPC, saponin, and tannin content. Similarly, flower samples were divided into three clusters, with Group 3 showing the highest antioxidant activity and saponin content. Overall, the findings highlight that the phytochemical traits of *D. ammoniacum*, including its essential oil and extracts, are influenced by ecological factors. The plant exhibited moderate to high antioxidant activity and TPC, suggesting its potential as a valuable medicinal plant compared to other species.

Abbreviations: Antioxidant activity (AA), 2,2-Diphenyl-2-picrylhydrazyl hydrate (DPPH), Electrical conductivity (EC), Essential oil (EO), Ferric reducing antioxidant power (FRAP), Soil acidity (pH), Principal component analysis (PCA), Radical scavenging capacity (RSC), Total flavonoid contents (TFC), Total phenolic contents (TPC)

Introduction

Medicinal plants are highly valued for their therapeutic properties and continue to play a vital role in modern medicine (Nazir et al., 2021; Sliwiska, 2018). These plants are rich sources of bioactive compounds with medicinal properties that can treat or alleviate a wide range of health conditions (Memarzadeh et al., 2020). The medicinal plant industry encompasses the

cultivation, harvesting, processing, and distribution of these plants and their derivatives (Hosseini et al., 2018). Numerous plant-derived chemical compounds have demonstrated significant pharmacological activities, serving as the basis for new drug development or the synthesis of pharmaceutical compounds (Hassan et al., 2019).

COPYRIGHT

© 2025 The author(s). This is an openaccess article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other medium is permitted, provided the original author(s) and source are cited, in accordance with accepted academic practice. No permission is required from the authors or the publishers.

Although the production of secondary metabolites in plants is primarily governed by genetic factors, their concentration and accumulation are significantly influenced by environmental conditions (Karimian et al., 2017). *Dorema ammoniacum* is a perennial herb native to arid and mountainous regions, such as Yazd, Isfahan, and Semnan provinces in Iran. The plant can grow up to 2 m tall and features a robust stem with large, pinnate leaves (Rechinger et al., 1987; Yousefzadi et al., 2011a). The resin, commonly known as gum ammoniacum, is obtained by making incisions in the stem to collect the exudate. In Iranian traditional medicine, this resin is used as an anthelmintic, for gastrointestinal disorders (Amin, 2005), and as a treatment for seizures (Motevalian et al., 2017). Additionally, *D. ammoniacum* exhibits antibacterial, vasodilatory, and anticonvulsant effects (Ghasemi et al., 2018).

Previous research on *D. ammoniacum* essential oils (EOs) from Hezar Mountain in the Rayen area, Kerman Province, showed that hydrodistillation of the gum, stem, seed, and fruit produced yellow oil yields of 0.4%, 0.5%, 0.3%, and 0.09% (w/w), respectively (Hosseini et al., 2018; Rajani et al., 2002; Yousefzadi et al., 2011b). Key chemical components of the gum include free salicylic acid, ammosesinol, doremin, doremene A, and ammodoremin (Rajani et al., 2002).

The major constituents of the flower oil were δ -cadinene (11.58%) and α -himachalene (7.71%). The stem oil contained δ -cadinene (16.24%), liguloxide (8.69%), and δ -amorphene (8.43%), while the root oil had 3-n-butyl phthalide (62.49%) as the predominant compound (Masoudi and Kakavand, 2017). Studies on volatile oils from the leaves of *D. ammoniacum* have also been conducted (Yousefzadi et al., 2011b). In fruit oil from *D. ammoniacum* in Birjand, (Z)- and (E)-ocimene, β -cyclocitral, and α -curcumene were the primary components, while α -gurjunene (49.5%), β -gurjunene (19.0%), and α -selinene (4.6%) were the dominant compounds in the leaves. Stem oil consisted primarily of hexadecanal (11.1%), α -cadinol (6.6%), sesquiceneol-2-one (6.6%), ethyl linoleate (6.3%), ledol (5.1%), and γ -eudesmol (4.4%). The essential oil from seeds contained 2-pentadecanone (19.1%), β -eudesmol (17.2%), germacrene D (5.8%), α -eudesmol (5.8%), and spathulenol (5.0%) (Hosseini et al., 2018).

The quality and quantity of essential oils are strongly influenced by climatic conditions (Moghaddam and Farhadi, 2015). Non-volatile compounds in the plant primarily consist of phenolic and flavonoid compounds synthesized

during the plant's lifecycle in response to various environmental factors (Masoudi and Kakavand, 2017). These compounds are known for their ability to prevent free radical formation during oxidative stress and enhance antioxidant activity. Clinical studies have shown that phenolics and flavonoids have not only high antioxidant capacities but also antimicrobial properties, including antiviral, antifungal, and antibacterial effects (Sim et al., 2019).

Reports on other bioactive compounds in *D. ammoniacum* extracts include salicylic acid, ammosesinol, ashamirone, and sesquiterpenes. Additionally, sesquiterpene coumarins, phenols, flavonoids, and phloroacetophenone glycosides have been identified in other *Dorema* species. The production and biological activity of these phytochemicals are influenced by ecological factors such as climate, soil composition, light availability, and interactions with other organisms (Norani et al., 2019).

This study aimed to investigate the variation in essential oil composition in *D. ammoniacum* plants from ten regions in Iran using gas chromatography-mass spectrometry (GC-MS). The essential oils were analyzed for total tannin content, saponin content, and antioxidant activity (AA), including 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and ferric-reducing antioxidant capacity (FRAP), as well as total phenol content (TPC) and total flavonoid content (TFC) from the roots and flowers of *D. ammoniacum*.

Material and methods

Plant materials

Fresh roots and flowers of *D. ammoniacum* were collected during the flowering stage (April of 2019) from ten regions in Iran, including Jiroft, Shahrood, Garmsar, Kerend, Birjand, Kashmar, Bardaskan, Bafq, Mehriz and Neyriz (Fig. 1). The samples were air-dried under shade conditions and room temperature. The botanical names of plants were confirmed by Dr. A. Sonboli, a taxonomist. A representative voucher specimen (MPH-2724) was archived in the Medicinal Plants and Drug Research Institute Herbarium (MPH) of Shahid Beheshti University, Iran.

Habitat characteristics

Site characteristics, including altitude, longitude, and latitude, were determined using GPS (Table 1). Data on average annual rainfall, temperature, and relative humidity were obtained from nearby weather stations (Table 1). Soil samples were collected at root depth (rhizosphere) from the study sites to analyze key physicochemical properties. Soil texture was determined using the

hydrometer method (Gangwar and Baskar, 2019). Soil acidity (pH) and electrical conductivity (EC) were measured using extracts from saturated soil paste. Soil organic carbon content was analyzed using the titration method (Gelman et al., 2012), while soil nitrogen was measured via the Kjeldahl method (Chakraborty

et al., 2019). Phosphorus content was determined using Olsen's method (Sims, 2000), potassium levels were assessed with a flame photometer (Ren et al., 2019), and microelements were analyzed through atomic absorption spectroscopy (Moron and Cozzolino, 2003) (Table 2).

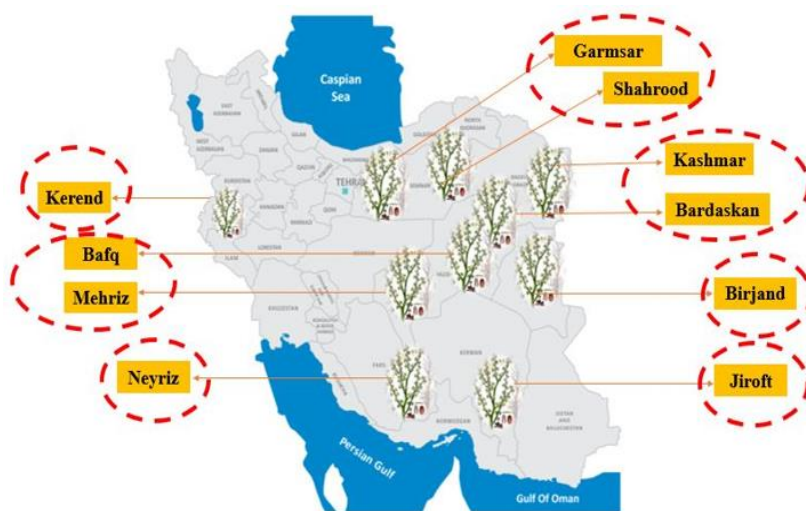


Fig. 1. Map of collection sites for ten *D. ammoniacum* populations.

Table 1. Geographical coordinates and collection sites of ten *D. ammoniacum* populations.

Sampling location	Province	Average rainfall (mm)	Average annual temperature (°C)	Relative humidity (%)	Latitude	Longitude	Altitude (m a.s.l.)*
Bardaskan	Razavi Khorasan	110	19.5	33	57°89'54"	35°97'83"	1479
Kashmar	Razavi Khorasan	170	17.5	40	58°46'85"	35°33'53"	1332
Birjand	South Khorasan	155	16.5	36	57°89'54"	35°97'83"	1468
Shahrood	Semnan	180	17	49	55°36'69"	36°44'99"	1297
Garmsar	Semnan	124	21.7	41	52°16'42"	35°28'11"	951
Jiroft	Kerman	239	27.1	38	57°26'57"	28°06'26"	1897
Kerend-e Gharb	Kermanshah	527	13.7	50	46°14'07"	34°16'50"	1553
Bafq	Yazd	96	24	37	55°42'48"	31°30'08"	1405
Mehriz	Yazd	149	22	40	53°50'12"	31°09'50"	2301
Neyriz	Fars	180	17.7	41.4	54°21'20"	29°14'40"	1636

* Meters above sea level.

Isolation and analysis of essential oils

Fifty grams of air-dried roots and flowers of *D. ammoniacum* were separately chopped and immersed in 500 mL of distilled water. EOs were extracted through hydro-distillation using a Clevenger-type apparatus for 3 h. The EOs were separated from the water, dried over anhydrous sodium sulfate, and stored at 4 °C for subsequent analysis. EO yields were calculated based on the dry weight of the plant material (Sařata et al., 2020).

GC analysis was conducted using an Agilent Technologies 7890B system (Santa Clara, CA, USA) equipped with a flame ionization detector. The system featured an HP-5 fused silica column (30 m length, 0.32 mm inner diameter, and 0.25 µm film thickness). Helium served as the carrier gas at a flow rate of 1.1 mL min⁻¹. Each EO sample (1 µL) was injected into a Thermoquest–Finnigan gas chromatograph coupled with a trace mass spectrometer, which used the same parameters for the fused silica column except for the inner

diameter (0.25 mm). The ionization voltage was set to 70 eV, while the ion source and interface temperatures were maintained at 200 °C and 250 °C, respectively.

Essential oil compounds were identified by comparing their mass spectra with those in the

Wiley 7.0 and Adams mass spectral libraries. Retention indices were also compared with those of a homologous series (C8 to C24) under identical operating conditions. Published data (Adams, 2007) were used as an additional reference (Fig. 2).

Table 2. Physical and chemical properties of soils (depth of 0–30 cm) at various sites.

Parameter	Measurement method	Jiroft	Shahrood	Garmsar	Kerend	Birjand	Kashmar	Bardaskan	Bafq	Mehriz	Neyriz
Clay (%)	Hydrometer method	8	12	22	12	12	12	22	10	10	10
Silt (%)	Hydrometer method	18	4	36	20	12	20	32	10	16	12
Sand (%)	Hydrometer method	74	84	42	68	76	68	46	80	74	78
Texture	-	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
EC (ds m ⁻¹)	Conductometer	1.9	0.89	0.57	1.86	0.66	0.49	0.55	0.75	0.65	0.51
pH	pH meter	7.55	7.65	7.54	7.48	7.89	7.81	7.85	7.55	7.71	8
OC (%)	Titration method	0.49	0.08	1.52	0.51	0.6	0.47	0.47	0.6	0.66	0.66
Nitrogen (%)	Kajeldal	0.04	.01	0.15	0.05	0.06	0.04	0.04	0.06	.06	0.06
Phosphorus (ppm)	Olsen method	6.4	5	15	7.8	8.4	4.2	8.4	6.4	6.2	6.2
Potassium (ppm)	Flame photometer	67	77	326.8	126.8	186.6	77	216.6	126.8	147	147
Fe (ppm)	Atomic	2.02	4.76	11.52	5.04	3.52	5.92	6.94	3.5	2.44	3.52
Zn (ppm)	Atomic	0.9	0.42	0.82	0.76	0.46	0.44	0.5	0.4	0.38	0.46
Cu (ppm)	Atomic	1.1	0.94	2.42	1.28	4.78	0.84	1.36	0.9	0.08	0.58
Mn (ppm)	Atomic	2.26	2.96	7.44	3.8	2.22	3.42	3.96	3.08	2.58	4.44

EC: Electrical conductivity.

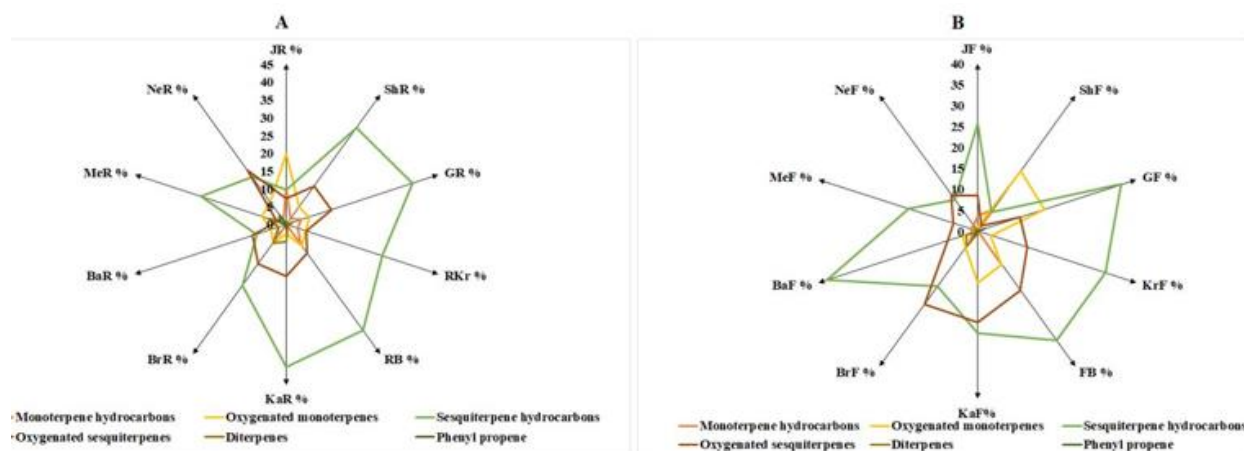


Fig. 2. Chemical groups of the essential oils compositions from organs of *D. ammoniacum*. A (root) and B (flower).

Preparation of different extracts

In the present study, leaf and stem extracts of *D. ammoniacum* were prepared by sonication, using an ultrasonic device with a 120 Hz frequency. Five g of dried plant material was sonicated for 30 min at 30 °C in 50 mL of methanol. All extracts were filtered using Whatman No.1 filter paper and then concentrated in a rotary evaporator at 40 °C in a vacuum. After drying the extracts, they were stored at 4 °C until further analysis (Shelke and Bhot, 2019).

Total tannin measurement

The tannin contents of methanolic extracts were determined according to Luthar and Kreft (1999). Accordingly, 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. Then, the samples were stored at 28 °C for 20 min. The absorbance of each sample was read at a wavelength of 520 nm. Tannic acid was used for drawing a standard curve. The TTC amount of rhizomes was reported

according to mg tannic acid equivalent per g dry weight (mg TA g⁻¹ DW).

Determination of saponin content

Five grams of powdered sample was mixed with 50 mL of 20% aqueous ethanol solution in a flask. The mixture was heated in a water bath at 55 °C for 90 min with periodic agitation and then filtered through Whatman No. 42 filter paper. The residue was re-extracted using 50 mL of 20% ethanol, and the two filtrates were combined. The combined extract was concentrated to approximately 40 mL at 90 °C and transferred to a separating funnel. To this, 40 mL of n-hexane was added, and the mixture was shaken vigorously. Repeated partitioning was performed until the aqueous layer became clear. Saponins were then extracted by adding 60 mL of normal butanol to the aqueous phase. The combined butanol extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. The drying process was carried out at 60 °C in an oven. Once dried, the samples were cooled in a desiccator and reweighed. This procedure was repeated two more times to ensure accuracy, and the average value was recorded. The saponin content was calculated as a percentage of the original sample using the following equation:

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

Where:

W₁ = Weight of evaporating dish

W₂ = Weight of evaporating dish + sample

Determination of total phenolic compounds

Total phenolic contents of *D. ammoniacum* were measured according to the Folin-Ciocalteu method (Slinkard and Singleton, 1977). A calibration curve was illustrated using a series of methanolic Gallic acid solutions (10, 30, 100, 250, 500, and 1000 µg mL⁻¹) combined with 0.1 mL Folin-Ciocalteu reagent. After 3 min, 0.3 mL sodium carbonate (7.5%) was added. The absorbance of the mixture was measured after 2 h at room temperature, at 765 nm, using a spectrophotometer (Smart Spec Plus, BIORAD). Twenty µL of each *D. ammoniacum* extract, with 0.05 g mL concentration, were combined with the reagents mentioned above in three technical replications to determine phenolic content. Gallic acid was used as a standard for a calibration curve, and the results were expressed as mg of Gallic acid equivalents g⁻¹ dry weight of extract (mg GAE g⁻¹ DW ext.).

The total phenolic compound was estimated using the following formula:

$$C = \frac{c \cdot V}{m}$$

where:

C: total phenolic content

c: The concentration of gallic acid established from the calibration curve, mg mL⁻¹

V: The volume of extract, mL;

m: The weight of pure plant methanolic extract

Determination of total flavonoid

Total flavonoid content (TFC) was measured using a colorimetric method described by Ordonez et al. (2006). Briefly, extracts of *D. ammoniacum* were prepared at 0.5 g mL⁻¹ in DMSO. The same amount of extract volume and aluminum chloride solution (2%, methanolic solution) was mixed in a test tube, and the absorbance was measured at 420 nm using a spectrophotometer after 10 min. Each extract was evaluated in triplicates. A calibration curve was prepared using methanolic quercetin solutions (10, 50, 100, 250, 500, and 1000 µg mL⁻¹). The results were expressed as mg of quercetin equivalents dry per gram dried weight of extract (mg QE g⁻¹ DW ext.).

Antioxidant capacity

DPPH method

Antioxidant activities of methanolic extracts were evaluated by DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) radical scavenging activity according to a previously described method (Bozin et al., 2007). It involved using IC₅₀ to compare the antioxidant properties. The absorbance of each sample was measured at 517 nm with an ELISA reader (Epoch, BioTek instrument). The radical scavenging capacity (RSC) was calculated as follows:

$$\text{Inhibition} = \frac{Ab - As}{Ab} \times 100$$

Where Inhibition is DPPH inhibition, Ab is the absorbance of the blank, As is the absorbance of the sample extract, and BHT is a positive control. IC₅₀ is a sample concentration from the equation where the inhibition percentage is 50%.

FRAP method

Reducing powers of the extracts were determined using a ferric reducing antioxidant power (FRAP) method (Tomasina et al., 2012). In this method, 50 µL methanolic extract, 3 mL fresh reagent of 190 FRAP [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 FeCl₃ 6H₂O (10:1:1, v/v/v)] were mixed. The resultant mixture was put in a hot water bath (37 °C) in the dark for 30 min. Then, the absorbance level was measured using an ELISA 193 reader (Epoch, BioTek instrument) at 593 nm wavelength. Each test was performed in triplicates and data were calculated using a standard curve of FeSO₄. The results were expressed as mg Fe²⁺ equivalent per g dry weight (mg Fe²⁺ g⁻¹ DW).

Statistical analysis

Data were analyzed according to the analysis of variance based on a randomized complete block design (RCBD) with three replications, using SAS Statistical Package Program version 9.0 and SPSS software version 20. The PROC UNIVARIATE in SAS was used for testing ANOVA assumptions, and residuals were normally distributed. Mean values were compared through Duncan's post hoc test ($P \leq 0.05$). A principal component analysis (PCA) was performed using XLSTAT software to determine the best relationships among samples and to measure the variables.

Results

Essential oil composition

Hydrodistillation of *D. ammoniacum* yielded 0.2–0.5% (v/w) from roots and 0.2–0.46% (v/w) from flowers, relative to the plant's dry weight. A total of 68 compounds were identified in the root oils of *D. ammoniacum* through GC/MS analysis (Table 3). In the root oils from populations in Jiroft, Shahrood, Garmsar, Kerend, Birjand, Kashmar, Bardaskan, Bafq, Mehriz, and Neyriz, more than 30 components were detected, accounting for 89.0%, 90.2%, 92.2%, 92.0%, 90.2%, 98.1%, 91.1%, 89.2%, 94.9%, and 91.8% of the total oil, respectively (Fig. S1A).

The root oil from Jiroft contained thymol (14.7%), heptacosane (12.8%), tridecanol (12.7%), and 4-methylene-5-hexenal (6.8%) as its major constituents. In Shahrood, the dominant components were β -bisabolene (23.1%), n-hexadecanol (7.5%), (6Z)-pentadecen-2-one (7.1%), and hexacosane (5.2%). The root oil of Garmsar had β -bisabolene (25.1%), tridecanol (11.5%), and heptacosane (4.8%) as key constituents. In Kerend, β -bisabolene (18.1%), n-dodecane (15.9%), and 2-pentadecanol (5.3%) were predominant. The root oil from Birjand was characterized by β -bisabolene (32.3%), carvacrol methyl ether (11.3%), and other notable compounds. In Kashmar, (6Z)-pentadecen-2-one (12.2%), δ -elemene (12.1%), β -bisabolene (9.4%), and (Z)- α -bisabolene (7.8%) were the

main components. Bardaskan root oil primarily contained (6Z)-pentadecen-2-one (13.6%), (Z)- α -bisabolene (7.9%), and (E)-phytol acetate (6.4%). In Bafq, n-eicosane (14.3%), (6Z)-pentadecen-2-one (13.5%), and n-dodecane (11.2%) were the dominant constituents. Mehriz root oil had δ -elemene (15.3%), (6Z)-pentadecen-2-one (13.2%), n-heptacosane (11.7%), and n-dodecane (10.4%). Finally, the root oil of Neyriz was composed mainly of n-heptacosane (15.3%), δ -elemene (14.2%), and (2Z,6Z)-farnesol (10.2%). These results highlight the regional variation in the chemical composition of *D. ammoniacum* root oils, with β -bisabolene and other bioactive compounds being significant across different populations

Thirty-six compounds were identified in the flower oils of the Jiroft ecotype, comprising 90.0% of the total oil (Table 4 and Fig. S1B). The major components in this oil were tridecanol (13.0%), δ -elemene (11.2%), and n-eicosane (8.2%). In the Shahrood flower oil, twenty-nine compounds were identified, accounting for 96.5% of the total oil. The dominant components were n-dodecane (46.6%), tridecanol (8.0%), and 4-methylene-5-hexenal (4.2%). In Garmsar plants, thirty-three compounds were identified, representing 92.5% of the total flower oil. The primary constituents included β -bisabolene (11.9%), p-cymen-8-ol (8.5%), n-dodecane (8.3%), and thymol (7.5%) (Table 4).

In Kerend plant samples, thirty-two compounds were detected, comprising 93.5% of the flower oil. Key components included n-heptacosane (27.3%), β -bisabolene (9.1%), and bicyclogermacrene (7.4%) (Table 5).

The flower oil from Birjand plants contained forty-one components, accounting for 89.9% of the oil. Major constituents included n-dodecane (11.4%), cuparene (7.8%), and p-cymen-8-ol (5.4%) (Table 4). In Kashmar plant samples, thirty-eight components were identified, comprising 96.3% of the flower oil. The main constituents were n-hexadecanol (11.2%), thymol (8.7%), n-heptacosane (6.3%), and (2E, 6E)-farnesol (6.2%) (Table 4). The flower oil of Bardaskan plant samples included twenty-eight identified components, which accounted for 90.3% of the total oil. The dominant compounds were (6Z)-pentadecen-2-one (8.7%), n-hexadecanol (8.2%), (Z)- α -bisabolene (8.1%), and (2Z, 6Z)-farnesol (7.5%) (Table 4). In Bafq plant samples, twenty-eight compounds were identified, comprising 89.9% of the flower oil. The main constituents were β -bisabolene (14.9%), δ -elemene (13.5%), (6Z)-pentadecen-2-one (9.2%), and n-pentadecanol (7.6%) (Table 4).

Table 3. Chemical composition (%) of root (R) essential oils of *D. ammoniacum*.

No	RT	Compounds	JR%	ShR%	GaR%	KrR%	BR%	KaR%	BrR%	BaR%	MeR%	NeR%	RI*
1	4.4	4-methylene-5-Hexenal	6.8+0.21^a									1.1+0.10	893
2	7.8	α -Pinene	0.6+0.06				0.7+0.07 ^a			0.2+0.01			932
3	8.1	Sabinene					0.4+0.03 ^a		0.3+0.04				961
4	8.5	β -Pinene	1.7+0.10										974
5	8.6	Myrcene				0.5+0.04 ^a	0.4+0.03		0.4+0.04			0.3+0.02	988
6	8.7	δ -Car-3-ene	4.7+0.17 ^a								3.1+0.24		1011
7	9.5	<i>p</i> -Cymene				0.3+0.02			1.1+0.13 ^a			0.6+0.05	1024
8	9.8	β -ocimene	0.8+0.06	0.7+0.09	1.0+0.09	2.5+0.18 ^a				0.8+0.07	1.2+0.10		1032
9	10.1	(Z)-Sabinene hydrate	0.7+0.07	0.5+0.04	1.1+0.08		3.2+0.24 ^a			0.2+0.01			1065
10	11.1	(E)-Sabinene hydrate			2.2+0.11 ^a		0.7+0.06		2.1+0.19			0.6+0.05	1086
11	11.2	Terpinolene	0.6+0.07	0.7+0.08 ^a									1086
12	11.5	iso-Pentyl isovalerate	0.5+0.04	1.2+0.10		1.1+0.10				1.5+0.12 ^a	0.2+0.01		1103
13	11.8	(E)-2-Nonenal				2.1+0.14 ^a					0.3+0.04		1150
14	12.9	<i>trans</i> -Pinocamphone	1.2+0.11				1.3+0.12 ^a			1.2+0.10		0.2+0.01	1158
15	13.1	Borneol	3.0+0.09										1163
16	13.4	<i>p</i> -Cymen-8-ol	0.5+0.04						4.1+0.24	1.4+0.11	5.4+0.27^a	1.3+0.11	1179
17	13.7	α -Terpineol	1.0+0.09				1.9+0.17 ^a						1186
18	13.9	<i>n</i> -Dodecane	0.8+0.07	0.9+0.10		15.9+0.21^a		0.4+0.03	0.1+0.01	11.2+0.09	10.4+0.31		1200
19	14.5	<i>endo</i> -Fenchyl acetate	1.20.11	3.6+0.21 ^a		0.1+11	3.6+0.22 ^a	3.2+0.24	0.5+0.04			0.3+0.01	1218
20	14.8	Thymol, methyl ether									0.2+0.01		1232
21	15.1	Carvacrol, methyl ether	0.6+0.05	4.3+0.25	2.2+0.17	5.3+0.21	11.3+0.25^a	2.7+0.21	2.3+0.21	1.3+0.09	1.7+0.21	1.2+0.11	1241
22	16.9	Thymol	14.7+0.20^a	1.7+0.18	4.4+0.22	0.3+0.20	0.7+0.06		0.1+0.01	1.8+0.21		5.4+0.26	1289
23	17.2	δ -Elemene		0.9+0.08			0.4+0.02	12.1+0.12^b	3.2+0.27	0.8+0.09	15.3+0.34^a	14.2+0.31^a	1335
24	19.2	α -Elemene	1.4+0.17				0.4+0.02	3.0+0.24	1.3+0.12	3.1+0.24 ^a			1389
25	19.6	Z-Caryophyllene	2.1+0.22 ^a	0.5+0.04	0.9+0.10	1.3+0.12	0.3+0.01	0.8+0.07	1.1+0.11		1.3+0.12		1408
26	19.9	2-Dodecanol									0.2+0.02	0.9+0.07	1410
27	20.4	<i>E</i> -Caryophyllene			1.3+0.11 ^a	0.8+0.07				0.5+0.04	0.2+0.01	0.2+0.02	1417
28	20.6	Dehydroaromadendrane		3.2+0.21	3.2+0.22	2.8+0.18	1.5+0.17	3.9+0.21 ^a	1.1+0.09	0.3+0.04	0.3+0.02	0.2+0.03	1460
29	20.7	<i>ar</i> -Curcumene				1.0+0.11			1.3+0.11 ^a	1.2+0.14		0.3+0.02	1475
30	21	γ -muurolene	1.5+0.21	1.9+0.18	2.9+0.14 ^a	0.5+0.04		1.8+0.16	2.3+0.21		0.2+0.20		1478
31	21.4	(Z)-Farnesene										1.3+0.12	1481
32	21.6	Germacrene D	0.6+0.06	1.4+0.12 ^a	1.4+0.12 ^a		0.6+0.05	1.3+0.21		0.9+0.09			1484
33	21.8	β -selinene							0.3+0.04				1489
34	21.9	2-Pentadecanol				5.3+0.24^a			0.2+0.03			5.9+0.22^a	-
35	22.2	Bicyclogermacrene				1.8+0.21 ^a			1.3+0.10		0.3+0.02	0.3+0.02	1502
36	22.4	β -Bisabolene	3.1+0.19	23.1+0.28^b	25.1+0.19^b	18.1+0.27^c	32.3+0.36^a	9.4+0.29^c		2.1+0.18			1505
37	22.7	(Z)- α -Bisabolene	1.1+0.10	2.5+0.19	2.5+0.21		1.1+0.10	7.8+0.35^b	9.3+0.36^a			7.9+0.36^b	1506
38	22.9	Elemicin						0.9+0.08			2.3+0.24	3.3+0.31 ^a	1560
39	23	(<i>E</i>)-Nerolidol					0.6+0.07	4.9+0.31	5.4+0.36^b	4.3+0.25		7.8+0.37^a	1561
40	23.2	Caryophyllene oxide	0.5+0.04			0.9+0.08	0.9+0.08	7.9+0.36^a	3.5+0.27	1.5+0.12			1567
41	23.4	Tridecanol	12.7+0.11^a	4.5+0.22	11.5+0.24^a		1.6+0.11	1.0+0.11		3.6+0.21	3.6+0.27	4.3+0.21	1570
42	23.6	<i>ar</i> -dihydro Turmerone	0.7+0.08	2.7+0.23	2.7+0.21 ^a		1.9+0.18	0.7+0.05	0.3+0.21				1595
43	23.8	Cedrol							1.9+0.20 ^a		0.2+0.21	0.2+0.05	1600

44	23.9	Junenol		2.3+0.021 ^a	2.3+0.20 ^a		1.4+0.12						1618
45	24.1	γ -Eudesmol				0.4+0.03				1.1+0.10 ^a			1630
46	24.2	α -Muurolol									0.3+0.02	0.5+0.04 ^a	1644
47	24.3	Cubenol	1.5+0.13	2.1+0.19 ^a	1.1+0.10	2.3+0.19 ^a	1.4+0.11		0.3+0.21	1.5+0.09	1.5+0.11		1645
48	24.4	<i>E</i> -sesqui-lavandulol									0.3+0.04		1646
49	24.7	(6 <i>Z</i>)-Pentadecen-2-one		7.1+0.34^b	2.1+0.19	3.1+0.27	1.7+0.13	12.2+0.11^a	13.6+0.29^a	13.5+0.11^a	13.2+0.24^a	3.6+0.23	1667
50	25.2	pentadecanal				1.2+0.18 ^a				0.6+0.05		0.5+0.04	1682
51	25.3	(2 <i>Z</i> ,6 <i>Z</i>)-Farnesal	0.9+0.08	1.6+0.11	2.6+0.21 ^a		2.2+0.25	0.6+0.02		1.2+0.11			1684
52	25.6	α -Bisabolol							0.9+0.07				1685
53	25.7	(2 <i>Z</i> ,6 <i>Z</i>)-Farnesol		4.5+0.26	1.5+0.14		2.1+0.19	0.4+0.02	1.4+0.21	0.5+0.04		10.2+0.37^a	1698
54	26	(2 <i>E</i> ,6 <i>E</i>)-Farnesol	3.7+0.24 ^a		3.1+0.21	2.3+0.21	1.3+0.11				0.5+0.04		1742
55	26.3	<i>n</i> -Pentadecanol	2.3+0.19		1.6+0.11		1.2+0.10	2.6+0.21 ^a	1.3+0.10				1773
56	26.5	<i>n</i> -Hexadecanol	4.2+0.27	7.5+0.27	3.3+0.27	4.0+0.34	1.1+0.09	8.5+0.40^a	5.4+0.31^b			5.0+0.31^b	1874
57	26.7	di- <i>n</i> -butyl phthalate							1.2+0.09	0.2+0.01	2.8+0.21 ^a		1906
58	26.9	Hexadecanoic acid			1.8+0.14 ^a	0.5+0.04	1.5+0.20						1959
59	27.2	<i>n</i> -Eicosane			2.2+0.18	1.3+0.12	0.6+0.06		1.8+0.20	14.3+0.32^a	4.4+0.30	2.8+0.23	2000
60	27.3	Heptadecanoic acid									0.2+0.01		2069
61	28.2	<i>n</i> -Octadecanol			1.5+0.12 ^a	0.5+0.04	1.3+0.09				0.2+0.03		2077
62	28.4	<i>n</i> -Heneicosane				1.4+0.11			1.3+0.17	1.7+0.14 ^a			2100
63	28.9	(<i>E</i>)-Phytol acetate			1.9+0.14	0.80.07	0.6+0.08	4.8+0.31	6.4+0.34^a	2.5+0.24	5.5+0.34^b	1.3+0.18	2218
64	29	<i>n</i> -Tricosane				1.4+0.011	1.4+0.11	4.6+0.27	5.9+0.33^a			2.7+0.19	2300
65	30.2	<i>n</i> -Tetracosane	0.5+0.04				1.8+0.21 ^a	0.2+0.18	1.2+0.13				2400
66	31.9	<i>n</i> -Pentacosane		2.4+0.21 ^a		0.7+0.06	2.4+0.19 ^a		1.1+0.10				2500
67	32	Hexacosane		5.2+0.31^a		4.4+0.32	1.2+0.09			1.1+0.12			2600
68	41.6	Heptacosane	12.8+0.30	3.2+0.28	4.8+0.31	5.1+0.30^d	1.2+0.08	3.3+0.31	4.9+0.32	13.1+0.28^b	11.7+0.35^c	15.3+0.32^a	2700
		Total compounds	89.1	90.2	92.2	92.0	90.2	98.1	91.1	89.2	94.9	91.8	

* RI: retention indices according to the normal alkanes between C8-C24. The bold type face means the compounds have the highest value. JR: Root of Jiroft, ShR: Root of Shahrood, GaR: Root of Garmsar, KrR: Root of Kerend, BR: Root of Birjand, KaR: Root of Kashmar, BrR: Root of Bardaskan, BaR: Root of Bafq, MeR: Root of Mehriz, NeR: Root of Neyriz.

In Mehriz plant samples, thirty-two components were identified, comprising 89.6% of the flower oil. The most abundant components were n-tridecanol (13.2%), δ -elemene (10.7%), and n-eicosane (7.9%) (Table 4). The flower oil of Neyriz plants contained thirty-three compounds, representing 89.9% of the oil. Important constituents included n-heptacosane (22.2%), n-hexadecanol (10.2%), n-eicosane (9.1%), and (6Z)-pentadecen-2-one (7.3%) (Table 4).

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) based on essential oils and ecological factors

Principal component analysis (PCA) of root and flower essential oil components and ecological factors of *D. ammoniacum* confirmed the relationships observed in the hierarchical cluster analysis (HCA) (Fig. 3A). For the root samples, 39 variables (20 essential oil compounds and 19 ecological factors) were reduced to two principal components, which collectively explained 97.6% of the total variance. The first principal component (PC1) accounted for 89.8% of the variance, while the second (PC2) explained 7.9%. A scatter plot was generated using these two components with the highest variance, providing a clear visualization of sample groupings and confirming the results of the cluster analysis (Fig. 3). In the scatter plot, the direction and angle of each composition axis indicate the contribution of that variable within the group. The samples were classified into three distinct groups, consistent with the cluster analysis findings, further validating the classification approach. According to Figure 4A-B, the flower oil of *D. ammoniacum* samples were grouped into three different clusters. In the principal component analysis, we determined 39 variables (20 essential oil compounds and 19 ecological factors) as 2 principal variables, which explained almost 97.6% of the total variance. To display the scatter plot, we used the relationship between the two components that had the highest variance among the total variance. We used the relationship between two components, which included the first component (PC), with 89.7%, and the second component with 7.9%, and a total of almost 97.6% of the total variance to check the scatter plot and confirm the cluster analysis (Fig. 4). In the scatter plot, the direction and angle of the composition axis showed the amount of that composition in that group. In this analysis, flower samples were placed in three groups similar to the cluster analysis, and the results of the cluster analysis were confirmed.

Relationship between essential oil and environmental factors

Root essential oil profile data and environmental factors (climate, soil, and topography) were analyzed through PCA and showed that the first and second principal components accounted for 89.8 and 7.9% of variations. According to data in Table 5, environmental factors such as organic carbon, nitrogen, and manganese positively correlated with the first axis, having correlation coefficient values ranging from 0.590 to 0.668. In contrast, longitude negatively correlated with the first axis (-0.683) (Table 5). Also, endo-fenchyl acetate and tridecanol had a negative and positive correlation with the first axis, having a correlation coefficient of -0.662 and 0.645, respectively. The average rainfall, relative humidity, and sand had a positive correlation ($r = 0.984$, $r = 0.720$, and $r = 0.536$) with the second axis, respectively. Latitude and clay had a negative correlation ($r = -0.666$ and $r = -0.559$) with the second axis. N-eicosane and heptacosane had a positive correlation with the second axis, having correlation coefficients of 0.799 and 0.596, respectively (Table 6). Iron, potassium, phosphorus, nitrogen, organic carbon, and clay showed correlations ($r = 0.524$, $r = 0.790$, $r = 0.708$, $r = 0.647$, and 0.606) with the third axis, respectively. Also, latitude and sand had negative correlations ($r = -0.5066$ and $r = -0.514$) with the third axis, respectively. Silt showed a negative correlation ($r = -0.507$) with the fourth axis (Table 5).

Flower oil profile analysis and environmental factors (climate, soil, and topography) were analyzed through PCA and showed that the first and second principal components accounted for 89.7 and 7.9% of variations. According to data in Table 6, environmental factors such as organic carbon, nitrogen, and manganese positively correlated with the first axis, with correlation coefficients of 0.630, 0.590, and 0.668, respectively. However, longitude correlated negatively with the first axis (-0.683) (Table 6). The average rainfall ($r = -0.984$), relative humidity ($r = 0.721$), and sand ($r = 0.536$) showed a positive correlation with the second axis. Latitude and clay had a negative correlation ($r = -0.667$ and $r = -0.558$) with the second axis. Moreover, iron, potassium, phosphorus, nitrogen, organic carbon, and clay showed different correlations ($r = 0.524$, $r = 0.790$, $r = 0.707$, $r = 0.647$, and 0.606) with the third axis, respectively, and relative humidity showed a positive correlation ($r = 0.608$) with the fourth axis (Table 7).

Table 4. Chemical composition (%) of flower (F) essential oils of *D. ammoniacum*.

No	RT	Components	JF%	ShF%	GaF%	KrF%	BF%	KaF%	BrF%	BaF%	MeF%	NeF%	RI*
1	4.4	4-methylene-5-Hexenal		4.2+0.28 ^a	3.2+0.21								893
2	7.8	α -Pinene	1.6+0.21 ^a							0.5+0.4			932
3	8.1	Sabinene	0.5+0.04 ^a				0.5+0.04 ^a				0.2+0.19		961
4	8.5	β -Pinene		2.6+0.24									974
5	8.6	Myrcene				0.2+0.01 ^a						0.1+0.10	991
6	9.5	<i>p</i> -Cymene	1.6+0.14 ^a			0.1+0.01			0.9+0.14	0.7+0.06	1.1+0.10	0.5+0.04	1024
7	9.8	β -ocimene		0.4+0.05			1.5+0.12 ^a		1.0+0.09		0.4+0.03	0.7+0.06	1032
8	10.1	(<i>Z</i>)-Sabinene hydrate		0.4+0.04			1.2+0.10 ^a						1065
9	11.1	ϵ -Sabinene hydrate		1.6+0.11			5.5+0.24^a		1.2+0.11		0.1+0.01	0.2+0.01	1086
10	11.5	iso-Pentyl isovalerate	0.5+0.04	0.5+0.04		0.4+0.02				1.3+0.12 ^a	0.2+0.02	0.2+0.02	1103
11	11.8	ϵ -2-Nonenal		1.2+0.12		1.8+0.14 ^a	0.5+0.04						1150
12	12.4	Iso-Isopulegol		0.4+0.03									1155
13	12.9	trans-Pinocamphone		1.5+0.16 ^a			0.5+0.04						1158
14	13.4	<i>p</i> -Cymen-8-ol	0.7+0.6	16+0.39^a	8.5+0.27^b		5.4+0.23^c	0.9+0.12		1.5+0.14			1179
15	13.9	<i>n</i> -Dodecane	1.1+0.10	46.6+0.48^a	8.3+0.37^c	2.4+0.21	11.4+0.21^b	1.3+0.11	2.3+0.19		0.4+0.03	0.7+0.06	1200
16	14.5	<i>endo</i> -Fenchyl acetate	0.4+0.03	1.6+0.20		4.2+0.31 ^a	1.0+0.09	0.6+0.05					1218
17	14.8	Thymol, methyl ether	0.4+0.04			3.2+0.31 ^a		1.5+0.12	2.7+0.14	1.3+0.11	0.3+0.02		1232
18	15.1	Carvacrol, methyl ether		0.3+0.02	0.9+0.08		2.4+0.21 ^a	0.9+0.08	2.1+0.15	0.5+0.04			1241
19	16.9	Thymol			7.5+0.33^a		1.1+0.14	8.7+0.38^a		0.5+0.04		0.1+0.01	1289
20	17.2	δ -Elemene	11.2+0.36^b	0.3+0.03			0.6+0.05	0.7+0.08	1.9+0.21	13.5+0.23^a	10.7+0.37^b	4.5+0.25	1335
21	17.7	α -Cubebene				1.1+0.10 ^a	0.80.07						1345
22	17.8	2-Undecanol		0.5+0.04		0.2+0.18			1.0+0.11 ^a		0.4+0.27	0.2+0.01	1366
23	19.2	α -Elemene			4.0+0.31	4.8+0.32 ^a	1.2+0.09		2.4+0.21	0.5+0.04			1389
24	19.6	<i>Z</i> -Caryophyllene	3.4+0.30 ^a	0.8+0.06	1.7+0.26	0.2+0.01	3.3+0.21 ^a	1.4+0.11					1408
25	19.9	2-Dodecanol								1.1+0.09			1410
26	20.4	<i>E</i> -Caryophyllene		1.1+0.9	1.4+0.12		1.4+0.12	1.5+0.12 ^a			0.9+0.07	0.7+0.06	1417
27	20.6	Dehydroaromadendrane	2.7+0.024	1.3+0.12	0.4+0.03	0.4+0.03	3.4+0.28 ^a	1.8+0.13	0.6+0.05		0.7+0.06		1460
28	20.7	ar-Curcumene					1.5+0.21	0.7+0.06	2.1+0.21 ^a		0.2+0.01	0.2+0.01	1475
29	21	γ -muurolene		0.5+0.04	3.7+0.31	1.3+0.11	3.2+0.23	3.9+0.24 ^a		0.7+0.05			1478
30	21.4	(<i>Z</i>)-Farnesene	2.6+0.21 ^a		0.8+0.07		0.6+0.15	0.7+0.06					1481
31	21.6	Germacrene D	0.8+0.07	0.6+0.05	1.8+0.23	0.4+0.03	1.2+0.14	0.8+0.07		2.0+0.21 ^a	1.2+0.12	1.5+0.12	1484
32	21.8	β -selinene			1.3+0.25	1.8+0.10 ^a		0.9+0.07	0.2+0.16				1489
33	21.9	2-Pentadecanol			2.2+0.24	6.1+0.36^a		4.2+0.21	5.9+0.24^a			4.4+0.023	-
34	22	(<i>E</i>)- β -Ionone	0.6+0.05		1.0+0.23	0.7+0.07	2.7+0.25	2.2+0.23	3.2+0.12 ^a		0.9+0.08		1490
35	22.2	Bicyclogermacrene	3.9+0.21	0.9+0.07	4.8+0.36	7.4+0.45^a	3.7+0.21	2.7+0.28		6.3+0.31^a	3.4+0.21	2.2+0.21	1502
36	22.4	β -Bisabolene			11.9+0.35^b	9.1+0.36^b	3.6+0.26	3.4+0.24		14.9+0.22^a			1505
37	22.7	(<i>Z</i>)- α -Bisabolene				4.2+0.32			8.1+0.32^a			0.1+0.01	1506
38	22.8	Cuparene	1.1+0.10	1.5+0.11	1.3+0.11	1.5+0.12	7.8+0.36^a	5.9+0.31^b					1508
39	22.9	Elemicin			1.8+0.12 ^a				1.3+0.11				1560
40	23	(<i>E</i>)-Nerolidol			1.3+0.13		0.7+0.06	1.1+0.10	5.9+0.23^a	4.0+0.21	2.2+0.21	5.2+0.35^a	1561
41	23.2	Caryophyllene oxide	1.7+0.14				1.2+0.11	3+0.21	4.3+0.23 ^a	0.5+0.04	1.2+0.11	0.2+0.18	1567
42	23.4	Tridecanol	13+0.10^a	8.0+0.25^b	1.9+0.14	0.2+0.01	1.8+0.23	4.9+0.25		5.7+0.32^c	13.2+0.21^a	4.9+0.22	1570
43	23.6	ar-dihydro Turmerone	1.1+0.09	0.6+0.05	0.9+0.08		1.9+0.21 ^a	0.9+0.08					1595

44	23.9	Junenol	1+0.01			0.8+0.06	1.1+0.10 ^a	0.9+0.07			0.2+0.01	1618	
45	24.1	γ -Eudesmol		0.5+0.04			0.7+0.08					1630	
46	24.2	α -Muurolol	0.3+0.02		1.2+0.09	3.4+0.26 ^a	2.8+0.21	3.3+0.28 ^a	0.3+0.02		0.8+0.07 ^a	1644	
47	24.3	Cubenol	0.5+0.04	0.5+0.04	0.8+0.07	0.9+0.08	2.3+0.21 ^a	1.1+0.10				1645	
48	24.7	(6Z)-Pentadecen-2-one	1.8+0.14		1.4+0.12		1.5+0.11	0.8+0.06	8.7+0.28^a	9.2+0.32^a	3.1+0.21	7.3+0.32^b	1667
49	25.2	Pentadecanal			1.7+0.15		0.6+0.05	2.2+0.19 ^a	2.2+0.21 ^a	0.9+0.08		0.8+0.21	1682
50	25.3	(2Z,6Z)-Farnesal	1.7+0.15		1.2+0.11	3.0+0.21 ^a	0.8+0.22	2+0.17		0.3+0.02	1.5+0.13	1.1+0.12	1684
51	25.6	α -Bisabolol	0.9+0.08				2.9+0.24 ^a			1.6+0.15	0.4+0.23	0.2+0.23	1685
52	25.7	(2Z,6Z)-Farnesal	0.7+0.07		4.6+0.32	0.7+0.06	3.1+0.25	3.3+0.21	7.5+0.24^a		0.5+0.04	3.1+0.25	1698
53	26	(2Z,6Z)-Farnesol	0.6+0.05		0.8+0.14	3.7+0.21		6.2+0.28^a	1.9+0.18	2.7+0.21			1742
54	26.3	<i>n</i> -Pentadecanol	4.4+0.30		3.7+0.32		0.6+0.06	1.5+0.18		7.6+0.34^a	3.8+0.21	4.4+0.24	1773
55	26.5	<i>n</i> -Hexadecanol	0.5+0.04	0.3+0.02	0.8+0.07			11.2+0.34^a	8.2+0.29^b	1.5+0.14		10.2+0.39^a	1874
56	26.7	di- <i>n</i> -butyl phthalate								1.2+0.11 ^a		0.7+0.07	1906
57	26.9	Hexadecanoic acid	4.9+0.24			0.5+0.04					5.1+0.11^a	0.2+0.02	1959
58	27.2	<i>n</i> -Eicosane	8.2+0.36	0.3+0.02	0.6+0.05		0.6+0.05	0.8+0.07		5.1+0.36^c	7.9+0.23^b	9.1+0.36^a	2000
59	27.3	Heptadecanoic acid	7.8+0.32								5.1+0.23^a		2069
60	28.4	<i>n</i> -Heneicosane	3.4+0.20			1.3+0.12			0.9+0.08	2.5+0.12	2.9+0.21 ^a		2100
61	28.9	(<i>E</i>)-Phytol acetate	0.3+0.02					0.5+0.04	4.3+0.21 ^a	2.9+0.31		1.4+0.11	2218
62	29	<i>n</i> -Tricosane	2.6+0.21								4.1+0.23 ^a		2300
63	30.2	<i>n</i> -Tetracosane	1.5+0.11					1.6+0.21		0.5+0.04	2.1+0.14 ^a	1.7+0.12	2400
64	41.6	Heptacosane		1.8+0.12	4.2+0.35	27.3+0.39^a	1.3+0.11	6.3+0.32^d	7.6+0.26^d		15+0.33^c	22.2+0.36^b	2700
		Total compounds	90	96.5	92.5	93.5	89.9	96.3	90.3	89.9	89.6	89.9	

* RI: retention indices according to the normal alkanes between C8-C24. The bold type face means the compounds have the highest value. JF: Flower of Jiroft, ShF: Flower of Shahrood, GaF: Flower of Garmsar, KrF: Flower of Kerend, BF: Flower of Birjand, KaF: Flower of Kashmar, BrF: Flower of Bardaskan, BaF: Flower of Bafq, MeF: Flower of Mehriz, NeF: Flower of Neyriz.

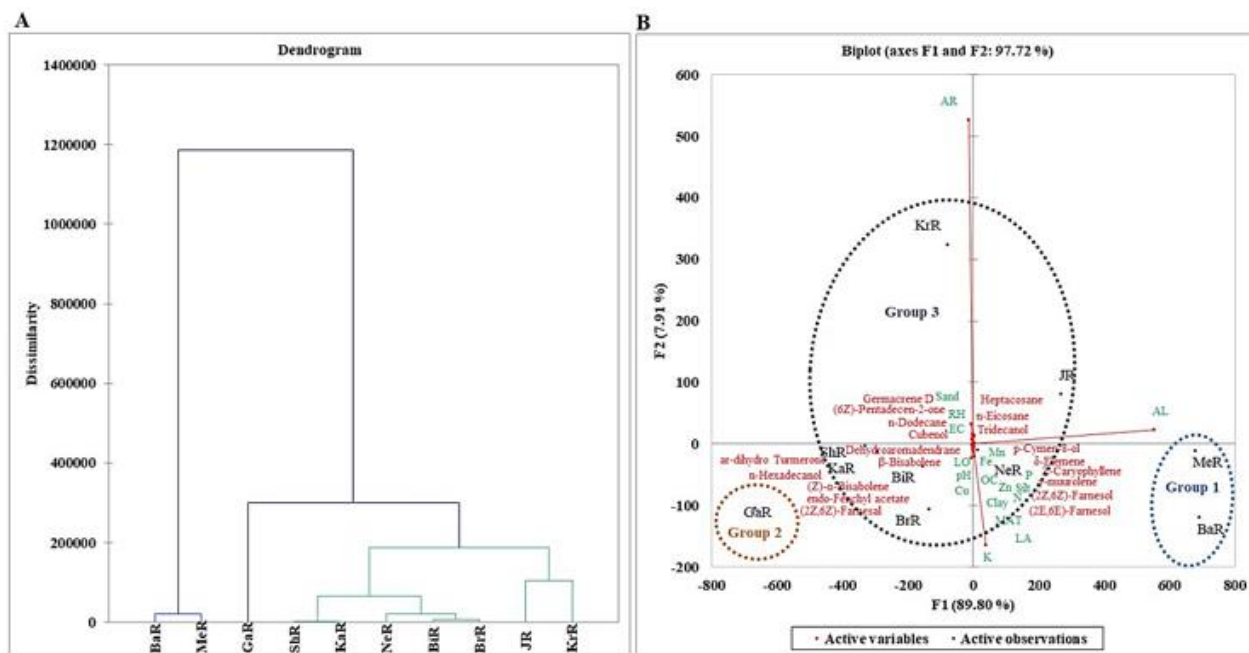


Fig. 3. Classification of 10 studied root samples of *D. ammoniacum* essential oil compounds based on 20 essential oil compounds and 19 ecological factors in the form of 2 principal variables in hierarchical cluster analysis (HCA) with Ward's based method (A). Classification and correlation of 10 studied samples of *D. ammoniacum* root essential oil compounds based on 20 essential oil compounds and 19 ecological factors scatter plot of samples drawn based on two first principal components (B). JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz.

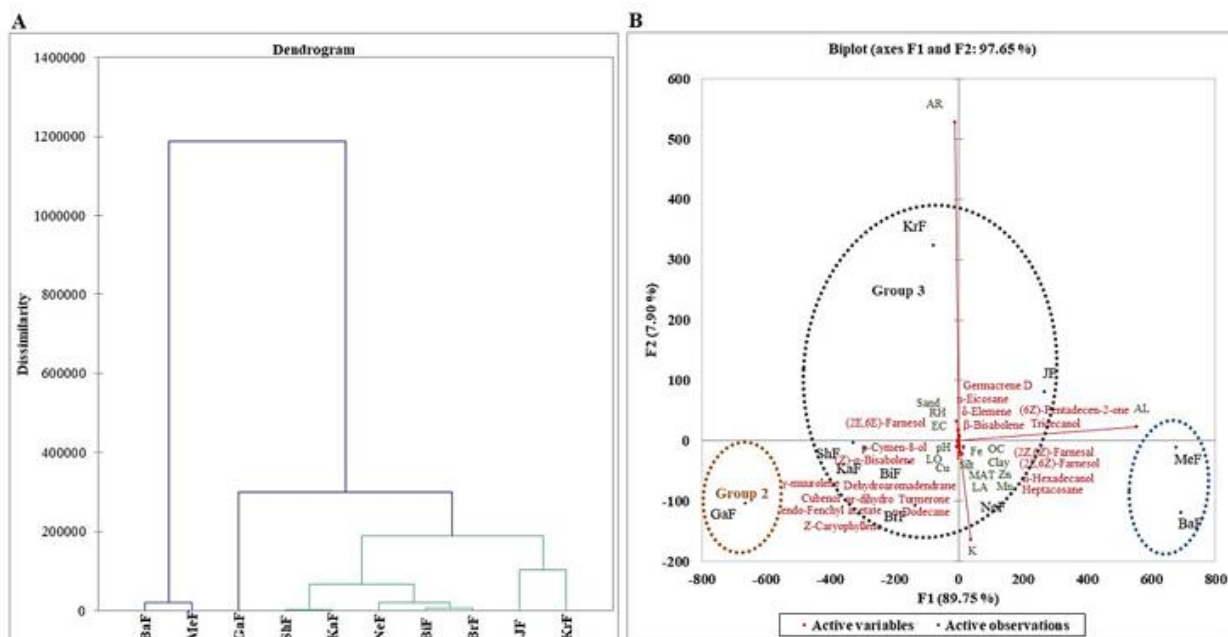


Fig. 4. Classification of 10 studied flower samples of *D. ammoniacum* essential oil compounds based on 20 essential oil compounds and 19 ecological factors in the form of 2 principal variables hierarchical cluster analysis (HCA) with Ward's based method (A). Classification and correlation of 10 studied samples of *D. ammoniacum* flower essential oil compounds based on 20 essential oil compounds and 19 ecological factors scatter plot of samples drawn based on two first principal components (B). JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Kashmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

Table 5. Correlation coefficient axes with essential oil compounds and environmental factors in root samples of *D. ammoniacum*.

Variables and Factors	Abbreviation	Axis 1	Axis 2	Axis 3	Axis 4
<i>p</i> -Cymen-8-ol	-	0.057	-0.004	0.130	<u>-0.636</u>
<i>n</i> -Dodecane	-	-0.143	0.427	0.091	-0.107
endo-Fenchyl acetate	-	<u>-0.662</u>	-0.215	-0.310	0.356
δ -Elemene	-	0.209	-0.079	-0.387	-0.339
Z-Caryophyllene	-	0.100	-0.208	-0.321	-0.379
Dehydroaromadendrane	-	-0.248	-0.420	-0.003	0.121
γ -muurolene	-	0.205	-0.409	0.092	-0.259
Germacrene D	-	-0.076	0.125	0.129	0.354
β -Bisabolene	-	-0.346	-0.443	0.262	<u>0.691</u>
(Z)- α -Bisabolene	-	-0.207	-0.352	0.004	<u>-0.772</u>
Tridecanol	-	<u>0.645</u>	0.102	-0.025	0.314
ar-dihydro Turmerone	-	-0.078	-0.399	0.214	0.592
Cubanol	-	-0.296	0.202	0.040	0.466
(6Z)-Pentadecen-2-one	-	-0.289	0.315	0.174	<u>-0.682</u>
(2Z,6Z)-Farnesal	-	-0.057	-0.083	0.489	<u>0.650</u>
(2Z,6Z)-Farnesol	-	0.382	-0.142	-0.262	0.380
(2E,6E)-Farnesol	-	0.343	-0.179	0.030	0.258
<i>n</i> -Hexadecanol	-	-0.020	-0.320	<u>-0.566</u>	-0.158
<i>n</i> -Eicosane	-	0.092	<u>0.799</u>	<u>0.505</u>	-0.084
Heptacosane	-	<u>0.604</u>	<u>0.596</u>	-0.214	-0.107
AR	Average rainfall	-0.076	<u>0.984</u>	0.163	-0.001
MAT	Mean annual temperature	0.490	-0.410	-0.137	0.195
RH	Relative humidity	-0.224	<u>0.720</u>	-0.083	0.469
LA	Latitude	0.029	<u>-0.666</u>	<u>-0.506</u>	-0.344
LO	Longitude	<u>-0.683</u>	-0.091	0.180	0.035
AL	Altitude	<u>1.000</u>	0.012	-0.009	0.000
Clay	-	0.170	<u>-0.559</u>	<u>0.613</u>	-0.307
Silt	-	0.386	-0.490	0.431	<u>-0.507</u>
Sand	-	-0.328	<u>0.536</u>	<u>-0.514</u>	0.460
EC	Electrical conductivity	-0.016	0.178	-0.403	0.123
pH	Potential of hydrogen	-0.093	-0.361	-0.147	-0.100
OC	Organic carbon	<u>0.629</u>	-0.238	<u>0.606</u>	0.091
N	Nitrogen	<u>0.590</u>	-0.226	<u>0.647</u>	0.161
P	Phosphorus	0.436	-0.422	<u>0.708</u>	0.161
K	Potassium	0.376	-0.485	<u>0.790</u>	0.009
Fe	Iron	0.306	-0.475	<u>0.524</u>	-0.090
Zn	Zinc	0.446	-0.183	-0.043	0.100
Cu	Copper	-0.352	-0.406	0.485	0.481
Mn	Manganese	<u>0.668</u>	-0.356	0.442	0.015

The bold and underlined values had significant correlation with the relevant axes.

Table 6. Correlation coefficient axes with essential oil compounds and environmental factors in flower samples of *D. ammoniacum*.

Variables and Factors	Abbreviation	Axis 1	Axis 2	Axis 3	Axis 4
<i>p</i> -Cymen-8-ol	-	-0.153	-0.179	0.080	<u>0.931</u>
<i>n</i> -Dodecane	-	-0.313	-0.158	-0.159	<u>0.887</u>
endo-Fenchyl acetate	-	-0.370	-0.156	-0.261	-0.027
δ -Elemene	-	0.201	<u>0.778</u>	-0.025	-0.171
Z-Caryophyllene	-	-0.154	-0.191	-0.075	0.129
Dehydroaromadendrane	-	-0.277	-0.287	0.003	-0.161
γ -muurolene	-	-0.177	-0.341	0.326	-0.015
Germacrene D	-	0.427	0.434	0.422	0.237
β -Bisabolene	-	0.104	0.406	<u>0.658</u>	-0.047
(Z)- α -Bisabolene	-	-0.156	-0.321	0.182	-0.462
Tridecanol	-	0.138	0.394	<u>-0.503</u>	0.187
ar-dihydro Turmerone	-	-0.314	-0.301	0.041	0.243
Cubanol	-	<u>-0.518</u>	-0.407	0.111	0.067
(6Z)-Pentadecen-2-one	-	0.236	0.416	0.358	-0.269
(2Z,6Z)-Farnesal	-	0.122	-0.141	-0.378	<u>-0.521</u>
(2Z,6Z)-Farnesol	-	0.140	<u>-0.610</u>	0.381	-0.317
(2E,6E)-Farnesol	-	-0.264	0.171	-0.076	<u>-0.573</u>
<i>n</i> -Hexadecanol	-	0.166	-0.132	-0.248	-0.399
<i>n</i> -Eicosane	-	<u>0.517</u>	0.443	-0.355	-0.112
Heptacosane	-	0.255	-0.261	-0.289	-0.382
AR	Average rainfall	-0.076	<u>0.984</u>	0.162	0.000
MAT	Mean annual temperature	0.490	-0.408	-0.136	0.029
RH	Relative humidity	-0.224	<u>0.721</u>	-0.090	<u>0.608</u>

LA	Latitude	0.029	<u>-0.667</u>	<u>-0.502</u>	-0.243
LO	Longitude	<u>-0.683</u>	-0.092	0.180	0.132
AL	Altitude	<u>1.000</u>	0.012	-0.009	0.000
Clay	-	0.170	<u>-0.558</u>	<u>0.616</u>	-0.023
Silt	-	0.386	-0.490	0.436	-0.448
Sand	-	-0.328	<u>0.536</u>	<u>-0.518</u>	0.320
EC	Electrical conductivity	-0.016	0.178	-0.406	-0.141
pH	Potential of hydrogen	-0.093	-0.362	-0.142	-0.098
OC	Organic carbon	<u>0.630</u>	-0.236	<u>0.606</u>	-0.082
N	Nitrogen	<u>0.590</u>	-0.223	<u>0.647</u>	-0.015
P	Phosphorus	0.436	-0.420	<u>0.707</u>	0.079
K	Potassium	0.376	-0.483	<u>0.790</u>	0.014
Fe	Iron	0.306	-0.474	<u>0.524</u>	0.091
Zn	Zinc	0.446	-0.181	-0.044	-0.135
Cu	Copper	-0.352	-0.403	0.485	0.150
Mn	Manganese	<u>0.668</u>	-0.356	0.441	0.081

The bold and underlined values had significant correlation with the relevant axes.

Table 7. Analysis of variance (mean squares) for the effects of site and plant part on total tannin content (TTC), saponins, total phenol content (TPC), total flavonoid content (TFC), DPPH, and FRAP in *D. ammoniacum* populations.

Source of Variation	df	TTC	Saponins	TPC	TFC	DPPH	FRAP
Block	2	0.1221 ^{ns}	0.0076 ^{**}	16.0145 ^{ns}	1.2795 ^{ns}	13.9520 ^{ns}	32.5166 ^{ns}
Site	9	0.3291 ^{**}	0.0024 ^{**}	29.5733 ^{**}	24.5121 ^{**}	10575.8871 ^{**}	712.8314 ^{**}
Plant part	1	0.000001 ^{ns}	0.0012 ^{ns}	199.1081 ^{**}	54.15 ^{**}	5908.3526 ^{**}	84.0166 ^{**}
Site × Plant part	9	0.2065 ^{ns}	0.002 ^{**}	45.7322 ^{**}	16.0825 ^{**}	2673.3874 ^{**}	147.9425 ^{**}
CV	-	24.6	18.5	21.4	14.4	3.7	10.2

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

Total tannin content (TTC)

The extracts from the roots and flowers of *D. ammoniacum* populations differed significantly in TTC (Table 7). The highest amount of total tannin occurred in samples that were designated as BaR with 1.8 mg TA g⁻¹ DW (Fig. 5), whereas the lowest total tannin content in ShF, GaF, and MeF were 0.5 and 0.6 mg TA g⁻¹ DW, respectively. As Figure 5 shows, the amount of TTC in the studied samples showed high levels of variation. It can be considered the first report about tannin contents in different organs of *D. ammoniacum*. On average, tannin content was more present in the roots than in the flowers.

Saponins

The analysis of variance revealed a significant difference ($P \leq 0.01$) in the saponin content between extracts from the roots and flowers of *D. ammoniacum* populations (Table 7). The highest saponin content was observed in the roots of Bafq plants (BaR), with a concentration of 0.16% (Fig. 6). Conversely, the flower extract from Garmsar (GaF) exhibited the lowest saponin levels. Similar to the tannin content, this study is the first to report variability in saponin levels across different organs of *D. ammoniacum*.

Antioxidant activity (DPPH) and (FRAP)

All extraction yields appear in supplementary data (Table S1). The results showed that methanolic extracts of GaR and BaR had 10.0 and 9.5% yields (w/w), with the lowest extraction yield occurring in NeF (1.2% W/W). Generally, the extracts of roots showed maximum yield. In the present work, the antioxidant activity of the samples appeared using the DPPH and FRAP methods. Significant differences occurred between the extracts from the roots and flowers of *D. ammoniacum* populations in antioxidant activity ($P \leq 0.01$) (Table 7). The results of comparison of antioxidant activity (DPPH) have been demonstrated in Figure 7. In DPPH assay, the highest radical scavenging activity (lowest IC₅₀) was observed in the BaR (roots of Bafq plants) and ShR (roots of Shahrood plants) samples with an IC₅₀ of 41.8 µg mL⁻¹ and 45.4 µg mL⁻¹ compared to BHT (26.5 µg mL⁻¹), a synthetic industrial antioxidant, respectively. The lowest activity (IC₅₀ 212.2 µg mL⁻¹) was associated with the KrF (flower of Kerend) sample. However, for the FRAP activity, samples BaR and ShR were higher than all the other samples, and the amount of antioxidant activity varied from 15.0 to 54.0 (Fig. 8).

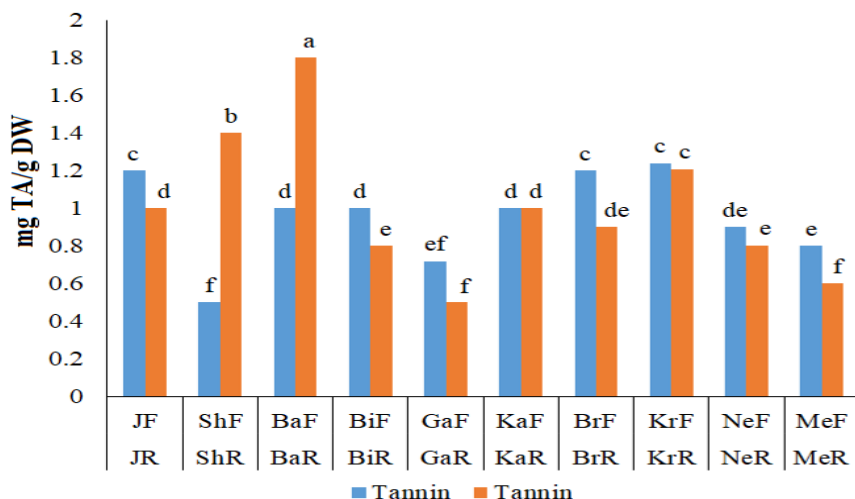


Fig. 5. Mean comparison of TTC (total tannin content; mg TA g⁻¹ DW) of *D. ammoniacum* methanolic extracts. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$). JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz. JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Kashmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

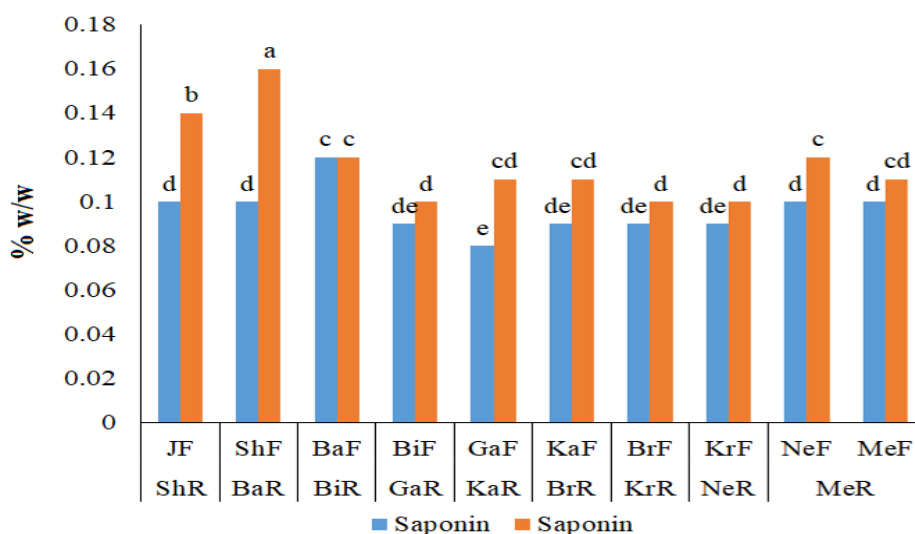


Fig. 6. Mean comparison of saponin content (%) of all *D. ammoniacum* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test ($p < 0.05$).

JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz. JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Kashmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

Total phenolic and flavonoid contents

The results showed that there was a significant difference ($P \leq 0.01$) between all extracts of *D. ammoniacum* in total phenolic and flavonoid content (Table 7). The root extract of the Bafq (BaR) had the highest total phenolic content (25.7 mg GAE g⁻¹ DW extract) (Fig. 9). On the

contrary, the ShF (flower of Shahrood) and Garmsar had a low total phenolic content, with 9.1 and 9.8 mg GAE g⁻¹ DW, respectively. Among the tested materials, the highest flavonoid content was recorded in Garmsar samples, 14.0 and 13.8 mg QE g⁻¹ (Fig. 10). On the other hand, Birjand samples exhibited the lowest levels of TFC (4.1 mg QE g⁻¹ DW).

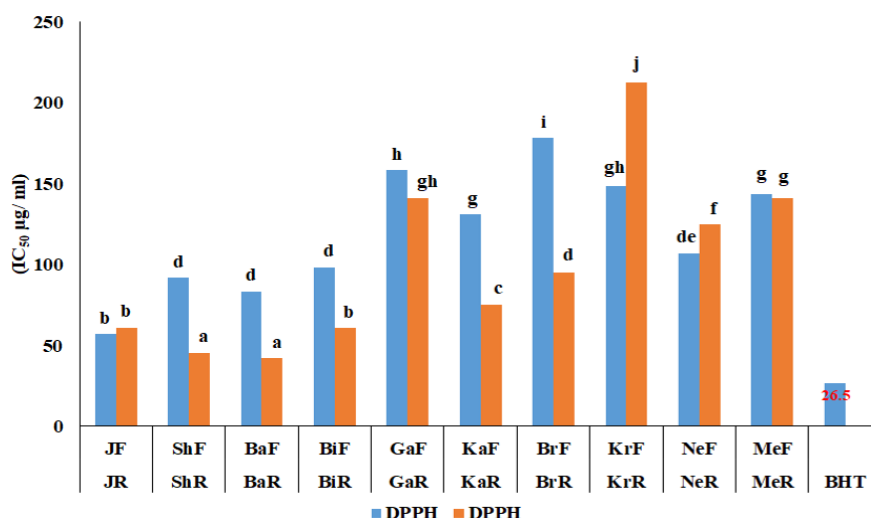


Fig. 7. Comparison of antioxidant capacity in all samples of *D. ammoniacum*. Mean comparison of DPPH (antioxidant activity by DPPH assay; IC₅₀) of all *D. ammoniacum* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$).

JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz. JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Kashmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

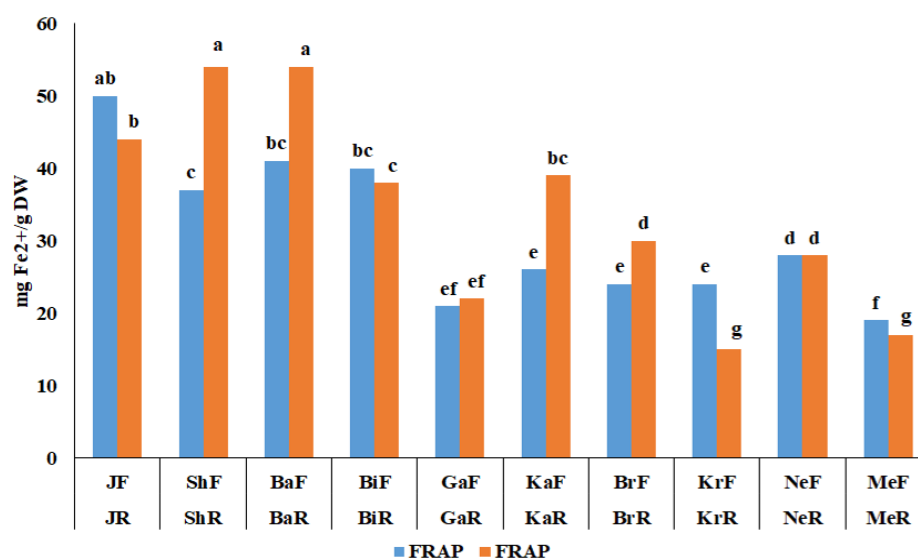


Fig. 8. Comparison of antioxidant capacity in all samples of *D. ammoniacum*. Mean comparison of FRAP (antioxidant activity by FRAP assay; mg Fe²⁺/g DW) of all *D. ammoniacum* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$). JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz. JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Kashmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) based on eco-phytochemical properties

Principal component analysis (PCA) of root and flower extracts of *D. ammoniacum* further confirmed the relationships obtained by the

hierarchical cluster analysis (HCA) of the samples (Fig. 11A-B). In root samples, we found 26 ecophytochemical variables as two new variables (two principal components), which justified almost 97.7% of the percentage of total variance,

of which the PC1 and PC2 accounted for 89.8 and 7.9% of total variance, respectively (Fig. 11A). For the flower samples, PCA accounted for 97.8% of the total variance, with PC1 and PC2 contributing 89.9% and 7.9%, respectively (Fig. 11B). Root samples were divided into three groups based on the PC1 and PC2 components. Group 3, comprising JR, KrR, ShR, KaR, BiR, BrR, and NeR, exhibited the highest levels of

antioxidant activity (FRAP and DPPH), total phenolic content (TPC), saponins, and tannins compared to the other groups. Group 2, which included GaR, had the lowest levels of tannins, saponins, and TPC. Group 1, consisting of MeR and BaR, showed moderate levels of antioxidant activity (FRAP and DPPH), TPC, saponins, and tannins.

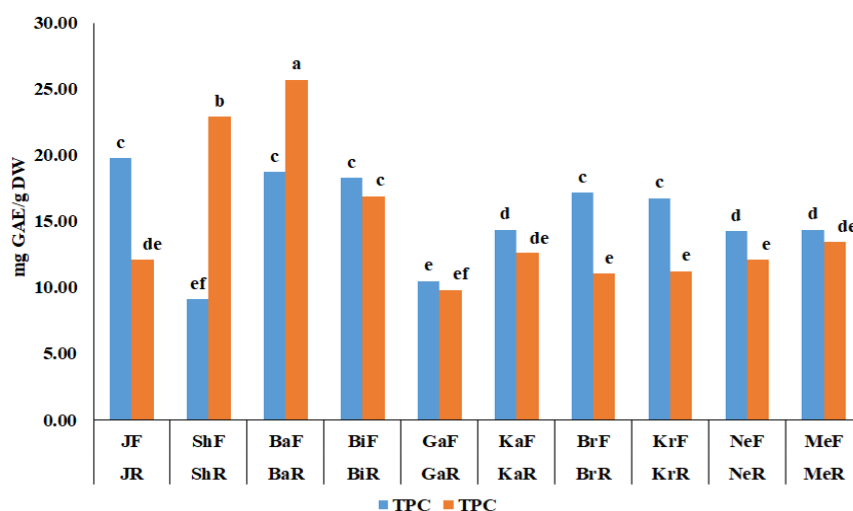


Fig. 9. Mean comparison of TPC (total phenolic content; mg GAE g⁻¹ DW) of methanolic extract of *D. ammoniacum*. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$). JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz. JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Kashmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

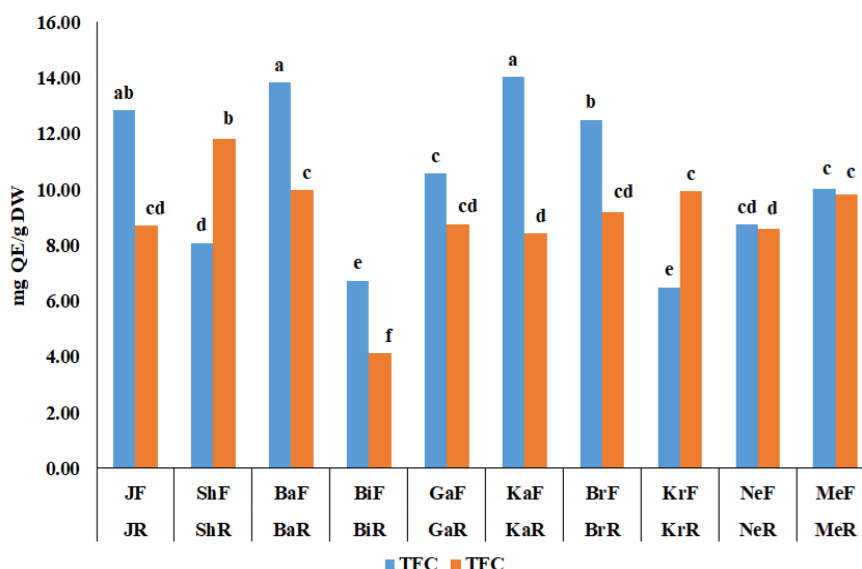


Fig. 10. Mean comparison of TFC (total flavonoid content; mg QE g⁻¹ DW) of methanolic extract of *D. ammoniacum*. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$). JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz. JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Kashmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

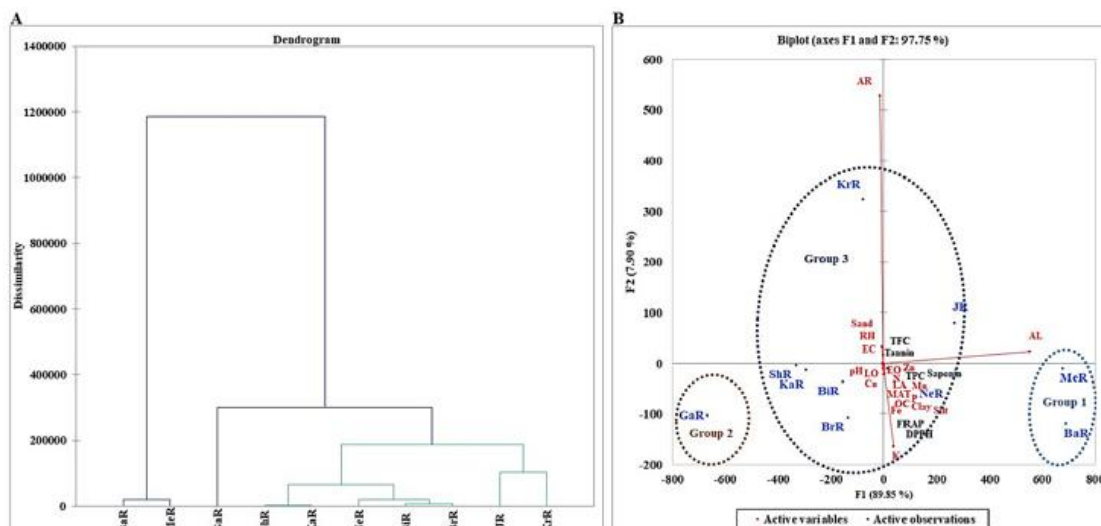


Fig. 11. Classification and correlation of 10 studied root samples of *D. ammoniacum* based on 26 ecophytochemical variables hierarchical cluster analysis (HCA) with Ward's based method (A). Classification and correlation of 10 studied samples of *D. ammoniacum* based on 26 ecophytochemical variables scatter plot of samples drawn based on two first principal components (B). JR: root of Jiroft, ShR: root of Shahrood, GaR: root of Garmsar, KrR: root of Kerend, BiR: root of Birjand, KaR: root of Kashmar, BrR: root of Bardaskan, BaR: root of Bafq, MeR: root of Mehriz, NeR: root of Neyriz.

Similarly, flower samples were grouped into three distinct clusters (Fig. 12A-B). Group 1, containing MeF and BaF, demonstrated low antioxidant activity compared to Group 3, which included JF, NeF, BrF, BiF, KaF, ShF, and KrF, and exhibited the highest antioxidant activity and saponin content. Group 2, represented by GaF, had the lowest levels of saponins, TPC, and DPPH activity. The PCA groupings closely aligned with the patterns observed in the HCA.

Relationship of eco-phytochemical properties

An eco-phytochemical analysis of the roots of *D. ammoniacum* and the environmental factors affecting its growth—such as climate, soil, and topography—revealed that the first and second principal components explained 89.8% and 7.9% of the variation, respectively (Table 8). According to the data in Table 8, several environmental factors positively correlated with the first principal component, including mean annual temperature, organic carbon, nitrogen, and manganese, with correlation coefficients ranging from 0.501 to 0.669. Conversely, longitude was negatively correlated with the first axis ($r = -0.683$). The second principal component showed positive correlations with average rainfall, relative humidity, and sand ($r = 0.984$, $r = 0.720$, and $r = 0.537$, respectively). In contrast, latitude and clay exhibited negative correlations with the second axis ($r = -0.668$ and $r = -0.559$). For the

third axis, iron, potassium, phosphorus, nitrogen, organic carbon, and clay showed positive correlations ($r = 0.520$, $r = 0.791$, $r = 0.704$, $r = 0.645$, and $r = 0.613$, respectively). Latitude and sand had negative correlations ($r = -0.507$ and $r = -0.514$) with the third axis (Table 8). Lastly, iron, tannin, total phenolic content (TPC), DPPH, and FRAP were positively correlated with the fourth axis, while pH showed a negative correlation.

The eco-phytochemical analysis of flower samples, along with the environmental factors (climate, soil, and topography), revealed that the first and second principal components explained 89.9% and 7.9% of the variation, respectively (Table 9). According to the data in Table 9, several environmental factors positively correlated with the first principal component, including mean annual temperature, organic carbon ($r = 0.630$), longitude ($r = -0.683$), and manganese ($r = 0.669$). Additionally, saponin and total phenolic content (TPC) showed positive correlations with the first axis, with correlation coefficients of 0.556 and 0.656, respectively.

The second principal component exhibited positive correlations with average rainfall, relative humidity, and sand ($r = 0.984$, $r = 0.721$, and $r = 0.536$, respectively). Latitude and clay showed negative correlations with the second axis ($r = -0.666$ and $r = -0.558$).

For the third principal component, iron, potassium, phosphorus, nitrogen, organic carbon, and clay all had positive correlations ($r = 0.521$, r

= 0.790, $r = 0.704$, $r = 0.645$, and $r = 0.614$). Latitude and sand were negatively correlated with the third axis ($r = -0.507$ and $r = -0.514$). Finally, the fourth axis showed positive correlations with sand and pH ($r = 0.529$ and $r =$

0.605), while latitude, silt, zinc, essential oil, TPC, and FRAP had negative correlations ($r = -0.536$, $r = -0.595$, $r = -0.624$, $r = -0.623$, $r = -0.684$, and $r = -0.652$).

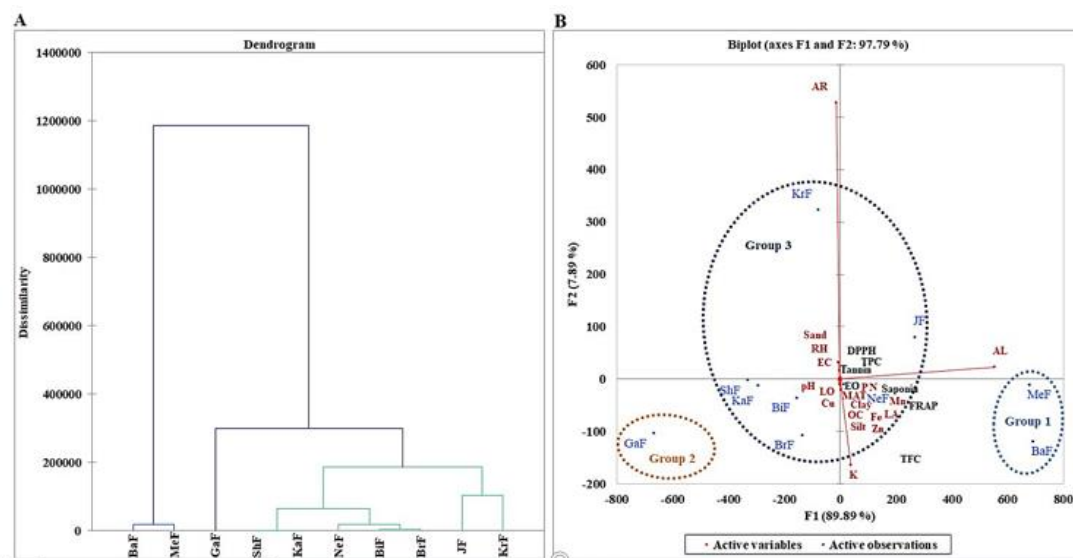


Fig. 12. Classification and correlation of 10 studied flower samples of *D. ammoniacum* based on 26 ecophytochemical variables hierarchical cluster analysis (HCA) with Ward's based method (A). Classification and correlation of 10 studied samples of *D. ammoniacum* based on 26 ecophytochemical variables scatter plot of samples drawn based on two first principal components (B). JF: flower of Jiroft, ShF: flower of Shahrood, GaF: flower of Garmsar, KrF: flower of Kerend, BiF: flower of Birjand, KaF: flower of Keshmar, BrF: flower of Bardaskan, BaF: flower of Bafq, MeF: flower of Mehriz, NeF: root of Neyriz.

Correlation between phytochemical properties and antioxidant activity

Correlations between phytochemical properties and antioxidant activity showed that antioxidant activity (i.e., DPPH and FRAP) correlated positively and significantly with TTC (0.33 and 0.32), saponin (0.31 and 0.36), and TPC (0.31 and 0.35) (Table 10).

Discussion

The oil yields from *Dorema ammoniacum* organs range from 0.2% to 0.5% v/w (relative to the dry weight of the plant). Studies on the Apiaceae family, including species such as *Oliveria decumbens*, *Trachyspermum ammi*, *Echinophora tenuifolia*, and *Heracleum persicum*, have demonstrated that EO content varies significantly among different plant organs (Hazrati et al., 2020). Specifically, *D. ammoniacum* stems and fruits contain EO yields of 0.3% and 0.5% v/w, respectively, while the aerial parts and roots yield 0.2% and 0.3% v/w, respectively (Hosseini et al., 2014; Delnavazi et al., 2014).

β -Bisabolene is a major component in the root and flower oils of *D. ammoniacum*, commonly utilized in personal care products and as a flavoring agent in beverages (Barton and Chickos,

2020). This compound, which has a balsamic aroma and is approved as a food additive in Europe, has also demonstrated cytotoxic activity against breast cancer cell lines in both in vitro and in vivo studies (Yeo et al., 2016). The chemical profile of *Dorema glabrum* oils highlights δ -cadinene (12.77%) as the primary compound in its root oil, followed by β -bisabolene (7.48%), α -fenchyl acetate (6.32%), and copaene (5.68%) (Asnaashari et al., 2011). Additionally, thymol, a principal component in some samples, is recognized as a food additive that enhances digestive secretion and supports improved digestive system functioning (Alagawany et al., 2021).

D. ammoniacum possesses various medicinal properties, including its use as an expectorant, carminative, antispasmodic, diaphoretic, mild diuretic, poultice, stimulant, antimicrobial, spleen and liver tonic, and anticancer agent (Mottaghipisheh et al., 2021). Phytochemical analyses of its EOs reveal variability in composition based on plant parts. For instance, Takalloo et al. (2013) identified δ -cadinene (16.24%), liguloxide (8.69%), and δ -amorphene (8.43%) as significant constituents in stem oil, whereas Yousefzadi et al. (2011) reported (Z)-ocimene (22.3%), (E)-ocimene (18.1%),

and β -cyclocitral (9.9%) as the main components in fruit oil. Similarly, Hosseini et al. (2014) noted 2-pentadecanone (19.1%), β -eudesmol (17.2%), germacrene D (5.8%), α -eudesmol (5.8%), and spathulenol (5.0%) as the dominant compounds in seed oil.

The chemical composition of *D. ammoniacum* oils predominantly consists of sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Hosseini et al., 2018; Masoudi and Kakavand, 2017). Variations in EO components are influenced by factors such as the plant's age, developmental stage, genetic diversity, climate, soil conditions, sampling location and time, as well as stressors from insects and microorganisms (Hazrati et al., 2020; Mutlu-Ingok et al., 2020; Norani et al., 2023). Environmental factors, including climatic conditions, topography, and soil fertility,

significantly impact the growth and EO yield of medicinal plants (Hassiotis et al., 2014). Therefore, identifying the environmental factors that enhance EO content and composition can provide insights into optimizing the medicinal and commercial applications of these plants (Ahmed and Tavaszi-Sarosi, 2019).

Among phytochemical properties, tannins are notable for their role as hydrolyzable or condensed forms, depending on their chemical structure. Tannin biosynthesis, which is closely linked to carbohydrate metabolism, is influenced by environmental conditions such as light stress, shading, atmospheric changes (CO_2 , N_2 , O_2 , and O_3), temperature fluctuations, exogenous plant hormones (e.g., abscisic acid, naphthaleneacetic acid, and ethylene), pathogen infections, solar radiation, and nutrient deficiencies (Qaderi et al., 2023).

Table 8. Correlation coefficient axes with phytochemical and environmental factors in root samples of *D. ammoniacum*.

Variables and Factors	Abbreviation	Axis 1	Axis 2	Axis 3	Axis 4
AR	Average rainfall	-0.076	<u>0.984</u>	0.162	0.007
MAT	Mean annual temperature	0.490	-0.409	-0.138	0.019
RH	Relative humidity	-0.224	<u>0.720</u>	-0.088	0.018
LA	Latitude	0.029	<u>-0.668</u>	<u>-0.507</u>	0.423
LO	Longitude	<u>-0.683</u>	-0.091	0.179	0.110
AL	Altitude	1.000	0.013	-0.009	0.000
Clay	-	0.170	<u>-0.559</u>	<u>0.613</u>	0.344
Silt	-	0.386	-0.491	0.431	0.357
Sand	-	-0.328	<u>0.537</u>	<u>-0.514</u>	-0.368
EC	Electrical conductivity	-0.016	0.176	-0.410	0.325
pH	Potential of hydrogen	-0.093	-0.359	-0.135	<u>-0.707</u>
OC	Organic carbon	<u>0.630</u>	-0.237	<u>0.604</u>	0.063
N	Nitrogen	<u>0.590</u>	-0.224	<u>0.645</u>	0.072
P	Phosphorus	0.436	-0.421	<u>0.704</u>	0.223
K	Potassium	0.376	-0.483	<u>0.791</u>	-0.009
Fe	Iron	0.306	-0.475	<u>0.520</u>	<u>0.504</u>
Zn	Zinc	0.446	-0.184	-0.051	0.489
Cu	Copper	-0.352	-0.403	0.484	-0.135
Mn	Manganese	<u>0.669</u>	-0.356	0.439	0.275
EO	Essential oil	0.038	-0.181	-0.289	0.438
Tannin	-	0.337	0.105	0.318	<u>0.794</u>
Saponin	-	0.031	-0.040	-0.469	0.426
TPC	Total phenol	0.362	-0.286	0.090	<u>0.600</u>
TFC	Total flavonoid	0.158	0.149	0.123	0.045
DPPH	-	0.220	-0.376	-0.138	<u>0.813</u>
FRAP	-	0.114	-0.374	-0.204	<u>0.855</u>

The bold and underlined values had significant correlation with the relevant axes.

Table 9. Correlation coefficient axes with phytochemical and environmental factors in flower samples of *D. ammoniacum*.

Variables and Factors	Abbreviation	Axis 1	Axis 2	Axis 3	Axis 4
AR	Average rainfall	-0.076	<u>0.984</u>	0.163	-0.004
MAT	Mean annual temperature	0.490	-0.409	-0.140	-0.125
RH	Relative humidity	-0.224	<u>0.721</u>	-0.086	0.372
LA	Latitude	0.029	<u>-0.666</u>	<u>-0.507</u>	<u>-0.536</u>
LO	Longitude	<u>-0.683</u>	-0.091	0.180	0.030
AL	Altitude	1.000	0.012	-0.009	0.000
Clay	-	0.170	<u>-0.558</u>	<u>0.614</u>	-0.331
Silt	-	0.386	-0.491	0.431	<u>-0.595</u>
Sand	-	-0.328	<u>0.536</u>	<u>-0.514</u>	<u>0.529</u>
EC	Electrical conductivity	-0.016	0.178	-0.411	-0.496
pH	Potential of hydrogen	-0.093	-0.362	-0.137	<u>0.605</u>
OC	Organic carbon	<u>0.630</u>	-0.237	<u>0.604</u>	-0.059
N	Nitrogen	0.590	-0.224	<u>0.645</u>	-0.025
P	Phosphorus	0.436	-0.420	<u>0.704</u>	-0.185
K	Potassium	0.376	-0.484	<u>0.790</u>	0.016
Fe	Iron	0.306	-0.474	<u>0.521</u>	-0.338
Zn	Zinc	0.447	-0.182	-0.051	<u>-0.624</u>
Cu	Copper	-0.352	-0.404	0.482	0.148
Mn	Manganese	<u>0.669</u>	-0.355	0.440	-0.134
EO	Essential oil	0.353	-0.071	-0.314	<u>-0.623</u>
Tannin	-	0.241	0.390	0.281	-0.652
Saponin	-	<u>0.656</u>	-0.292	0.427	-0.184
TPC	Total phenol	<u>0.556</u>	0.162	0.161	<u>-0.684</u>
TFC	Total flavonoid	0.262	-0.488	-0.011	-0.376
DPPH	-	0.357	0.056	-0.392	-0.481
FRAP	-	0.251	-0.021	-0.253	<u>-0.652</u>

Bold and underlined values had significant correlation with the relevant axes.

Table 10. Correlation between six main traits on *D. ammoniacum* 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^{**}	0.31[*]	0.31[*]	0.08 ^{ns}	1	
FRAP	0.33^{**}	0.36^{**}	0.35^{**}	0.02 ^{ns}	0.89^{**}	1

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

In contrast, saponins—another key phytochemical—are categorized as triterpenoid or steroid types, with the latter being common in medicinal herbs. Saponins exhibit a wide range of pharmacological and medicinal activities while typically displaying low oral toxicity in humans (Sparg et al., 2004). Their presence in plants is linked to tonic and stimulating properties, further emphasizing their therapeutic potential (Ezeabara et al., 2014).

Previous studies on hydroalcoholic extracts from the aerial parts of *Dorema aitchisonii* and ethanolic extracts from the aerial parts of *Dorema aucheri* demonstrated weak antioxidant activity based on the DPPH assay, with IC₅₀ values of 488 µg mL⁻¹ and 200 µg mL⁻¹, respectively (Khanahmadi et al., 2012; Nabavi et al., 2012). In a comparative radical scavenging test (DPPH) conducted on several Apiaceae species (*Falcaria vulgaris*, *Smyrniopsis aucheri*, *Smyrniopsis munzurdagensis*, *Smyrniium cordifolium*, and *Actinolema macrolema*), Zengin et al. (2019) reported the highest radical scavenging activity in the methanolic extract of *Smyrniium cordifolium* (59.2 mg TE g⁻¹ extract), while *Smyrniopsis munzurdagensis* exhibited low antioxidant activity (2.29 mg TE g⁻¹). Differences in antioxidant capacities among samples from various populations are likely attributable to variations in their polyphenolic compound profiles (Rostaei et al., 2018).

This study records, for the first time, the phenolic and flavonoid contents in different organs of *D. ammoniacum*. Nazir et al. (2021) reported that the total phenolic and flavonoid contents of *D. ammoniacum* aerial parts (methanolic extract) from Pakistan were 68.2 mg GAE g⁻¹ and 66.97 mg QE g⁻¹, respectively. Phenolic and flavonoid compounds, which are effective free radical scavengers found in fruits and vegetables, play a crucial role in neutralizing oxidizing molecules such as singlet oxygen and various free radicals implicated in several diseases (Owen et al., 2003). The polyphenolic content of plant extracts varies depending on several factors, including their role in plant defense mechanisms against predators, oxidative stress, drought, infections, and extreme weather conditions (Tuladhar et al., 2021). Iran's natural habitats provide valuable resources for the production of medicinal plants, and the domestication and large-scale cultivation of native or ecologically adapted species hold significant potential for commercial exploitation (Jamshidi-Kia et al., 2017; Sharafzadeh and Alizadeh, 2012). Environmental factors substantially influence the growth of medicinal plants and their phytochemical properties, often enhancing the production of desirable

metabolites (Biondi et al., 2021). By selecting appropriate environmental conditions and plant varieties, the production of secondary metabolites can be optimized (Yang et al., 2018). In this study, the correlation between phytochemical properties and antioxidant activity was evaluated. However, no significant correlation was observed between total antioxidant activity and total flavonoid content in *D. ammoniacum*. Generally, antioxidant activity in plants is significantly related to total tannin content (Medda et al., 2021), saponin content (Punkte-Garza et al., 2017), and total phenolic content (Zhang et al., 2023). For example, species such as *Myrtus communis* L., *Agave salmiana*, *Hyssopus officinalis*, *Portulaca oleracea*, and *Origanum vulgare* have shown relatively high antioxidant capacities corresponding to their phenolic content (Nile et al., 2017). Antioxidant capacity reflects the presence of valuable bioactive compounds, including phenolics and flavonoids. Identifying such bioactive resources could pave the way for the discovery of traditional medicines to address critical diseases (Basgedik et al., 2014).

Conclusions

Dorema ammoniacum exhibits relatively low essential oil yields in its roots and flowers. The EO content and chemical composition of *D. ammoniacum* samples vary significantly across different ecosystems, highlighting the importance of identifying chemotypes with potentially higher pharmacological activity within natural populations. This study provides new insights into the antioxidant activity, total tannin content, saponin levels, total phenolic content, and total flavonoid content of *D. ammoniacum*. Root extracts were found to contain higher levels of phytochemicals compared to flower extracts, with variations influenced by the ecosystems in which the plants were grown. Among the studied populations, the Garmsar population demonstrated the highest eco-physiological diversity. Overall, *D. ammoniacum* exhibited moderate levels of phenols, flavonoids, and antioxidant capacity. To address concerns about overharvesting, we recommend that breeders and cultivators prioritize the domestication and expansion of *D. ammoniacum* cultivation in pasturelands. Such practices could ensure sustainable use of this valuable plant while reducing pressure on wild populations.

Acknowledgments

The authors would like to thank the Iran National Science Foundation (Grant Number: 98002070)

and Modares Science and Technology Park (MSTPARK) at Tarbiat Modares University for financially supporting this research.

Funding

This study was funded by Iran National Science Foundation (Grant Number: 98002070) and Tarbiat Modares University.

Author contributions

Conceptualization and Supervision: MA; Methodology: MN; investigation, writing and original draft: MN, writing, review and editing: All authors. Data collection: MN and MEi. Data analysis: All authors. Funding acquisition and Resources: MA.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Aboukhalid K, Al Faiz C, Douaik A, Bakha M, Kursa K, Agacka-Moldoch M, Machon N, Tomi F, Lamiri A. 2017. Influence of environmental factors on essential oil variability in *Origanum compactum* Benth. growing wild in Morocco. *Chemistry & Biodiversity* 14, e1700158.

Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. 4th edn. Allured Publishing Corporation, Carol Stream.

Ahmed HM, Tavaszi-Sarosi S. 2019. Identification and quantification of essential oil content and composition, total polyphenols, and antioxidant capacity of *Perilla frutescens* (L.) Britt. *Food Chemistry* 275, 730-738.

Alagawany M, Farag MR, Abdelnour SA, Elnesr SS. 2021. A review on the beneficial effect of thymol on health and production of fish. *Reviews in Aquaculture* 13, 632-641.

Amin G. 2005. Popular medicinal plants of Iran. Tehran: Tehran University Press.

Asnaashari S, Dadizadeh E, Talebpour AH, Eskandani M, Nazemiyeh H. 2011. Free radical scavenging potential and essential oil composition of the *Dorema glabrum* Fisch. CA Mey roots from Iran. *BioImpacts: BI* 1, 241.

Barton D, Chickos J. 2020. The vapor pressure and vaporization enthalpy of (–) β -Elemene and (–) β -Bisabolene by correlation gas chromatography. *The Journal of Chemical Thermodynamics* 148, 106139.

Basgedik B, Ugur A, Sarac N. 2014. Antimicrobial,

antioxidant, antimutagenic activities, and phenolic compounds of *Iris germanica*. *Industrial Crops and Products* 61, 526-530.

Biondi F, Balducci F, Capocasa F, Visciglio M, Mei E, Vagnoni M, Mezzetti B, Mazzoni L. 2021. Environmental conditions and agronomical factors influencing the levels of phytochemicals in *Brassica* vegetables responsible for nutritional and sensorial properties. *Applied Sciences* 11, 1927.

Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. 2007. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. *Journal of Agricultural and Food Chemistry* 55, 7879-7885.

Chakraborty P, Sarker RK, Roy R, Ghosh A, Maiti D, Tribedi P. 2019. Bioaugmentation of soil with *Enterobacter cloacae* AKS7 enhances soil nitrogen content and boosts soil microbial functional-diversity. *3 Biotech* 9, 1-10.

Delnavazi M, Tavakoli S, Rustaie A, Batooli H, Yassa N. 2014. Antioxidant and antibacterial activities of the essential oils and extracts of *Dorema ammoniacum* roots and aerial parts. *Res J Pharmacogn* 1, 11-18.

Ezeabara CA, Okeke C, Aziagba BO, Ilodibia CV, Emeka AN. 2014. Determination of saponin content of various parts of six *Citrus* species. *International Research Journal of Pure and Applied Chemistry* 4, 137.

Gangwar D, Baskar M. 2019. Texture determination of soil by hydrometer method for forensic purpose. Central Forensic Science Laboratory: Chandigarh, India.

Gelman F, Binstock R, Halicz L. 2012. Application of the Walkley-Black titration for the organic carbon quantification in organic rich sedimentary rocks. *Fuel* 96, 608-610.

Ghasemi F, Tamadon H, Hosseinmardi N, Janahmadi M. 2018. Effects of *Dorema ammoniacum* gum on neuronal epileptiform activity-induced by pentylenetetrazole. *Iranian Journal of Pharmaceutical Research: IJPR* 17, 735.

Hassan SS, Abdel-Shafy HI, Mansour MS. 2019. Removal of pharmaceutical compounds from urine via chemical coagulation by green synthesized ZnO-nanoparticles followed by microfiltration for safe reuse. *Arabian Journal of Chemistry* 12, 4074-4083.

Hassiotis C, Ntana F, Lazari D, Poulis S, Vlachonasios K. 2014. Environmental and

- developmental factors affect essential oil production and quality of *Lavandula angustifolia* during flowering period. *Industrial Crops and Products* 62, 359-366.
- Hazrati S, Mollaei S, Angourani HR, Hosseini SJ, Sedaghat M, Nicola S. 2020. Guide to harvesting *Heracleum persicum*: How essential oil composition and phenolic acid profile fluctuate at different phenological stages? *Food Sci. Nutr.* 8(11), 6192-6206.
- Hosseini SAR, Naseri HR, Azarnivand H, Jafari M, Rowshan V, Panahian AR. 2014. Comparing stem and seed essential oil in *Dorema ammoniacum* D. Don. from Iran. *Journal of Essential Oil Bearing Plants* 17, 1287-1292.
- Hosseini SS, Nadjafi F, Asareh MH, Rezadoost H. 2018. Morphological and yield related traits, essential oil and oil production of different landraces of black cummin (*Nigella sativa*) in Iran. *Scientia Horticulturae* 233, 1-8.
- Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. 2017. Medicinal plants: Past history and future perspective. *Journal of Herbmед Pharmacology* 7, 1-7.
- Karimian V, Vahabi MR, Roustakhiz J, Nodehi N. 2017. Identification of some ecological factors affecting essential oil of *Verbascum songaricum* Schrenk shoots (Case Study: Rangelands of Isfahan and Kohgiluyeh and Buyerahmad Provinces, Iran). *Journal of Rangeland Science* 7, 183-194.
- Khanahmadi M, Miraghaee SS, Karimi I. 2012. Evaluation of the antioxidant and antimicrobial properties of *Dorema aucheri* plant. *Iranian Red Crescent Medical Journal* 14, 1.
- Luthar Z, Kreft I. 1999. Influence of temperature on tannin content in different ripening phases of buckwheat (*Fagopyrum esculentum* Moench) seeds. *Fagopyrum* 16, 61-65.
- Masoudi S, Kakavand S. 2017. Volatile constituents of the aerial parts of *Terataenium lasiopentalum* (Boiss.) Manden., stems and leaves of *Dorema ammoniacum* D. Don. and leaves, fruits and stems of *Leutea petiolare* (DC.) M. Pimen from Iran. *Journal of the Chilean Chemical Society* 62, 3311-3314.
- Medda S, Fadda A, Dessena L, Mulas M. 2021. Quantification of total phenols, tannins, anthocyanins content in *Myrtus communis* L. and antioxidant activity evaluation in function of plant development stages and altitude of origin site. *Agronomy* 11, 1059.
- Memarzadeh SM, Gholami A, Pirbalouti AG, Masoum S. 2020. Bakhtiari savory (*Satureja bachtiarica* Bunge.) essential oil and its chemical profile, antioxidant activities, and leaf micromorphology under green and conventional extraction techniques. *Industrial Crops and Products* 154, 112719.
- Moghaddam M, Farhadi N. 2015. Influence of environmental and genetic factors on resin yield, essential oil content and chemical composition of *Ferula assa-foetida* L. populations. *Journal of Applied Research on Medicinal and Aromatic Plants* 2, 69-76.
- Moron A, Cozzolino D. 2003. Exploring the use of near infrared reflectance spectroscopy to study physical properties and microelements in soils. *Journal of Near Infrared Spectroscopy* 11, 145-154.
- Motevalian M, Mehrzadi S, Ahadi S, Shojaii A. 2017. Anticonvulsant activity of *Dorema ammoniacum* gum: evidence for the involvement of benzodiazepines and opioid receptors. *Research in Pharmaceutical Sciences* 12, 53.
- Mottaghipisheh J, Vitalini S, Pezzani R, Iriti M. 2021. A comprehensive review on ethnobotanical, phytochemical and pharmacological aspects of the genus *Dorema*. *Phytochemistry Reviews* 20, 945-989.
- Mutlu-Ingok A, Devecioglu D, Dikmetas DN, Karbancioglu-Guler F, Capanoglu E. 2020. Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review. *Molecules* 25, 4711.
- Nabavi SM, Nabavi SF, Ebrahimzadeh MA. 2012. Free radical scavenging and antioxidant activities of *Dorema aitchisonii*. *Journal of Food and Drug Analysis* 20, 8.
- Nazir N, Nisar M, Zahoor M, Uddin F, Ullah S, Ullah R, Ansari SA, Mahmood HM, Bari A, Alobaid A. 2021. Phytochemical analysis, in vitro anticholinesterase, antioxidant activity and in vivo nootropic effect of *Ferula ammoniacum* (*Dorema ammoniacum*) D. Don. in scopolamine-induced memory impairment in mice. *Brain Sciences* 11, 259.
- Nile SH, Nile AS, Keum Y-S. 2017. Total phenolics, antioxidant, antitumor, and enzyme inhibitory activity of Indian medicinal and aromatic plants extracted with different extraction methods. *3 Biotech* 7, 1-10.
- Norani M, Crawford A, Tahamtani Y, Ebadi M, Ayyari M. 2023. Extraction and essential oils profiling of different *Dorema ammoniacum* D.

- Don. organs and evaluation of antioxidant capacity. *Journal of Agricultural Science and Technology*, 701-717.
- Norani M, Ebadi M-T, Ayyari M. 2019. Volatile constituents and antioxidant capacity of seven *Tussilago farfara* L. populations in Iran. *Scientia Horticulturae* 257, 108635.
- Ordóñez A, Gómez J, Vattuone M. 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chemistry* 97, 452-458.
- Owen R, Haubner R, Mier W, Giacosa A, Hull W, Spiegelhalder B, Bartsch H. 2003. Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and Chemical Toxicology* 41, 703-717.
- Puente-Garza CA, Meza-Miranda C, Ochoa-Martínez D, García-Lara S. 2017. Effect of in vitro drought stress on phenolic acids, flavonols, saponins, and antioxidant activity in *Agave salmiana*. *Plant Physiology and Biochemistry* 115, 400-407.
- Qaderi MM, Martel AB, Strugnell CA. 2023. Environmental factors regulate plant secondary metabolites. *Plants* 12, 447.
- Rajani M, Saxena N, Ravishankara M, Desai N, Padh H. 2002. Evaluation of the antimicrobial activity of ammoniacum gum from *Dorema ammoniacum*. *Pharmaceutical Biology* 40, 534-541.
- Rechinger K, Lemond J, Hedge I. 1987. *Flora Iranica* (Umbelliferae). Akademische Druck Verlagsanstalt, Graz, Austria 162, 236-257.
- Ren J, Yang W, Li Z, Hu Y. 2019. Influence of matrix effect on determination accuracy of potassium content in soil and plant samples by flame photometer. *Acta Agriculturae Zhejiangensis* 31, 955-962.
- Rostaei M, Fallah S, Lorigooini Z, Surki AA. 2018. Crop productivity and chemical compositions of black cumin essential oil in sole crop and intercropped with soybean under contrasting fertilization. *Industrial Crops and Products* 125, 622-629.
- Sharafzadeh S, Alizadeh O. 2012. Some medicinal plants cultivated in Iran. *Journal of Applied Pharmaceutical Science* 134-137.
- Sałata A, Buczkowska H, Nurzyńska-Wierdak R. 2020. Yield, essential oil content, and quality performance of *Lavandula angustifolia* leaves, as affected by supplementary irrigation and drying methods. *Agriculture* 10, 590.
- Sim YY, Ong WTJ, Nyam KL. 2019. Effect of various solvents on the pulsed ultrasonic-assisted extraction of phenolic compounds from *Hibiscus cannabinus* L. leaves. *Industrial Crops and Products* 140, 111708.
- Sims JT. 2000. Soil test phosphorus: Olsen P. *Methods of phosphorus analysis for soils, sediments, residuals, and waters* 20.
- Singh B, Kaur A. 2018. Control of insect pests in crop plants and stored food grains using plant saponins: A review. *LWT* 87, 93-101.
- Shelke V, Bhot M. 2019. HPTLC fingerprint profile of leaf and stem methanolic extract of *Asclepias curassavica* L. *International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN)* 12, 4713-4717.
- Slinkard K, Singleton VL. 1977. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture* 28, 49-55.
- Sliwinska E. 2018. Flow cytometry—a modern method for exploring genome size and nuclear DNA synthesis in horticultural and medicinal plant species. *Folia Horticulturae* 30, 103.
- Sparg S, Light M, Van Staden J. 2004. Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology* 94, 219-243.
- Takalloo M, Sami S, Avvalb M. 2013. Chemical composition of the essential oils from flowers, stems and roots of *Dorema ammoniacum* D. Don from Iran. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4, 640-644.
- Tomasina F, Carabio C, Celano L, Thomson L. 2012. Analysis of two methods to evaluate antioxidants. *Biochemistry and Molecular Biology Education* 40, 266-270.
- Tuladhar P, Sasidharan S, Saudagar P. 2021. Role of phenols and polyphenols in plant defense response to biotic and abiotic stresses. In: *Biocontrol Agents and Secondary Metabolites*. Elsevier, pp. 419-441.
- Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q. 2018. Response of plant secondary metabolites to environmental factors. *Molecules* 23, 762.
- Yeo SK, Ali AY, Hayward OA, Turnham D, Jackson T, Bowen ID, Clarkson R. 2016. β -Bisabolene, a sesquiterpene from the essential oil extract of *Opoponax* (*Commiphora guidottii*), exhibits cytotoxicity in breast cancer cell lines. *Phytotherapy Research* 30, 418-425.
- Yousefzadi M, Mirjalili HM, Alnajjar N, Zeinali A,

Parsa M. 2011a. Composition and in vitro antimicrobial activity of the essential oil of *Dorema ammoniacum* D. Don fruit from Iran. Journal of the Serbian Chemical Society 76, 857-863.

Yousefzadi M, Mirjalili MH, Alnajjar N, Zeinali A, Parsa M. 2011b. Composition and in vitro antimicrobial activity of the essential oil of *Dorema ammoniacum* D. Don fruit from Iran. Journal of Serbian Chemical Society 76, 857-863.

Zengin G, Mahomoodally MF, Paksoy MY, Picot-Allain C, Glamocilja J, Sokovic M, Diuzheva A, Jekő J, Cziáky Z, Rodrigues MJ. 2019. Phytochemical characterization and bioactivities of five *Apiaceae* species: Natural sources for novel ingredients. Industrial Crops and Products 135, 107-121.

Zhang Y, Li Y, Ren X, Zhang X, Wu Z, Liu L. 2023. The positive correlation of antioxidant activity and prebiotic effect about oat phenolic compounds. Food Chemistry 402, 134231.

Supplementary Files

Table S1. Extraction yield of all samples of *D. ammoniacum*.

	Jiroft	Shahrood	Garmsar	Kerend	Birjand	Kashmar	Bardaskan	Bafq	Mehriz	Neyriz
R	2.6±0.09 ^r	7.2±0.07 ^f	10.0±0.10^a	9.3±0.09 ^b	7.4±0.17 ^c	7.5±0.26 ^d	8.0±0.10 ^d	9.5±0.20^{ab}	8.0±0.17 ^d	7.0±0.23 ^e
F	3.9±0.06 ^{op}	2.6±0.12 ^r	5.6±0.09 ^j	2.7±0.07 ^r	6.7±0.12 ^h	4.2±0.07 ⁿ	5.7±0.09 ^j	4.9±0.20 ^k	1.7±0.15 ^t	1.2±0.09 ^u

R: root, F: flower. The bold type face means the compounds have the highest value.

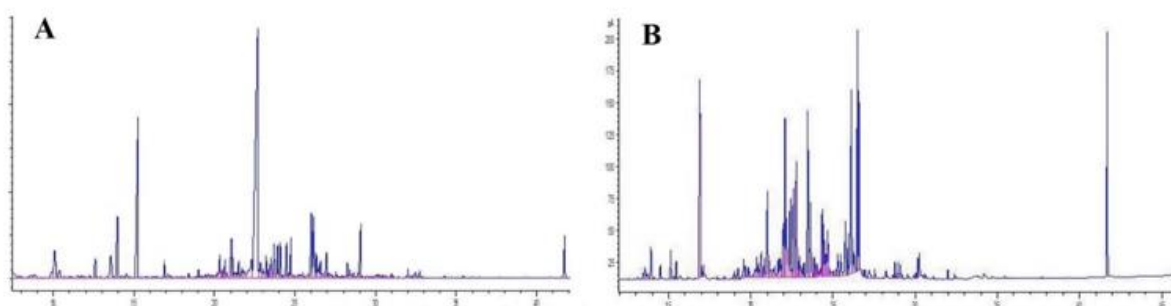


Figure S1. GC profile of essential oil from stem (A) and leaf (B) of *D. ammoniacum*.

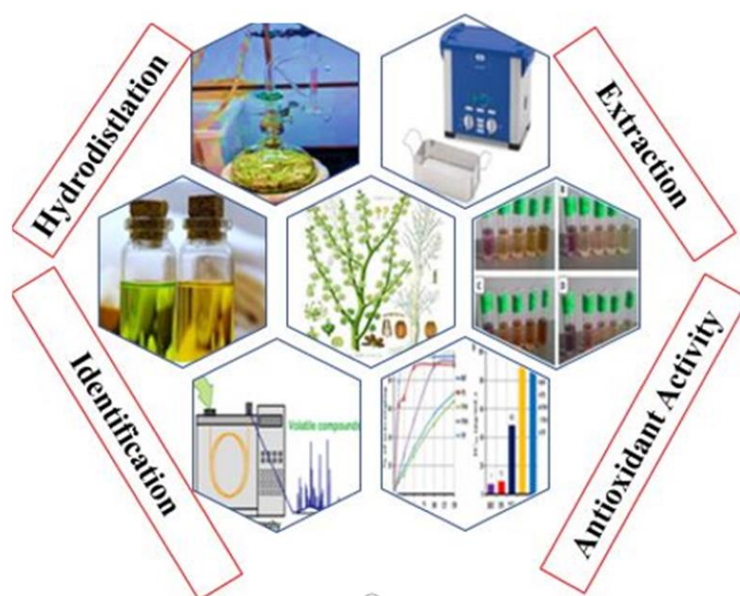


Figure S2. Graphical abstract.