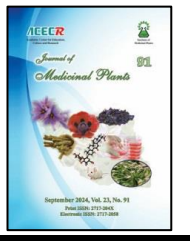
J. Med. Plants 2024; 23(91): 42-63

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Volatile compounds variation and phytochemicals of leaf and stem of *Dorema ammoniacum* D. Don. Wild populations

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ARTICLE INFO

Keywords:
Volatile compounds
Vasha
GC-MS
Antioxidant activity
β-bisabolene

ABSTRACT

Background: *Dorema ammoniacum* D. Don. is an important perennial medicinal plant which is belonging to the Apiaceae family. **Objective:** We aimed to compere Essential Oils (EOs) and antioxidant activity along with other phytochemical properties of *Dorema ammoniacum* of different populations. **Methods:** Leaves and stems of 10 populations of *D. ammoniacum* were collected from Jiroft, Shahrood, Garmsar, Kerend, Birjand, Kashmar, Bardaskan, Bafq, Mehriz and Neyriz. The essential oil contents were investigated with gas chromatography techniques and compared with the commercial gum-resin essential oil. **Results:** The EO yield of *D. ammoniacum* were 0.2-0.3 (stem and leaf) and 1.0 (gum) v/w %, respectively. Essential oils from different organs showed some variation that was correlated to the plant part but also similarities in the EO profiles were easily observed. The major compounds in the EO of gum were (2E,6E)-farnesol, cuparene, (2Z,6Z)-farnesol and *β*-bisabolene. Endo-Fenchyl acetate, p-cymen-8-ol, cuparene, and *β*-bisabolene were identified in stem and leaf oil. Analysis of variance of phytochemical characteristics showed that there was a significant difference among all extracts of *D. ammoniacum* in terms of the Antioxidant Activity (AA), Total Tannin Content (TTC), saponin, Total Phenol Content (TPC) and Total Flavonoid Content ($P \leq 0.01$). **Conclusion:** Gum essential oil composition resembles more to leaf-derived *D. ammoniacum oil*. This study provides new insights into the antioxidant capacity and other phytochemical properties of *D. ammoniacum*.

1. Introduction

The use of medicinal plants as traditional sources of medicinal drugs has a long history [1, 2], hence, medicinal plants are used various industrial products [3]. These herbal products are popular as they are natural and have healthy properties [4]. *Dorema ammoniacum* (family Apiaceae) is an important perennial medicinal plant that is endemic plant in Iran [5]. *D. ammoniacum* grows to a height of 1-2 m in central areas of Iran such as the Yazd, Isfahan and Semnan provinces and its local names are Vasha, Kandal and Koma-kandal [6]. This species exudes a gum-resin that has medicinal and industrial applications. For example, it used as an antihelmintic agent and for gastrointestinal

Abbreviations: AA, antioxidant activity; DPPH, 2,2-diphenyl-2 picrylhydrazyl hydrate; EO, essential oil; FRAP, ferric reducing antioxidant power; LSD, least significant difference; RSC, radical scavenging capacity; TFC, Total flavonoid contents; TPC, Total phenolic contents

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doi: 10.61186/jmp.23.91.42

Received 10 February 2024; Received in revised form 21 October 2024; Accepted 29 October 2024

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[DOI: 10.61186/jmp.23.91.42]

disorders in Iranian traditional medicine [7]. It is also recommended for the treatment of seizures [8]. Moreover, antibacterial, vasodilatory and anticonvulsant protective effects of this herbal plant have also been recognized [9]. The hydrodistillation of *D. ammoniacum* gum, stem, seed and fruit gave a yellow oil in 0.4 (w/w), 0.5 % (w/w), 0.3 % (w/w) and 0.09 % (w/w) yield, respectively [4, 5, 10]. Some of the chemical constituents of *D. ammoniacum* gum include free salicylic acid, ammoresinol, doremin, doremine A and ammodoremin [10]. The stem oils have δ-cadinene (16.24 %), liguloxide (8.69 %) and δ-amorphene (8.43 %) as the major components, while the root oil has been shown to accumulate high levels of 3-*n*-butyl phthalide, reported at 62.49 % [11]. Different plant parts of the species show quantitative and qualitative accumulation of volatile compounds. In a study by Yousefzadi et. al [5], the major constituents of the fruit oil of *D. ammoniacum*, collected from Birjand were (*Z*)- and (*E*)-ocimenone, *β*-cyclocitral and *ar*-curcumene, and the main components of the leaves were *α*-gurjunene (49.5 %), *β*-gurjunene (19.0 %) and *α*-selinene (4.6 %). However, seeds contain 2-pentadecanone (19.1 %), *β*-eudesmol (17.2 %), germacrene D (5.8 %), *α*-eudesmol (5.8 %) and spathulenoll (5.0 %) [4]. 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 [11]. Phenolics and flavonoids are well known to prevent the generation of free radical during oxidative stress, and enhance the antioxidant activities. Many clinical studies have shown the value of these compounds not only as high antioxidant capacity but also for their antimicrobial activities [12]. It is well established that the biological activity is linked to the phytochemicals produced by medicinal plants and the latter depends on many factors such as the cultivation area, climatic conditions, inherent genetic variation amongst different populations, and the collection period [13]. The present study was designed to identify the essential oil variation of plants of *D. ammoniacum* found in 10 different populations of Iran using gas chromatography-mass spectrometry (GC-MS) and also to evaluate the total tannin content, saponin, antioxidant activity (AA) including 2,2-diphenyl-2 picrylhydrazyl hydrate (DPPH) and ferric reducing antioxidant power (FRAP), total phenol content (TPC) and total flavonoid content (TFC) of *D. ammoniacum* leaves and stems. Finally, the compounds of the commercial gum resin were also compared with phytochemicals from other plant organs in order to identify the best organ that had similar chemistry to the commercial gum resin.

2. Materials and methods

2.1. Plant materials

Fresh leaves and stems of *D. ammoniacum* were collected, during the leafing and flowering stage stages (2019), from 10 different populations of Iran including Jiroft, Shahrood, Garmsar, Kerend, Birjand, Kashmar, Bardaskan, Bafq, Mehriz and Neyriz (Table 1) and then air-dried under shade conditions and room temperature. Plant identities were confirmed by Dr. A. Sonboli as a taxonomist and a representative voucher specimen (MPH-2724) was placed in the Medicinal Plants and Drug Research Institute Herbarium (MPH) of Shahid Beheshti University. The collected plant samples are kept in a private herbarium collection. Also, gum-resin of *D. ammoniacum* was bought from a local authentic harvester in Birjand, which is commercially available in the market.

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[DOI: 10.61186/jmp.23.91.42]

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

Sampling location	Province	Average rainfall (mm)	Mean annual temperature (ºC)	Relative humidity (%)	Latitude	Longitude	Altitude (m a.s.l.)*
Bardaskan	Razavi Khorasan	110	19.5	33	57º89′54″	35º97′83″	1479
Kashmar	Razavi Khorasan	170	17.5	40	58º46′85″	35º33′53″	1332
Birjand	South Khorasan	155	16.5	36	57º89′54″	35º97′83″	1468
Shahrood	Semnan	180	17	49	55º36′69″	36º44′99″	1297
Garmsar	Semnan	124	21.7	41	52º16′42″	35º28′11″	951
Jiroft	Kerman	239	27.1	38	57º26′57″	28º06′26″	1897
Kerend-e Gharb	Kermanshah	527	13.7	50	46º14′07″	34º16′50″	1553
Bafq	Yazd	96	24	37	55º42′48″	31º30′08″	1405
Mehriz	Yazd	149	22	40	53º50′12″	31º09′50″	2301
Neyriz	Fars	180	17.7	41.4	54º21′20″	29º14′40″	1636

* Meters above sea level

2.2. Isolation and analysis of essential oils

About 50 grams air-dried leaves, stems and gum of *D. ammoniacum* was 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-type 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 [13].

Analysis using GC was performed using an 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. The injection temperature was set at 250 °C and the detector temperature was set at 280 °C. The oven temperature was programmed from 60 °C (2 min hold) to 250 °C with the ramp of 5 °C/min. Selected essential oil (1 μl) was also 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 identification of essential oil compounds was based on the mass spectrum of each respective compound that was 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 [14].

2.3. Preparation of different extracts

In the present work, leaf and stem extracts of *D. ammoniacum* were also prepared by sonicating (using an ultrasonic device-120 Hz frequency). About five gram of dried plant material extracted for 30 min at 30 °C in 50 mL of methanol. All the extracts were filtered using

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Whatman No.1 filter paper, and then concentrated in rotary evaporator at 40 °C in vacuo. Once the extracts were finally dried, they were then stored at 4 °C until analysis [13].

2.4. Determination of Total Tannin Content

The total tannin content (TTC) of each methanolic extract was determined using the method of Luthar and Kreft [15].

2.5. Determination of Saponins

Five grams of the powdered samples were mixed with 50 ml of 20 % (v/v) aqueous ethanol solution in a flask. The mixtures were heated with periodic agitation in a water bath for 90 minutes at 55 ºC; and filtered through Whatman filter paper (No. 42). The filtrate was extracted with 50 ml of 20 % ethanol and both extract were poured together and the combined extract was reduced to a volume of 40 ml by heating it at 90 ºC before it was transferred to a separating funnel where 40 ml of n hexane was added and shaken vigorously. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5 % NaCl solution and evaporated to dryness in a pre-weighed evaporation dish [16] before drying at 60 ºC in the oven. All extracts were then re-weighed after cooling in a dessicator. The process was repeated two more times to get an average. The saponin content was determined according to the following calculation:

$$\text{Percentage (\%) Saponin} = \frac{W2-W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

W1 = Weight of evaporating dish

W2 = Weight of evaporating dish + sample

2.6. Determination of total phenolic compounds

The total phenolic content of each extracts was measured by using the Folin–Ciocalteu method [17].

2.7. Determination of Total Flavonoid

The colorimetric method of Ordonez et al. [18] was used to determine the total flavonoid content (TFC). Briefly, extracts of *D. ammoniacum* were resuspended in DMSO to yield a concentration of 0.5 g/ml. Equal amount of extract volume and aluminum chloride solution (2 %, methanolic solution) were mixed together in a test tube and the absorbance was measured at 420 nm using a spectrophotometer after 10 min. The experiment for each extract was done in triplicate. A calibration curve was prepared using a series of methanolic quercetin solutions. The results were expressed as mg of quercetin equivalents dry per gram dried weight of extract (mg QE/g DW Ext).

2.8. Antioxidant Capacity

2.8.1. DPPH method

The antioxidant activities of methanolic extracts were evaluated with 2,2-diphenyl-2 picrylhydrazyl hydrate (DPPH) radical scavenging activity based on the previously described method of Bozin et al. [19], using the IC_{50} to compare the antioxidant properties.

2.8.2. FRAP method

The reducing powers of the extracts were determined using ferric reducing antioxidant power (FRAP) method [20].

2.9. Statistical analysis

The data was analyzed according to the analysis of variance for factorial experiments 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

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within SAS was used to test the assumptions of ANOVA, and residuals were normally distributed. The means were compared through the least significant difference (LSD) at the 5 % probability level. The correlation analysis results of phytochemical properties and antioxidant activity were expressed as Pearson correlation coefficients using SPSS software version 20.

3. Results

3.1. Essential oil composition

The hydrodistillation of *D. ammoniacum* oil gave in 0.2-0.3, 0.2-0.3 and 1.0 v/w % (relative to dry weight of plant) yield for stem, leaves and gum, respectively. In the stem oil of *Dorema ammoniacum*, a total of 62 components were identified, ranging from a minimum value of 87.8 % in the stem oil of Shahrood to a maximum value of 97.2 % in the stem oil of Bardskan (Table 2). Among the ten different stem samples, several common compounds were identified, including *p*-cymen-8-ol, *endo*-fenchyl acetate, 2-pentadecanol, *β*-bisabolene, elemicin, *n*-eicosane, and heptacosane. The stem oil of Garmsar (SG) exhibited the highest amount of *p*-cymen-8-ol at 22.4 %, while the stem of Birjand (SB) showed the lowest amount at 0.3 %. The highest value of *endo*-fenchyl acetate was found in the stem oil of Shahrood (SSh) at 55.6 %, whereas the stem oils of SB and SNe had the lowest amount at 0.2 %. Furthermore, the highest values of 2-pentadecanol, *β*-bisabolene, elemicin, *n*-eicosane, and heptacosane were observed in SBr (11.2 %), SKr (21.3 %), SB (16.7 %), SMe (12.7 %), and SNe (25.7 %) respectively.

In leaf oil of *D. ammoniacum*, 59 compounds were identified, ranging from 90.3 % in leaves of Bafq to 95.6 % in leaves of Jiroft (Table 3). *n*-Dodecane, *δ*-elemene, *β*-bisabolene, cuparene, *n*-hexadecanol, and heptacosane were identified as a common major compounds between leaf samples. The highest values of dodecane, *δ*-elemene, *β*-bisabolene, cuparene, *n*-hexadecanol, and heptacosane were obtained in LG (56.1 %), LBr (15.2 %), LKr (7.0 %), LJ (8.2 %), LNe (13.4 %), and LB (51.7 %), respectively. Also, the lowest of dodecane (1.1 %), *δ*-elemene (0.1 %), *β*-Bisabolene (0.1 %), cuparene (0.1 %), *n*-hexadecanol (1.0 %), and heptacosane (0.8 %) were obtained in LJ, LB, and LJ, respectively.

Thirty six compounds were identified in the gum EO and the percentage of identified compounds was 89.7 % (Table 4). The major compounds detected in the EO of gum were (2*E*, 6*E*)- farnesol (12.2 %), cuparene (11.5 %), (2*Z*,6*Z*)-farnesol and *β*-bisabolene (8.7 %). *β*-Bisabolene as the common major component was shown in leaves and gum oil of *D. ammoniacum*. The chemical compositions of oils of *D. ammoniacum* consisted of mainly sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Fig. 1, 2, 3).

Table 2. Chemical composition (% relative abundance) of stem (S) essential oils of *D. ammoniacum*

No	RT	Components	SJ %	SSh %	SG %	SKr %	SB %
1	7.8	*α*-Pinene	1.4 ± 0.09[y]				
2	8.1	Sabinene	0.6 ± 0.04	0.5 ± 0.02	3.2 ± 0.12		
3	8.6	*β*-Pinene		0.7 ± 0.01	0.4 ± 0.04		0.3 ± 0.01
4	9.5	*p*-Cymene					
5	9.8	*β*-ocimene	0.6 ± 0.04	0.8 ± 0.04	1.0 ± 0.09	1.2 ± 0.04	0.2 ± 0.01
6	10.1	(*Z*)-Sabinene hydrate		0.4 ± 0.05		0.5 ± 0.01	0.2 ± 0.02
7	11.1	(*E*)-Sabinene hydrate					0.2 ± 0.01
8	11.5	*iso*-Pentyl isovalerate		0.3 ± 0.05		0.7 ± 0.02	
9	11.8	(*E*)-2-Nonenal	0.4 ± 0.03	1.2 ± 0.11	0.9 ± 0.07		0.2 ± 0.04

Table 2. Chemical composition (% relative abundance) of stem (S) essential oils of *D. ammoniacum* (Continued)

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No	RT	Components	SJ %	SSh %	SG %	SKr %	SB %
10	12.9	*trans*-Pinocamphone		0.9 ± 0.09	0.6 ± 0.04		
11	13.4	*p*-Cymen-8-ol	**6.8 ± 0.11**	**19.5** ± 0.48	**22.4 ± 0.71**	0.9 ± 0.08	0.3 ± 0.04
13	14.5	*endo*-Fenchyl acetate	**13.5 ± 0.34**	**55.6 ± 0.87**	**47.2 ± 0.58**	2.3 ± 0.9	0.2 ± 0.02
14	14.8	Thymol, methyl ether	0.3 ± 0.04	1.9 ± 0.01	0.4 ± 0.01	2.9 ± 0.08	
15	15.1	Carvacrol, methyl ether	1.2 ± 0.1				0.1 ± 0.01
16	17.2	*δ*-Elemene	**6.1 ± 0.09**		0.7 ± 0.02		0.7 ± 0.05
17	17.7	*α*-Cubebene				0.7 ± 0.04	0.2 ± 0.04
18	17.8	2-Undecanol		0.6 ± 0.04	0.3 ± 0.04	0.8 ± 0.03	
19	19.2	*α*-Elemene	3.2 ± 0.09				0.8 ± 0.04
20	19.6	*Z*-Caryophyllene	0.4 ± 0.05	0.3 ± 0.07	0.5 ± 0.04	0.4 ± 0.04	1.1 ± 0.08
21	19.9	2-Dodecanol	0.4 ± 0.02				
22	20.4	*E*-Caryophyllene	2.0 ± 0.07		1.0 ± 0.08		0.6 ± 0.06
23	20.6	Dehydroaromadendrane	0.8 ± 0.04			0.7 ± 0.05	2.6 ± 0.11
24	20.7	*ar*-Curcumene	0.5 ± 0.04			0.4 ± 0.06	
25	21	γ-muurolene	3.0 ± 0.09		0.3 ± 0.02		3.9 ± 0.13
26	21.4	(*Z*)-Farnesene	0.6 ± 0.03		0.5 ± 0.02	0.7 ± 0.01	0.3 ± 0.04
27	21.6	Germacrene D	2.2 ± 0.07		0.6 ± 0.02	0.1 ± 0.01	1.6 ± 0.06
28	21.8	β-selinene				2.9 ± 0.9	
29	21.9	2-Pentadecanol	4.2 ± 0.04	0.3 ± 0.07	0.5 ± 0.04	4.1 ± 0.1	1.7 ± 0.09
30	22	(*E*)- β –Ionone				2.3 ± 0.13	
31	22.2	Bicyclogermacrene			0.3 ± 0.02		
32	22.4	β-Bisabolene	**5.2 ± 0.12**	0.5 ± 0.04	0.3 ± 0.02	**21.3** ± 0.41	**5.0 ± 0.14**
33	22.7	(*Z*)-α-Bisabolene	1.3 ± 0.1		0.6 ± 0.06	4.5 ± 0.14	2.7 ± 0.09
34	22.8	Cuparene				0.4 ± 0.04	
35	22.9	Elemicin	**11.8 ± 0.34**	0.2 ± 0.01	4.3 ± 0.11	**5.1** ± 0.12	**16.7 ± 0.55**
36	23.2	Caryophyllene oxide	0.3 ± 0.03				1.2 ± 0.01
37	23.4	Tridecanol	2.4 ± 0.09			1.0 ± 0.02	
38	23.6	*ar*-dihydro Turmerone	1.5 ± 0.1		0.4 ± 0.09		
39	23.8	Cedrol					
40	23.9	Junenol	0.5 ± 0.01			0.3 ± 0.04	
41	24.1	γ-Eudesmol	0.3 ± 0.02		2.0 ± 0.09		
42	24.2	α-Muurolol	0.4 ± 0.04		0.6 ± 0.05	**21.2 ± 0.46**	**30.5 ± 0.84**
43	24.3	Cubenol	1.5 ± 0.07			0.4 ± 0.02	
44	24.7	(6*Z*)-Pentadecen-2-one	0.6 ± 0.04		0.4 ± 0.04	3.3 ± 0.05	
45	25.2	Pentadecanal	0.9 ± 0.03				
46	25.3	(2*Z*,6*Z*)-Farnesal	1.4 ± 0.1		0.9 ± 0.07		3.7 ± 0.17
47	25.6	α-Bisabolol					
48	25.7	(2*Z*,6*Z*)-Farnesol	1.1 ± 0.04		0.6 ± 0.04	0.7 ± 0.03	2.3 ± 0.08
49	26	(2*E*,6*E*)-Farnesol	3.4 ± 0.22		0.5 ± 0.02	2.7 ± 0.09	2.0 ± 0.09
50	26.3	*n*-Pentadecanol	0.3 ± 0.04		0.3 ± 0.04		**11.6 ± 0.56**
51	26.5	*n*-Hexadecanol	3.9 ± 0.15		0.6 ± 0.04		1.8 ± 0.11
52	26.7	di-*n*-butyl phthalate					
52	27.2	*n*-Eicosane	0.3 ± 0.04	0.2 ± 0.01	0.2 ± 0.01	1.2 ± 0.07	1.2 ± 0.08
53	28.2	*n*-Octadecanol					
54	28.4	*n*-Heneicosane				0.3 ± 0.04	0.2 ± 0.04
55	28.5	Ethyl linoleate				1.2 ± 0.04	
56	28.7	Phytol	0.5 ± 0.03				
57	28.9	(*E*)-Phytol acetate					
58	29	*n*-Tricosane	0.5 ± 0.03			1.5 ± 0.09	0.2 ± 0.04
59	30.2	*n*-Tetracosane	0.5 ± 0.01				0.3 ± 0.02
60	31.9	*n*-Pentacosane				2.1 ± 0.05	0.2 ± 0.02
61	32	Hexacosane		0.3 ± 0.09			0.2 ± 0.04
62	41.6	Heptacosane	2.7 ± 0.8	4.3 ± 0.24	0.3 ± 0.04	**6.1** ± 0.14	1.6 ± 0.07
		Total compounds	**89.6**	**87.8**	**92.8**	**97.3**	**96.75**

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Table 2. Chemical composition (% relative abundance) of stem (S) essential oils of *D. ammoniacum* (Continued)

No	RT	Components	SKa %	SBr %	SBa %	SMe %	SNe %	RI* (Kovats index)
1	7.8	*α*-Pinene			0.9 ± 0.07			932
2	8.1	Sabinene		0.9 ± 0.04				961
3	8.6	*β*-Pinene		1.1 ± 0.04			1.1 ± 0.09	974
4	9.5	*p*-Cymene			0.3 ± 0.01	0.4 ± 0.02	0.3 ± 0.02	1024
5	9.8	*β*-ocimene		0.2 ± 0.02		1.9 ± 0.07		1032
6	10.1	(*Z*)-Sabinene hydrate			0.3 ± 0.04			1065
7	11.1	(*E*)-Sabinene hydrate			3.0 ± 0.12	0.2 ± 0.02	2.5 ± 0.09	1086
8	11.5	*iso*-Pentyl isovalerate		1.3 ± 0.09	2.0 ± 0.09			1103
9	11.8	(*E*)-2-Nonenal						1150
10	12.9	*trans*-Pinocamphone		0.6 ± 0.04				1158
11	13.4	*p*-Cymen-8-ol	**5.6 ± 0.21**	2.9 ± 0.07	2.0 ± 0.09	1.7 ± 0.07	**5.1 ± 0.21**	1179
13	14.5	*endo*-Fenchyl acetate	**14.4 ± 0.51**	**14.2 ± 0.29**	**9.1 ± 0.33**	**10.8 ± 0.04**	0.2 ± 0.01	1218
14	14.8	Thymol, methyl ether		1.3 ± 0.04	1.3 ± 0.09	0.2 ± 0.04		1232
15	15.1	Carvacrol, methyl ether	1.8 ± 0.09	0.3 ± 0.01	2.4 ± 0.13	0.8 ± 0.05	0.4 ± 0.02	1241
16	17.2	*δ*-Elemene		1.3 ± 0.07	**6.7 ± 0.27**	**12.7 ± 0.41**	**9.8 ± 0.32**	1335
17	17.7	*α*-Cubebene						1345
18	17.8	2-Undecanol						1366
19	19.2	*α*-Elemene		1.3 ± 0.04				1389
20	19.6	*Z*-Caryophyllene	2.5 ± 0.08	1.7 ± 0.08				1408
21	19.9	2-Dodecanol			0.3 ± 0.02		0.5 ± 0.03	1410
22	20.4	*E*-Caryophyllene	2.1 ± 0.07		0.9 ± 0.01	0.7 ± 0.02	0.1 ± 0.01	1417
23	20.6	Dehydroaromadendrane	0.9 ± 0.07	1.3 ± 0.7	0.8 ± 0.02	0.2 ± 0.01		1460
24	20.7	*ar*-Curcumene				1.5 ± 0.09		1475
25	21	*γ*-muurolene	3.6 ± 0.11	0.6 ± 0.04				1478
26	21.4	*(Z)*-Farnesene		0.1 ± 0.01	2.4 ± 0.10	0.7 ± 0.05	1.3 ± 0.07	1481
27	21.6	Germacrene D	0.7 ± 0.08	1.4 ± 0.1		0.8 ± 0.04		1484
28	21.8	*β*-selinene			0.2 ± 0.04			1489
29	21.9	2-Pentadecanol	**9.8 ± 0.32**	**11.2 ± 0.39**	**7.9 ± 0.13**	3.7 ± 0.11	**7.3 ± 0.28**	-
30	22	*(E)- β* –Ionone						1490
31	22.2	Bicyclogermacrene			1.2 ± 0.07			1502
32	22.4	*β*-Bisabolene	1.0 ± 0.08	0.4 ± 0.01	1.6 ± 0.1	**5.3 ± 0.27**	**5.4 ± 0.22**	1505
33	22.7	*(Z)-α*-Bisabolene	3.6 ± 0.13			0.5 ± 0.02		1506
34	22.8	Cuparene		1.4 ± 0.04				1508
35	22.9	Elemicin	**5.7 ± 0.31**	**9.4 ± 0.14**	**10.1 ± 0.2**	1.3 ± 0.08	3.8 ± 0.12	1560
36	23.2	Caryophyllene oxide						1567
37	23.4	Tridecanol	4.9 ± 0.18	2.4 ± 0.09	4.0 ± 0.09		1.6 ± 0.03	1570
38	23.6	*ar*-dihydro Turmerone	1.2 ± 0.1	3.9 ± 0.07		0.2 ± 0.04		1595
39	23.8	Cedrol	1.0 ± 0.07			0.7 ± 0.02		1600
40	23.9	Junenol		0.2 ± 0.04	0.5 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	1618
41	24.1	*γ*-Eudesmol	0.7 ± 0.04	1.3 ± 0.04	0.9 ± 0.04	0.2 ± 0.02	0.7 ± 0.05	1630
42	24.2	*α*-Muurolol						1644
43	24.3	Cubenol	2.4 ± 0.09		1.5 ± 0.08	2.7 ± 0.17	3.7 ± 0.12	1645
44	24.7	*(6Z)*-Pentadecen-2-one	1.1 ± 0.01	2.3 ± 0.09	**9.7 ± 0.36**	3.7 ± 0.24		1667
45	25.2	Pentadecanal	1.4 ± 0.04					1682
46	25.3	*(2Z,6Z)*-Farnesal	3.6 ± 0.09	1.3 ± 0.02	0.5 ± 0.03		1.9 ± 0.09	1684
47	25.6	*α*-Bisabolol		2.3 ± 0.07				1685
48	25.7	*(2Z,6Z)*-Farnesol	1.7 ± 0.02	1.9 ± 0.02	0.8 ± 0.04		4.6 ± 0.21	1698
49	26	(2*E*,6*E*)-Farnesol	1.0 ± 0.01	2.3 ± 0.04	3.9 ± 0.14	2.0 ± 0.12		1742
50	26.3	*n*-Pentadecanol	1.2 ± 0.04	1.6 ± 0.06				1773
51	26.5	*n*-Hexadecanol	2.1 ± 0.04	2.2 ± 0.08			4.2 ± 0.19	1874
52	26.7	di-*n*-butyl phthalate	1.4 ± 0.07		2.8 ± 0.11			1906
52	27.2	*n*-Eicosane	0.8 ± 0.05	0.4 ± 0.02	**9.4 ± 0.74**	**12.7 ± 0.49**	**7.1 ± 0.34**	2000
53	28.2	*n*-Octadecanol	0.8 ± 0.05			0.6 ± 0.03		2077
54	28.4	*n*-Heneicosane	0.7 ± 0.05	0.9 ± 0.02				2100
55	28.5	Ethyl linoleate						-
56	28.7	Phytol			0.4 ± 0.01	0.6 ± 0.07	0.2 ± 0.01	2122
57	28.9	(*E*)-Phytol acetate		4.3± 0.08				2218
58	29	*n*-Tricosane	1.3 ± 0.01					2300
59	30.2	*n*-Tetracosane		1.8 ± 0.12	0.7 ± 0.02		2.1 ± 0.09	2400

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Table 2. Chemical composition (% relative abundance) of stem (S) essential oils of *D. ammoniacum* (Continued)

No	RT	Components	SKa %	SBr %	SBa %	SMe %	SNe %	RI* (Kovats index)
60	31.9	*n*-Pentacosane						2500
61	32	Hexacosane						2600
62	41.6	Heptacosane	**10.0 ± 0.34**	**14.9 ± 0.45**	4.3 ± 0.13	**22.6 ± 0.71**	**25.7 ± 0.81**	2700
		Total compounds	**89.0**	**97.2**	**90.8**	**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.

SJ: Stem of Jiroft, SSh: Stem of Shahrood, SG: Stem of Garmsar, SKr: Stem of Kerend, SB: Stem of Birjand, SKa: Stem of Kashmar, SBr: Stem of Bardaskan, SBa: Stem of Bafq, SMe: Stem of Mehriz, SNe: Stem of Neyriz

y: Data are mean ± SE.

Table 3. Chemical composition (% relative abundance) of leaf (L) essential oils of *D. ammoniacum*

No	RT	Components	LJ %	LSh %	LG %	LKr %	LB %
1	7.8	*α*-Pinene	0.5 ± 0.04[y]				0.1 ± 0.02
2	8.1	Sabinene			**5.6 ± 0.04**		0.1 ± 0.02
3	8.5	Myrcene				1.0 ± 0.09	
4	8.6	*β*-Pinene			0.7 ± 0.17	0.6 ± 0.05	
5	9.5	*p*-Cymene				1.1 ± 0.18	0.1 ± 0.03
6	9.8	*β*-ocimene			**1.4 ± 0.13**	0.5 ± 0.04	2.1 ± 0.20
7	10.1	*(Z)*-Sabinene hydrate				0.3 ± 0.04	
8	11.1	*(E)*-Sabinene hydrate					1.3 ± 0.12
9	11.5	*iso*-Pentyl isovalerate					0.5 ± 0.05
10	12.9	*trans*-Pinocamphone			0.4 ± 0.04	1.7 ± 0.12	1.0 ± 0.09
11	13.9	*n*-Dodecane	1.1 ± 0.16	**8.7 ± 0.47**	**56.1 ± 0.41**	**14.2 ± 0.17**	**8.9 ± 0.29**
12	14.3	*β*-citronellol				0.3 ± 0.02	
13	14.5	*endo*-Fenchyl acetate			1.7 ± 0.11		
14	15.1	Carvacrol, methyl ether				2.1 ± 0.11	
15	17.2	*δ*-Elemene	**7.2 ± 0.23**	2.7 ± 0.12	2.3 ± 0.14	0.6 ± 0.05	0.2 ± 0.02
16	17.7	*α*-Cubebene				1.6 ± 0.11	
17	17.8	2-Undecanol					
18	19.2	*α*-Elemene	0.9 ± 0.11			4.8 ± 0.15	
19	19.6	*Z*-Caryophyllene	1.4 ± 0.17	3.4 ± 0.23	0.3 ± 0.02		
20	20.4	*E*-Caryophyllene	1.1 ± 0.16	1.7 ± 0.15	0.7 ± 0.06	0.8 ± 0.07	
21	20.6	Dehydroaromadendrane	0.6 ± 0.05	1.0 ± 0.09	0.9 ± 0.08	2.6 ± 0.14	
22	20.7	*ar*-Curcumene					
23	20.8	*α*-selinene	3.2 ± 0.34				
24	21	*γ*-muurolene	1.5 ± 0.11	4.6 ± 0.24	1.0 ± 0.11	0.5 ± 0.08	
25	21.4	*(Z)*-Farnesene	0.5 ± 0.04		0.5 ± 0.05	0.7 ± 0.07	
26	21.6	Germacrene D	1.3 ± 0.14	1.5 ± 0.18	0.6 ± 0.04	1.2 ± 0.21	
27	21.9	2-Pentadecanol				0.7 ± 0.12	
28	22	*(E)*- *β* -Ionone	2.6 ± 0.19	1.1 ± 0.11	0.5 ± 0.06		
29	22.2	Bicyclogermacrene	**5.4 ± 0.24**	1.7 ± 0.12	1.1 ± 0.09	4.3 ± 0.17	
30	22.4	*β*-Bisabolene	**5.2 ± 0.24**	3.3 ± 0.21	0.5 ± 0.04	**7.0± 0.19**	0.1 ± 0.09
31	22.7	*(Z)-α*-Bisabolene			1.0± 0.10		
32	22.8	Cuparene	**8.2 ± 0.29**	1.8 ± 0.22	1.8 ± 0.12	4.6 ± 0.12	0.1 ± 0.08
33	22.9	Elemicin					0.2 ± 0.14
34	23	*(E)*-Nerolidol					0.1 ± 0.07
35	23.2	Caryophyllene oxide	1.1 ± 0.1				
36	23.4	Tridecanol	1.0 ± 0.09	1.5 ± 0.15	1.8 ± 0.12		0.2 ± 0.02
37	23.6	*ar*-dihydro Turmerone	0.7 ± 0.07	4.0 ± 0.23	0.9 ± 0.08	2.1 ± 0.14	0.2 ± 0.01
38	23.8	Cedrol	1.2 ± 0.11	1.4 ± 0.21			
39	23.9	Junenol	1.5 ± 0.12	1.0 ± 0.09	0.3 ± 0.04	0.7 ± 0.08	
40	24.1	*γ*-Eudesmol			0.7 ± 0.05		
41	24.2	*α*-Muurolol	1.1 ± 0.1		0.5 ± 0.04	11.5 ± 0.14	4.1 ± 0.24
42	24.3	Cubenol	**5.9 ± 0.24**	4.2 ± 0.27	0.9 ± 0.09	0.9 ± 0.06	0.1 ± 0.01
43	24.7	*(6Z)*-Pentadecen-2-one	4.6 ± 0.21	1.6 ± 0.21	0.5 ± 0.03		0.1 ± 0.02
44	25.2	Pentadecanal	0.9 ± 0.08				1.9 ± 0.14
45	25.3	*(2Z,6Z)*-Farnesal	1.9 ± 0.14	1.0 ± 0.19	0.9 ± 0.08	0.2 ± 0.03	0.3 ± 0.04
46	25.6	*α*-Bisabolol					

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Table 3. Chemical composition (% relative abundance) of leaf (L) essential oils of *D. ammoniacum* (Continued)

No	RT	Components	LJ %	LSh %	LG %	LKr %	LB %
47	25.7	*(2Z,6Z)*-Farnesol	0.7 ± 0.08	2.2 ± 0.20	0.8 ± 0.07	2.2 ± 0.21	1.3 ± 0.14
48	26	*(2E,6E)*-Farnesol	1.8 ± 0.12	1.6 ± 0.17	1.5 ± 0.14		2.2 ± 0.20
49	26.5	*n*-Hexadecanol	**13.0 ± 0.55**	7.7 ± 0.42	4.7 ± 0.2	4.4 ± 0.21	1.0 ± 0.09
50	26.7	di-*n*-butyl phthalate	**6.6 ± 0.24**				
51	26.9	Hexadecanoic acid		4.2 ± 0.25			
52	27.2	*n*-Eicosane	**6.6 ± 0.24**	3.4 ± 0.21	0.5 ± 0.04	1.5 ± 0.14	0.5 ± 0.04
53	28.2	*n*-Octadecanol					0.8 ± 0.06
54	28.4	*n*-Heneicosane					
55	28.5	Ethyl linoleate					2.7 ± 0.11
56	28.9	(*E*)-Phytol acetate	2.1 ± 0.14	1.9 ± 0.17	0.4 ± 0.03		
57	29	*n*-Tricosane	1.7 ± 0.15	3.9 ± 0.29	0.3 ± 0.02		
58	30.2	*n*-Tetracosane	1.0 ± 0.12				
59	41.6	Heptacosane	0.8 ± 0.09	**20.9 ± 0.75**	1.4 ± 0.09	**17.3 ± 0.14**	**51.7 ± 0.47**
		Total compounds	**95.6**	**92.0**	**93.2**	**92.0**	**92.7**

Table 3. Chemical composition (% relative abundance) of leaf (L) essential oils of *D. ammoniacum* (Continued)

No	RT	Components	LKa %	LBr %	LBa %	LMe %	LNe %	RI* (Kovats index)
1	7.8	*α*-Pinene				0.2 ± 0.02	0.1 ± 0.04	932
2	8.1	Sabinene		0.5 ± 0.04		0.3 ± 0.03		961
3	8.5	Myrcene						991
4	8.6	*β*-Pinene		0.2 ± 0.02				974
5	9.5	*p*-Cymene	0.4 ± 0.03	2.1 ± 0.17	0.8 ± 0.16	1.2 ± 0.16	0.9 ± 0.09	1024
6	9.8	*β*-ocimene				0.8 ± 0.10	0.4 ± 0.03	1032
7	10.1	*(Z)*-Sabinene hydrate		0.1 ± 0.09	0.3 ± 0.02			1065
8	11.1	*(E)*-Sabinene hydrate						1086
9	11.5	*iso*-Pentyl isovalerate	0.7 ± 0.09	1.7 ± 0.12		0.5 ± 0.03	1.3 ± 0.12	1103
10	12.9	*trans*-Pinocamphone						1158
11	13.9	*n*-Dodecane	**21.7 ± 0.22**	3.1 ± 0.42	**19.4 ± 0.32**	2.7 ± 0.22	2.4 ± 0.11	1200
12	14.3	*β*-citronellol						1200
13	14.5	*endo*-Fenchyl acetate		1.3 ± 0.17				1218
14	15.1	Carvacrol, methyl ether		2.4 ± 0.23	0.5 ± 0.03	1.0 ± 0.09	0.2 ± 0.01	1241
15	17.2	*δ*-Elemene	1.0 ± 0.09	**15.2 ± 0.45**	1.0 ± 0.09	2.8 ± 0.15	**12.2 ± 0.32**	1335
16	17.7	*α*-Cubebene						1345
17	17.8	2-Undecanol	1.2 ± 0.08		1.2 ± 0.13		0.5 ± 0.03	1366
18	19.2	*α*-Elemene		0.1 ± 0.02				1389
19	19.6	*Z*-Caryophyllene	0.7 ± 0.06		0.7 ± 0.07	2.7 ± 0.23		1408
20	20.4	*E*-Caryophyllene	0.6 ± 0.04		0.6 ± 0.05		0.5 ± 0.04	1417
21	20.6	Dehydroaromadendrane			1.2± 0.14	0.4 ± 0.03		1460
22	20.7	*ar*-Curcumene		1.2 ± 0.17		2.0 ± 0.21		1475
23	20.8	α-selinene						1476
24	21	γ-muurolene	0.5 ± 0.04	1.8 ± 0.20	1.1 ± 0.08			1478
25	21.4	(*Z*)-Farnesene		3.4 ± 0.11			0.2 ± 0.02	1481
26	21.6	Germacrene D				0.2 ± 0.03	0.4 ± 0.04	1484
27	21.9	2-Pentadecanol		**5.4 ± 0.24**			4.9 ± 0.31	-
28	22	(*E*)- β -Ionone						1490
29	22.2	Bicyclogermacrene		2.1 ± 0.14		7.5 ± 0.11		1502
30	22.4	β-Bisabolene	0.6 ± 0.05	1.1 ± 0.09	3.4 ± 0.09	**6.8 ± 0.22**	4.3 ± 0.20	1505
31	22.7	(*Z*)-α-Bisabolene				1.8± 0.09		1506
32	22.8	Cuparene	1.6 ± 0.14	4.3 ± 0.27	0.4 ± 0.01	1.9 ± 0.5	2.3 ± 0.21	1506
33	22.9	Elemicin						1560
34	23	(*E*)-Nerolidol		2.2 ± 0.09			1.1 ± 0.10	1561
35	23.2	Caryophyllene oxide						1567
36	23.4	Tridecanol		0.3 ± 0.01			0.4 ± 0.04	1570
37	23.6	*ar*-dihydro Turmerone	0.5 ± 0.04	1.8 ± 0.10	0.5 ± 0.04			1595
38	23.8	Cedrol		0.3 ± 0.03				1600
39	23.9	Junenol			0.4 ± 0.02	0.5 ± 0.04		1618
40	24.1	*γ*-Eudesmol				0.4 ± 0.02	0.8 ± 0.07	1630
41	24.2	*α*-Muurolol	0.3 ± 0.02		0.3 ± 0.02		0.2 ± 0.02	1644
42	24.3	Cubenol	1.6 ± 0.14		1.0 ± 0.09	1.2 ± 0.14		1645
43	24.7	*(6Z)*-Pentadecen-2-one	2.4 ± 0.24	3.1 ± 0.11	2.4 ± 0.14	**7.1 ± 0.34**	3.4 ± 0.21	1667
44	25.2	Pentadecanal	1.2 ± 0.21	1.3 ± 0.09				1682

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Table 3. Chemical composition (% relative abundance) of leaf (L) essential oils of *D. ammoniacum* (Continued)

No	RT	Components	LKa %	LBr %	LBa %	LMe %	LNe %	RI* (Kovats index)
45	25.3	(2*Z*,6*Z*)-Farnesal	2.1 ± 0.21	3.0 ± 0.22	2.1 ± 0.11	1.9 ± 0.11	1.4 ± 0.15	1684
46	25.6	α-Bisabolol		1.5 ± 0.11				1685
47	25.7	(2*Z*,6*Z*)-Farnesol	**7.6 ± 0.27**		**5.4 ± 0.23**		**8.3 ± 0.11**	1698
48	26	(2*E*,6*E*)-Farnesol	4.2 ± 0.22	3.1 ± 0.14	**6.2 ± 0.25**			1742
49	26.5	*n*-Hexadecanol	8.2 ± 0.41	**10.3 ± 0.24**	8.7 ± 0.37	2.5 ± 0.20	**13.4 ± 0.24**	1874
50	26.7	di-*n*-butyl phthalate				2.2± 0.17		1906
51	26.9	Hexadecanoic acid						1959
52	27.2	*n*-Eicosane		1.7 ± 0.12		**6.3 ± 0.24**	**7.7 ± 0.17**	2000
53	28.2	*n*-Octadecanol						2077
54	28.4	*n*-Heneicosane		1.8 ± 0.09				2100
55	28.5	Ethyl linoleate						-
56	28.9	(*E*)-Phytol acetate		**5.1 ± 0.22**		4.6 ± 0.14	**5.1 ± 0.14**	2218
57	29	*n*-Tricosane						2300
58	30.2	*n*-Tetracosane		1.1 ± 0.09			0.4 ± 0.04	2400
59	41.6	Heptacosane	**35.2 ± 0.31**	**10.3 ± 0.14**	**32.7 ± 0.34**	**31.2 ± 0.25**	**17.8 ± 0.16**	2700
		Total compounds	**90.7**	**92.9**	**90.3**	**90.7**	**90.6**	

* RI: retention indices according to the normal alkanes between C8-C24. The bold type face means the compounds have the highest value.
LJ: Leaves of Jiroft, LSh: Leaves of Shahrood, LG: Leaves of Garmsar, LKr: Leaves of Kerend, LB: Leaves of Birjand, LKa: Leaves of Kashmar, LBr: Leaves of Bardaskan, LBa: Leaves of Bafq, LMe: Leaves of Mehriz, LNe: Leaves of Neyriz
Y: Data are mean ± SE.

Table 4. Chemical composition (% relative abundance) of gum essential oils of *D. ammoniacum*

No	RT	Essential oil component	Gum %	RI* (Kovats index)
1	7.8	*α*-Pinene	0.9 ± 0.05 [y]	932
2	9.8	*β*-ocimene	0.9 ± 0.08	1032
3	10.1	*(Z)*-Sabinene hydrate	0.6 ± 0.10	1065
4	11.1	*(E)*-Sabinene hydrate	0.8 ± 0.16	1086
5	15.1	Carvacrol, methyl ether	2.2 ± 0.20	1241
6	16.9	Thymol	2.3 ± 0.21	1289
7	19.2	*α*-Elemene	3.2 ± 0.25	1389
8	19.6	*Z*-Caryophyllene	0.8 ± 0.07	1408
9	20.4	*E*-Caryophyllene	0.9 ± 0.08	1417
10	20.6	Dehydroaromadendrane	1.0 ± 0.09	1460
11	20.7	*ar*-Curcumene	0.8 ± 0.07	1475
12	21	*γ*-muurolene	1.1 ± 0.09	1478
13	21.4	*(Z)*-Farnesene	0.8 ± 0.07	1481
14	21.6	Germacrene D	0.6 ± 0.05	1484
15	22	*(E)*- β -Ionone	1.3 ± 0.02	1490
16	22.2	Bicyclogermacrene	4.3 ± 0.04	1502
17	22.4	*β*-Bisabolene	**6.1 ± 0.19**	1505
18	22.8	Cuparene	**11.5 ± 0.25**	1508
19	22.9	Elemicin	1.1 ± 0.08	1560
20	23	*(E)*-Nerolidol	3.2 ± 0.21	1561
21	23.2	Caryophyllene oxide	1.4 ± 0.10	1567
22	23.4	Tridecanol	0.4 ± 0.04	1570
23	23.6	*ar*-dihydro Turmerone	1.6 ± 0.12	1595
24	23.9	Junenol	0.7 ± 0.06	1618
25	24.2	*α*-Muurolol	2.3 ± 0.18	1644
26	24.3	Cubenol	1.7 ± 0.09	1645

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Table 4. Chemical composition (% relative abundance) of gum essential oils of *D. ammoniacum* (Continued)

No	RT	Essential oil component	Gum %	RI* (Kovats index)
27	24.7	(6*Z*)-Pentadecen-2-one	4.0 ± 0.03	1667
28	25.2	Pentadecanal	1.8 ± 0.08	1682
29	25.3	(2*Z*,6*Z*)-Farnesal	1.4 ± 0.07	1684
30	25.7	(2*Z*,6*Z*)-Farnesol	**8.7 ± 0.24**	1698
31	26	(2*E*,6*E*)-Farnesol	**12.2 ± 0.28**	1742
32	26.3	*n*-Pentadecanol	1.8 ± 0.09	1773
33	26.5	*n*-Hexadecanol	2.5 ± 0.16	1874
34	28.2	*n*-Octadecanol	0.4 ± 0.03	2077
35	28.9	(*E*)-Phytol acetate	3.3 ± 0.02	2218
36	32	Hexacosane	0.4 ± 0.03	2600
		Total compounds	**89.0**	

* RI: retention indices according to the normal alkanes between C8-C24. The bold type face means the compounds have the highest value. y: Data are mean ± SE.

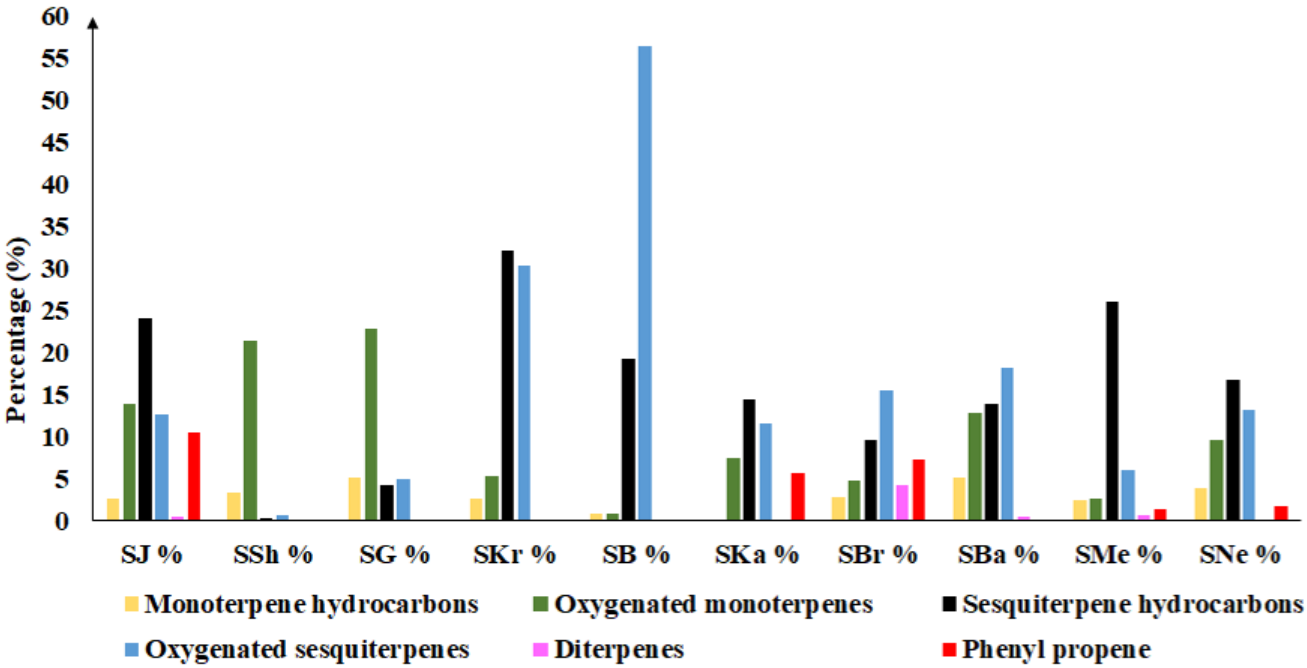

Fig. 1. Chemical groups of the essential oils compositions from stems of *D. ammoniacum*. SJ: Stem of Jiroft, SSh: Stem of Shahrood, SG: Stem of Garmsar, SKr: Stem of Kerend, SB: Stem of Birjand, SKa: Stem of Kashmar, SBr: Stem of Bardaskan, SBa: Stem of Bafq, SMe: Stem of Mehriz, SNe: Stem of Neyriz

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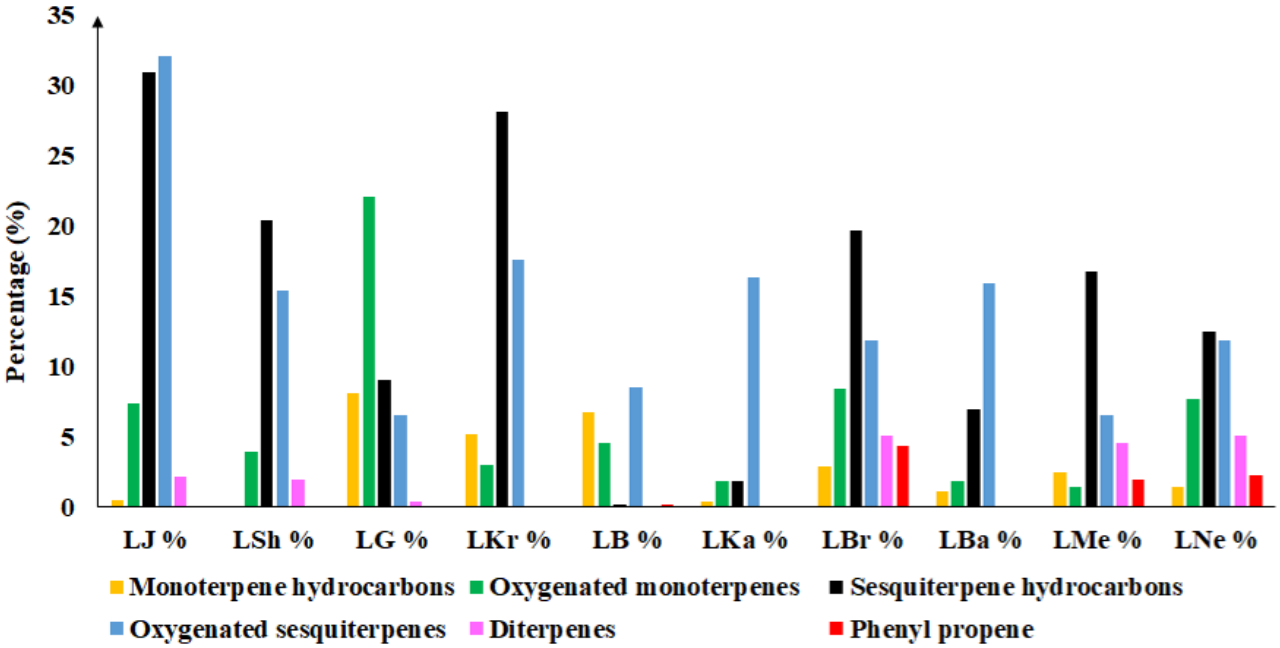

Fig. 2. Chemical groups of the essential oils compositions from leaves of *D. ammoniacum*. LJ: Leaves of Jiroft, LSh: Leaves of Shahrood, LG: Leaves of Garmsar, LKr: Leaves of Kerend, LB: Leaves of Birjand, LKa: Leaves of Kashmar, LBr: Leaves of Bardaskan, LBa: Leaves of Bafq, LMe: Leaves of Mehriz, LNe: Leaves of Neyriz

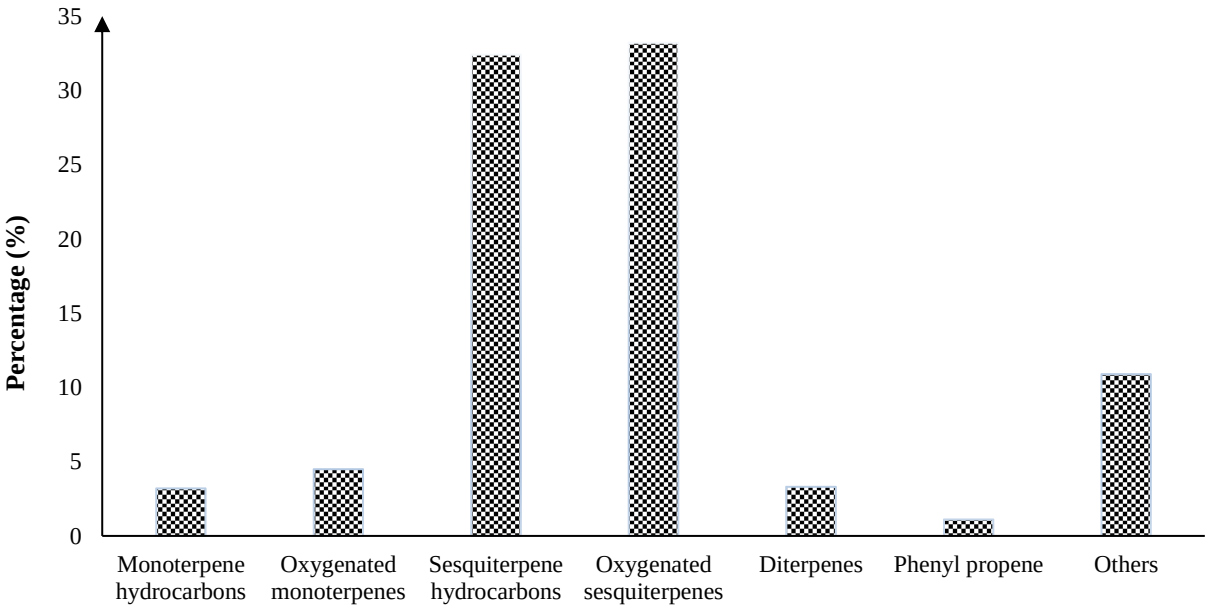

Fig. 3. Chemical groups of the essential oils compositions of *D. ammoniacum* gum.

3.2. Total Tannin Content (TTC)

The extracts from the stems and leaves of *D. ammoniacum* populations with regards to the tested TTC content were significantly different. The highest amount of total tannin was found in the samples that were designated as LBr, LBi and LNe with 1.3 and 1.2 mg TA/g DW, respectively (Fig. 4) whereas the lowest amount

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of total tannin those samples designated as SJ, SGa, SKa and SKr and the TTC values were recorded as 0.4 mg TA/g DW. As Fig. 4 shows, the amount of TTC in the studied samples shows high levels of variation and high diversity. This is the first report about tannin contents in different organ of *D. ammoniacum*.

3.3. Saponins

The analysis of variance showed that there was a significant difference ($P \leq 0.01$) between the extracts from the stems and leaves of *D. ammoniacum* populations as the highest level of saponin was contained in the SBa (stem of Bafq) with 0.13 % (Fig. 5). Also, l sample LBi (leaf of Birjand) has low levels of saponins and similarly to the tannin content, this is the first report about saponins being variable in different organs of *D. ammoniacum*.

3.4. Antioxidant Activity (DPPH) and (FRAP)

The results show that the methanolic extracts of LNe and LMe with 8.7 % and 8.6 % (W/W) had the highest yield and the lowest yield of extraction also was in KrS (1.0 % W/W). Generally, the extracts of leaves showed the maximum yield. In the present work, the antioxidant activity of the samples was evaluated using the DPPH and FRAP methods. Significant differences were noted between the extracts from the stems and leaves of *D. ammoniacum* populations in antioxidant activity ($P \leq 0.01$). The results of comparison of antioxidant activity have been demonstrated in Figure 6. In DPPH assay, the highest radical scavenging activity (lowest IC_{50}) was observed in the SBi (Stem of Birjand) and LJ (leaf of Jiroft) samples with an IC_{50} of 70.3 µg/ml and 79.9 µg/ml compared to BHT (26.0 µg/ml), a synthetic industrial antioxidant, respectively. The lowest activity (IC_{50} 300 µg/ml) was associated with the SKr (Stem of Kerend) samples. However, for the FRAP activity, samples LJ and LBa were higher than all the other samples and, the amount of antioxidant activity varied from 45.1 to 10.0 (Fig. 6A).

3.5. Total phenolic and flavonoid contents

The results showed that there was a significant difference ($P \leq 0.01$) between the all extracts of *D. ammoniacum* in total phenolic and flavonoid content. The stems extract of the Birjand, Neyriz (SBi, SNe) and LBa (leaf of Bafq) had the highest total phenolic content (19.3, 18.7 and 18.4 mg GAE/g DW extract; Fig. 7A). On the contratry, the LKr (leaves of Kerend) had a low total phenolic content with 8.3 mg GAE/g DW. Out of the test materials, the highest flavonoid content was recorded for the SGa samples (stem of Garmsar) and this was at a value of 11.8 mg QE/g (Fig. 7B). On the other hand, LGa (leaf of Garmsar) exhibited the lowest levels of TFC (4.1 mg QE/g DW).

3.6. Correlation between Phytochemical Properties and Antioxidant Activity

In this study, the correlation between phytochemical properties and antioxidant activity was investigated (Table 5). The results showed that there is a positive and significant correlation between the TPC (0.38 and 0.46) with antioxidant activity (DPPH and FRAP). However, the correlation between total antioxidant activity and TFC of *D. ammoniacum* was not significant.

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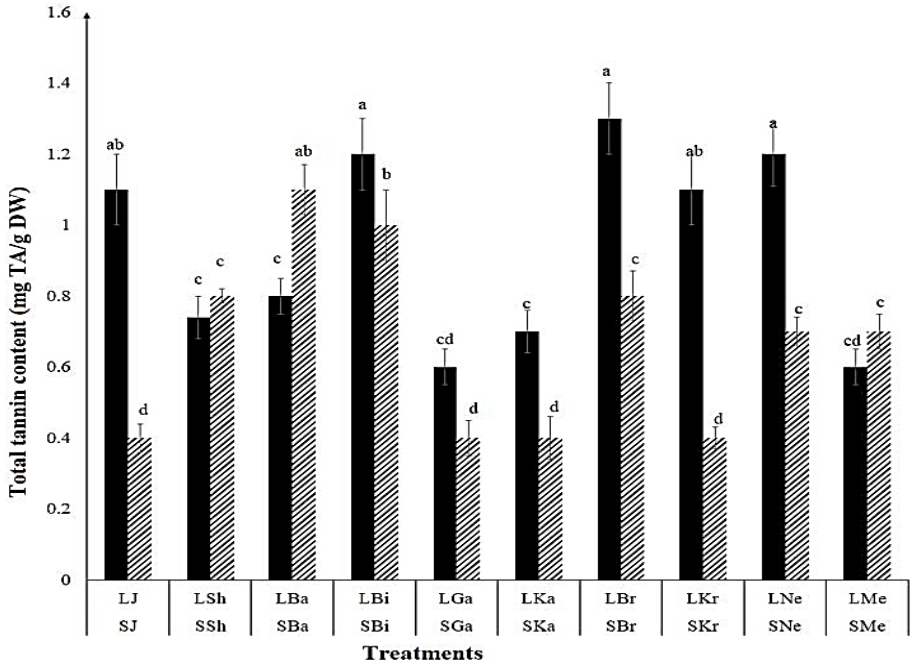

Fig. 4. Mean comparison of TTC (total tannin content; mg TA/g DW) of *D. ammoniacum* methanolic extracts. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$).

Black bars: SJ: Stem of Jiroft, SSh: Stem of Shahrood, SG: Stem of Garmsar, SKr: Stem of Kerend, SB: Stem of Birjand, SKa: Stem of Kashmar, SBr: Stem of Bardaskan, SBa: Stem of Bafq, SMe: Stem of Mehriz, SNe: Stem of Neyriz

Pattern fill bars: LJ: Leaves of Jiroft, LSh: Leaves of Shahrood, LG: Leaves of Garmsar, LKr: Leaves of Kerend, LB: Leaves of Birjand, LKa: Leaves of Kashmar, LBr: Leaves of Bardaskan, LBa: Leaves of Bafq, LME: Leaves of Mehriz, LNe: Leaves of Neyriz

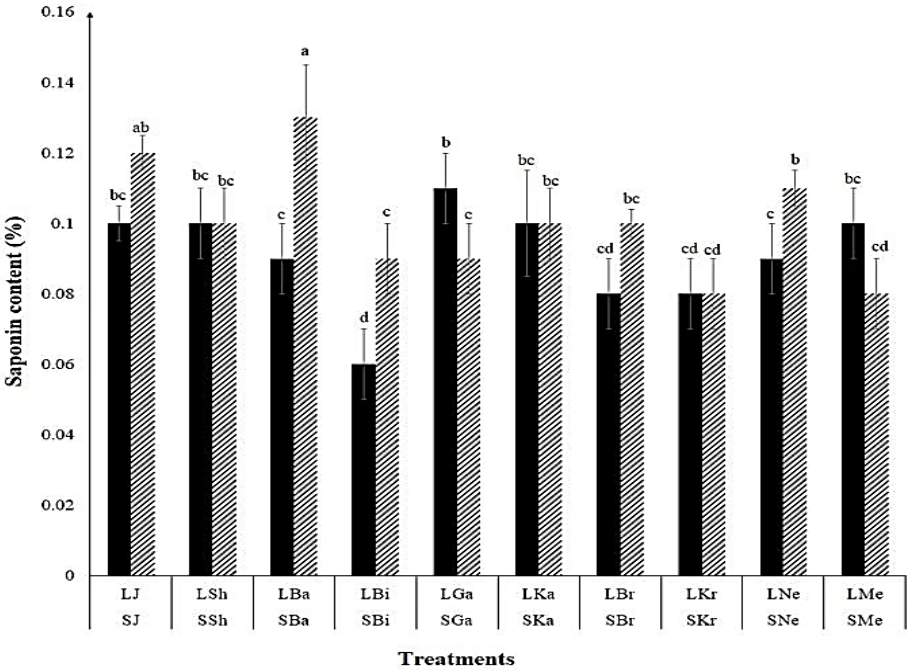

Fig. 5. Mean comparison of Saponin content (%) of all *D. ammoniacum* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$).

Black bars: SJ: Stem of Jiroft, SSh: Stem of Shahrood, SG: Stem of Garmsar, SKr: Stem of Kerend, SB: Stem of Birjand, SKa: Stem of Kashmar, SBr: Stem of Bardaskan, SBa: Stem of Bafq, SMe: Stem of Mehriz, SNe: Stem of Neyriz

Pattern fill bars: LJ: Leaves of Jiroft, LSh: Leaves of Shahrood, LG: Leaves of Garmsar, LKr: Leaves of Kerend, LB: Leaves of Birjand, LKa: Leaves of Kashmar, LBr: Leaves of Bardaskan, LBa: Leaves of Bafq, LME: Leaves of Mehriz, LNe: Leaves of Neyriz

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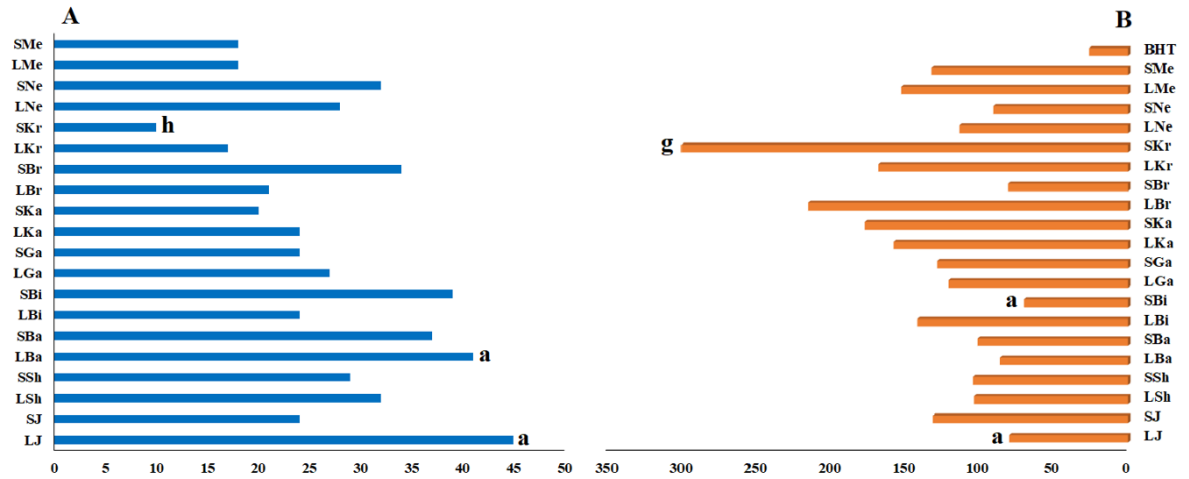

Fig. 6. Mean comparison of antioxidant activity by FRAP assay; mg Fe++/g DW (A: FRAP) and antioxidant activity by DPPH assay; IC_{50} (B: DPPH) of all *D. ammoniacum* extracts. Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$).

SJ: Stem of Jiroft, SSh: Stem of Shahrood, SG: Stem of Garmsar, SKr: Stem of Kerend, SB: Stem of Birjand, SKa: Stem of Kashmar, SBr: Stem of Bardaskan, SBa: Stem of Bafq, SMe: Stem of Mehriz, SNe: Stem of Neyriz

LJ: Leaves of Jiroft, LSh: Leaves of Shahrood, LG: Leaves of Garmsar, LKr: Leaves of Kerend, LB: Leaves of Birjand, LKa: Leaves of Kashmar, LBr: Leaves of Bardaskan, LBa: Leaves of Bafq, LMe: Leaves of Mehriz, LNe: Leaves of Neyriz

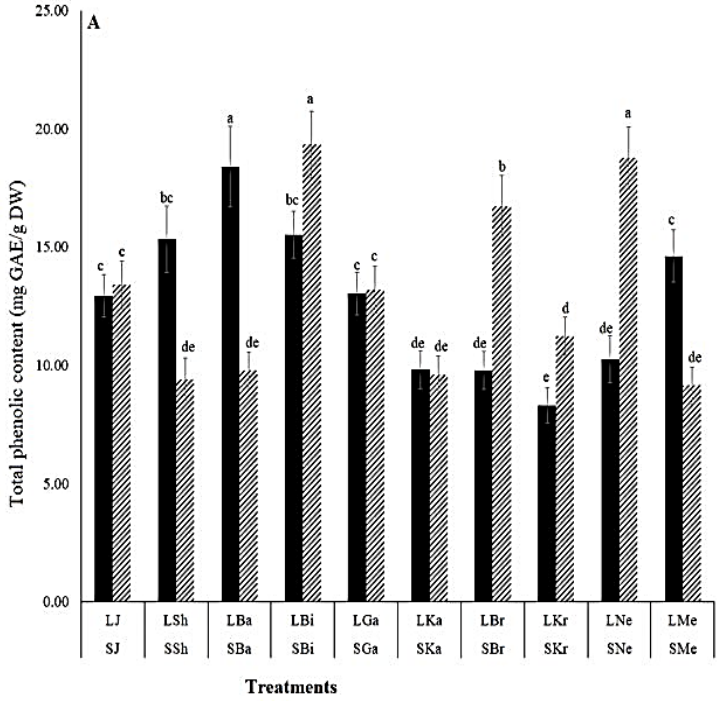

Fig. 7A. Mean comparison of phytochemical traits of methanolic extract of *D. ammoniacum*. A: TPC (total phenolic content; mg GAE/g DW) Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$).

Black bars: SJ: Stem of Jiroft, SSh: Stem of Shahrood, SG: Stem of Garmsar, SKr: Stem of Kerend, SB: Stem of Birjand, SKa: Stem of Kashmar, SBr: Stem of Bardaskan, SBa: Stem of Bafq, SMe: Stem of Mehriz, SNe: Stem of Neyriz

Pattern fill bars: LJ: Leaves of Jiroft, LSh: Leaves of Shahrood, LG: Leaves of Garmsar, LKr: Leaves of Kerend, LB: Leaves of Birjand, LKa: Leaves of Kashmar, LBr: Leaves of Bardaskan, LBa: Leaves of Bafq, LMe: Leaves of Mehriz, LNe: Leaves of Neyriz

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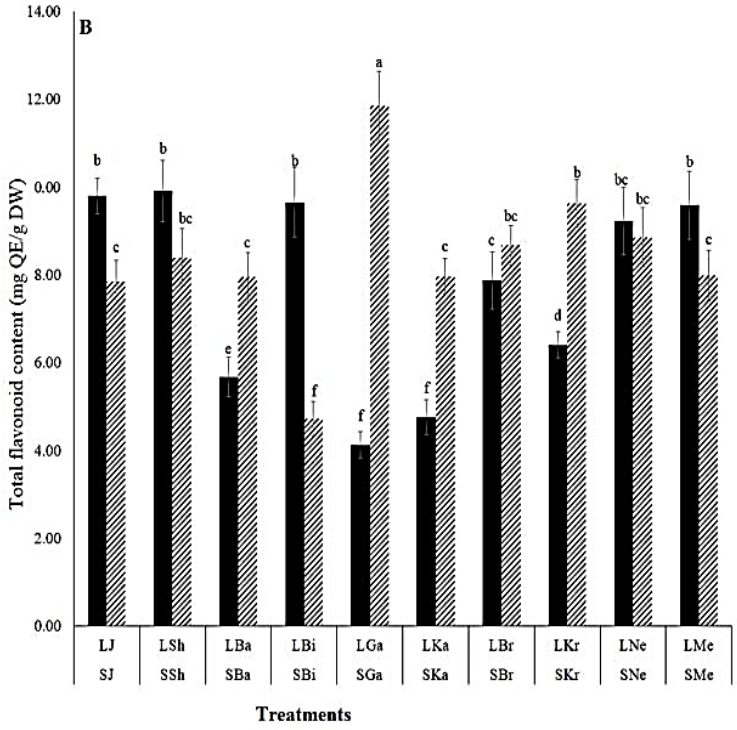

Fig. 7B. Mean comparison of phytochemical traits of methanolic extract of *D. ammoniacum*. B: TFC (total flavonoid content; mg QE/g DW). Different letters indicate statistical significance based on least significant difference (LSD) test ($P < 0.05$).

Black bars: SJ: Stem of Jiroft, SSh: Stem of Shahrood, SG: Stem of Garmsar, SKr: Stem of Kerend, SB: Stem of Birjand, SKa: Stem of Kashmar, SBr: Stem of Bardaskan, SBa: Stem of Bafq, SMe: Stem of Mehriz, SNe: Stem of Neyriz

Pattern fill bars: LJ: Leaves of Jiroft, LSh: Leaves of Shahrood, LG: Leaves of Garmsar, LKr: Leaves of Kerend, LB: Leaves of Birjand, LKa: Leaves of Kashmar, LBr: Leaves of Bardaskan, LBa: Leaves of Bafq, LMe: Leaves of Mehriz, LNe: Leaves of Neyriz

Table 5. Correlation between six main traits on studied *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

Traits	Tannin	Saponin	TPC	TFC	DPPH	FRAP
Tannin	1					
Saponin	**0.28***	1				
TPC	0.04^{ns}	0.02^{ns}	1			
TFC	0.09^{ns}	-0.05^{ns}	0.09^{ns}	1		
DPPH	0.2^{ns}	0.12^{ns}	**0.38***	0.18^{ns}	1	
FRAP	0.08^{ns}	0.22^{ns}	**0.46***	**0.27***	**0.88****	1

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

4. Discussion

Hosseini et al [22] reported the EO content of leaves of *D. aucheri* from several areas were 0.67 and 2.6 w/w %, respectively. There are several previous studies indicate the essential oil content can vary amongst different plant organs such as the Apiaceae family, including *Oliveria decumbens*, *Trachyspermum ammi*,

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Echinophora tenuifolia and *Heracleum persicum* [21]. In comparison, Hosseini et. al [22] reported the EO content of stem and fruit of *D. ammoniacum* were 0.3 and 0.5 v/w%. Also, the EO yields of areal part and root of *D. ammoniacum* were 0.2 and 0.3 v/w %, respectively [23].

β-bisabolene as a main component in leaf and gum of *D. ammoniacum* is used in personal care products and citrus flavors found in beverages [24]. It has a balsamic aroma and is approved as a food additive in Europe [25]. It has also been shown to exhibit cytotoxicity in breast cancer cell lines, both in vitro and in vivo [26]. Cuparene was one of the most dominant essential oil constituents in the leaf and gum oils [27]. In comparison, the chemical component of the essential oil from *Dorema Glabrum* also revealed δ-cadinene (12.77 %) as the main compound of the root that is followed by *β*-bisabolene (7.48 %), *α*-fenchyl acetate (6.32 %), and copaene (5.68 %) [28]. However, in previous phytochemical studies on the EOs of *D. ammoniacum*, Takalloa et. al [29] reported the major components in stem oil to be *δ*-cadinene (16.24 %), liguloxide (8.69 %) and *δ*-amorphene (8.43 %). Otherwise, Yousefzadi et. al [30] 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. [22] indicated that the most important components in EOs of stem were hexadecanal (11.1 %), α-cadinol (6.6 %), sesquicineol-2-one (6.6 %), ethyl linoleate (6.3 %), ledol (5.1 %) and *γ*-eudesmol (4.4 %). The volatile components of *D. ammoniacum* consisted of mainly sesquiterpene hydrocarbons and oxygenated sesquiterpenes. This result is consistent with previous reports [4, 11]. The change in the essential oil components is also influenced by factors such as the age and the development stage of medicinal plants. Soil type, nutrient availability, pH, and other edaphic factors can affect the uptake of essential oil precursors and the subsequent biosynthesis of these compounds [21]. The reasons for these differences can be due to many factors such as genetic diversity, climate, soils, location and time of sampling, insect and microorganisms stress, and other geological and environmental conditions. Temperature, precipitation, sunlight exposure, and other climatic variables can influence the synthesis and concentration of essential oil components. Changes in these environmental factors can lead to variations in the plant's metabolic processes and essential oil profiles. [31, 32]. In fact, the amount of tannin in the leaf was more than the stem. This result is not necessarily surprising as tannins as the formation and accumulation of these tannins generally occur in young, and actively growing leaves [33]. Tannins are categorized as hydrolyzable or condensed tannins, depending on their chemical structure. Therefore, The environmental factors such as light stress and shading, atmospheric change (CO_2, N_2, O_2, and O_3), temperature (day and night), exogenous plant hormone (abscisic acid, naphthaleneacetic acid, and ethylene), infection of pathogens (bacterial and fungal), solar radiation, nitrogen, water, and phosphorus deficiency [34] may affect the tannin biosynthesis.

In contrast to triterpenoid saponins, steroid saponins are common in plants used as medicinal herbs or that are exploited for their health-promoting properties [35]. Saponins have been reported to have a vast range of pharmacological and medicinal activities and are thus indicated as usually have low oral toxicity in humans [36]. The presence of saponin in plants have been reported to be responsible for the tonic

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and stimulating activities observed in various medical herbs [16].

Previous studies of the hydroalcoholic extracts from *D. aitchisonii* aerial parts and ethanolic extracts of *D. aucheri* aerial parts confirmed that these species had weak antioxidant activity based on the DPPH assay as IC_{50} values of 488 and 200 µg/mL, respectively [37, 38]. As a radical scavenging investigation (DPPH) on several species of the Apiaceae (*Falcaria vulgaris*, *Smyrniopsis aucheri*, *Smyrniopsis munzurdagensis*, *Smyrnium cordifolium*, and *Actinolema macrolema*), Zengin et al. [39] reported the highest radical scavenging activity was observed in *Smyrnium cordifolium* methanolic extract with 59.2 mg TE/g extract and *Smyrniopsis munzurdagensis* extract showed low antioxidant activity with 2.29 mg TE/g. The difference in antioxidant capacity among the samples of various populations could be attributed to the differences in their polyphenolic compounds.

This paper presents the first recorded data on the phenolic and flavonoid contents in different organs of *D. ammoniacum*. The study conducted by Nazir et. al [2] reported the total phenolic content and flavonoid content of *D. ammoniacum* aerial parts (extracted with methanol) from Pakistan as 68.2 mg GAE/g and 66.97 mg QE/g, respectively. Also, the total phenols ranged from 52 ± 7 to 67 ± 2 mg/g was showed in D. aucheri extracts [2]. Furthermore, the ferric reducing antioxidant power (FRAP) was also found to be maximum in the stem and flower Phenolics and flavonoids are known for their antioxidant properties and their ability to scavenge free radicals implicated in various diseases [2]. The composition of polyphenol compounds in plant extracts can vary depending on factors such as defense mechanisms against threats, oxidative stress, and environmental conditions [41]. Abiotic factors, such as temperature, light intensity, water availability, and soil characteristics, can modulate the plants' metabolic pathways and the production of polyphenols [41]. There is generally a significant correlation between total phenolic content and antioxidant activity in plants. For example, *H. officinalis*, *P. oleracea*, and *O. vulgare* have shown high antioxidant capacity despite having comparable phenolic content [42]. The presence of antioxidants, including phenolic and flavonoid compounds, indicates the potential for various valuable bioactive resources. Exploring and identifying these compounds can lead to the discovery of traditional medicines and remedies for critical diseases [43].

5. Conclusion

This study highlights the variations in the essential oil content and chemical compositions of *D. ammoniacum* samples across different ecosystems, underscoring the significance of selecting chemotypes with potentially higher pharmacological activity from natural populations. Furthermore, the essential oil composition of the commercial gum closely resembled the leaf-derived essential oil of *D. ammoniacum*, suggesting the feasibility of substituting leaf-derived oil for gum. Also, *D. ammoniacum* had a moderate content of phenol and flavonoid and antioxidant capacity. It is recommended to expand the cultivation of this valuable plant in pastures to compensate for the excessive harvesting of the plant.

Author contribution

Conceptualization and Supervision: M. A; Methodology: M. N; Investigation, Writing - original draft: M. N; Writing - review and editing: All authors; Data collection: M. N and

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M-T. E; Data analysis: All authors; Funding acquisition and Resources: M. A.

Conflicts of interest

The authors declared no conflict of interest.

Acknowledgment

The authors would like to thank the Iran National Science Foundation (Grant Number: 98002070) and Tarbiat Modares University for the financial support of this research.

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How to cite this article: Norani M, Ebadi M-T, Ayyari M. Volatile compounds variation and phytochemicals of leaf and stem of *Dorema ammoniacum* D. Don. Wild populations. ***Journal of Medicinal Plants*** 2024; 23(91): 42-63.
doi: 10.61186/jmp.23.91.42

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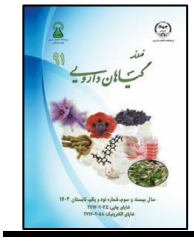

مقاله تحقیقاتی

تغییرات ترکیبات اسانس‌ها و ترکیبات فیتوشیمیایی برگ و ساقه جمعیت‌های طبیعی وشا *Dorema ammoniacum* D. Don

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اطلاعات مقاله

گل‌واژگان:
ترکیبات فرار
وشا
کروماتوگرافی گازی
فعالیت آنتی‌اکسیدانی
β-bisabolene

چکیده

مقدمه: وشا گیاه دارویی چند ساله مهم از خانواده Apiaceae می‌باشد. **هدف:** هدف ما مقایسه اسانس (EOs) و فعالیت آنتی‌اکسیدانی همراه با سایر خواص فیتوشیمیایی *Dorema ammoniacum* با منشاء مختلف بود. **روش بررسی:** در این تحقیق برگ و ساقه ۱۰ جمعیت گیاه وشا از مناطق مختلف ایران شامل جیرفت، شاهرود، گرمسار، کرند، بیرجند، کاشمر، بردسکن، بافق، مهریز و نیریز جمع آوری گردید. محتویات و ترکیبات اسانس بدست آمده با تکنیک‌های کروماتوگرافی گازی مورد بررسی قرار گرفت و با اسانس صمغ-رزین تجاری مقایسه شد. **نتایج:** بازده اسانس به ترتیب ۰/۲-۰/۳ (ساقه)، ۰/۲-۰/۳ (برگ) و ۱/۰ (صمغ) درصد وزنی-حجمی بود. ترکیبات اسانس اندام‌های مختلف تفاوت‌هایی را نشان دادند که با اندام گیاه مرتبط بود، اما شباهت‌هایی در پروفایل اسانس نیز به وضوح مشاهده شد. ترکیبات اصلی در اسانس صمغ وشا (2*E*,6*E*)-farnesol، cuparene-(2*Z*,6*Z*)، farnesol و β-bisabolene بودند. *Endo*-fenchyl acetate ، *p*-cymen-8-ol و β-bisabolene به عنوان ترکیبات مهم در اسانس ساقه شناسایی شد. ترکیبات اصلی اسانس برگ‌ها شامل dodecane، heptacosane، *n*- cuparene و β-bisabolene بودند. نتایج تجزیه واریانس خصوصیات فیتوشیمیایی نشان داد که در بین تمام عصاره‌های وشا از نظر فعالیت آنتی‌اکسیدانی، محتوای تانن کل، ساپونین، محتوای فنل کل و محتوای فلاونوئید کل ($P \leq 0.01$) تفاوت معنی‌داری وجود دارد. **نتیجه‌گیری:** ترکیبات اسانس صمغ *D. ammoniacum* شباهت زیادی به اسانس برگ نشان داد. مطالعه حاضر اطلاعات جدیدی در مورد AA، TTC، Saponin، TPC و TFC وشا ارائه کرد و این عصاره‌ها دارای محتوای متوسط فنل و فلاونوئید و ظرفیت آنتی‌اکسیدانی بودند.

مخففها: AA، فعالیت آنتی‌اکسیدانی؛ DPPH، دی فنیل هیدرازیل هیدرات؛ EO، اسانس؛ RSC، رادیکال آزاد؛ FRAP، آنتی‌اکسیدان احیاکننده آهن؛ LSD، حداقل اختلاف معنی‌دار؛ TPC، محتوای فنل کل؛ TFC، محتوای فلاونویید کل

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تاریخ دریافت: ۲۱ بهمن ۱۴۰۲؛ **تاریخ دریافت اصلاحات:** ۳۰ مهر ۱۴۰۳؛ **تاریخ پذیرش:** ۸ آبان ۱۴۰۳

doi: 10.61186/jmp.23.91.42

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