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Original article

Antioxidant and antibacterial activities of the essential oils and extracts of *Dorema ammoniacum* roots and aerial parts

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Abstract

Background and objectives*: Dorema ammoniacum* D. Don (Apiaceae) is a monocarpic plant endemic to central Iran. The gum resin of this species is a known therapeutic agent in Iranian, Indian and Western traditional medicines. The aim of the present study was to investigate essential oil constituents and evaluation of antioxidant and antibacterial activities of the essential oils and extracts of *D. ammoniacum* aerial parts and roots. **Methods**: Essential oils were analyzed using GC and GC/MS. The oils together with *n*-hexane, chloroform, ethyl acetate and methanol extracts of the plant samples were subjected to antioxidant evaluation by DPPH and FRAP assays and antibacterial screening using disk diffusion and micro-well dilution methods. **Results:** Thirty-four compounds were identified in the aerial parts oil, among them β-himachalene (9.3%) and β-chamigrene (8.7%) were the main constituents. Thirty-five compounds were also characterized in the roots oil, of which β-bisabolene (15.1%) and hexadecanal (13.2%) were the main components. Ethyl acetate extract of the roots showed the highest antioxidant activity in both DPPH (IC_{50} 21.3 ± 2.7 μg/mL) and FRAP (112.7 ± 8.1 mmol $FeSO_4.7H_2O$ equivalent/g) assays. In antibacterial assay, the ethyl acetate and chloroform extracts of the roots exhibited strong antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Essential oils were also found to be active against *Shigella dysenteriae*. **Conclusion**: Considerable antioxidant and antibacterial activities of *D. ammoniacum* make it an appropriate candidate for further studies and identification of its bioactive principles.

Keywords*:* antibacterial, antioxidant, Apiaceae, essential oil, *Dorema ammoniacum*

Introduction

The genuse *Dorema* D. Don belonging to the Apiaceae family is represented by seven species in flora of Iran, among them *D. ammoniacum* and *D. aucheri* are endemic [1]. *D. ammoniacum* is a perennial monocarpic plant growing up to 2.5 m in height in arid and semi-arid regions of central Iran [1]. This species exudes a medicinal gum resin, commonly known as "Ushaq" or "Vasha" in Iranian Traditional Medicine and also as "Persian ammoniacum" in Greek and Latin medicinal literatures [2-4].

The Persian ammoniacum is an ancient therapeutic agent described by "Abu-Mansur Movafagh Heravi" and "Isaac Judaeus" in 10th century [3,4]. In Iranian folk medicine, this gum resin has been considered useful in treatment of

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spastic pains, gastric disorders, intestinal parasitic infections and skin inflammations and as analgesic, stimulant, expectorant and laxative [2,5-7]. It has also been traditionally used in Western and Indian medicines as antispasmodic, expectorant, diaphoretic and emmenagogue and also for treatment of catarrh, asthma, chronic bronchitis and persistent coughing [8,9].

D. ammoniacum gum resin has been reported to exhibit some bioactivities including antibacterial, antifungal and acetylcholinesterase (AChE) inhibitory effects [10-12]. Free salicylic acid, ammoresinol, dshamirone and some sesquiterpene chromandiones (e.g. ammodoremin and doremin A) have also been isolated and identified from Persian ammoniacum [11-13]. There are also some reports about the essential oils obtained from *D. ammoniacum* [14-16]. The fruits oil of this species has been found to possess potent cytotoxic activity on SW-480 and MCF-7 cells, two human cancerous cell lines, as well as high antimicrobial effects against *Bacillus subtilis* and *Staphylococcus epidermidis* [14,15]. Moreover, it has been reported to contain ocimenone stereoisomers (40.4%) and β-cyclocitral (9.9%) as the main compounds [15]. A previous study on the essential oil of *D. ammoniacum* leaves has also introduced the it be a sesquiterpene rich oil (90.2%) which contained high amounts of α-gurjunene (49.5%) and β-gurjunene (19.0%) [16].

The present study was designed to identify the essential oil composition and also evaluation of the antioxidant and antibacterial potentials of the essential oils and extracts obtained from the aerial parts and roots of *D. ammoniacum*. To the best of our knowledge, this is the first report on the antioxidant and antibacterial properties of *D. ammoniacum* aerial parts and roots.

Experimental

Plant material

The aerial parts and roots of *D. ammoniacum* were collected from Kashan region (Isfahan province, center of Iran) at its flowering stage in June 2012. The voucher specimen of the authenticated plant (voucher no. KBGH 1890) was deposited in the Herbarium of the Kashan Research Botanical Garden, Research Institute of Forests and Rangelands, Kashan, Iran.

Extraction

The air-dried and ground aerial parts and roots (100 g each) were individually macerated with *n*-hexane, chloroform, ethyl acetate and methanol (3×0.5 L each), successively at room temperature. All of the extracts were concentrated by a rotary evaporator below 45 °C.

Essential oil extraction

Essential oils were obtained from air-dried and comminuted aerial parts and roots (100 g each) by hydrodistillation using a Clevenger-type apparatus for 4 h. The oils were then dried over anhydrous sodium sulfate and stored in umber glasses at 4 °C until analysis.

GC and GC/MS analysis

The essential oils were analyzed on a Hewlett-Packard 6890 gas chromatograph with HP-5MS column (30 m × 0.25 mm *id*, 0.25 μm film thickness) equipped with a mass detector (Hewlett-Packard model 5973 HP). The flow rate of the carrier gas (Helium) was 1 mL/min. The initial oven temperature was 40 °C and was raised at a rate of 3 °C per minute to 250 °C. The injection temperature was 250 °C and the oil samples (1 μL) were injected with a split ratio of 1:90. The mass spectra were obtained by electron ionization at 70 eV. and the retention indices (RI) of the compounds were calculated using a homologous series of *n*-alkanes injected in conditions equal to the samples.

Identification of compounds was carried out using computer matching with the wiley7n.l library, and also by comparison of the retention indices and fragmentation pattern of the mass spectra with those published in the literature for standard compounds [17].

An Agilent HP-6890 gas chromatograph coupled with a FID detector was also applied for essential oils analysis to achieve relative amounts of the

separated compounds. The FID detector temperature was 290 °C and the operation was performed under the same conditions as described for GC/MS analyses.

Antioxidant activity

DPPH free radical scavenging assay

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) method was used to assess the free radical-scavenging potential of the essential oils and extracts [18]. Briefly, 2 mL of sample solution (10 mg/mL in methanol) was serially diluted with methanol to reach concentrations ranging from 0.5 to 3.1×10^{-2} mg/mL. 2 mL of DPPH solution (80 µg/mL in methanol) was then added to the diluted solutions and they were kept 30 min at the room temperature in the dark. UV absorptions were then recorded at 517 nm. Vitamin E was used as the positive control. The test was performed in triplicate and the IC_{50} values were reported as means ± SD.

FRAP assay

The reducing powers of the extracts were determined using ferric reducing antioxidant power (FRAP) method [19]. FRAP reagent was freshly prepared by mixing 5 mL of $FeCl_3$ solution (20 mM) and 5 mL of 2,4,6-tripyridyl-*s*-triazine solution (10 mM in HCl (40 mM)) with 50 mL of acetate buffer (0.3 M, pH 3.6). 100 µL of sample solution (500 µg/mL in methanol) was added to 3 mL of FRAP reagent and the mixture was incubated at 37 °C for 10 min. The absorption of the solution was then recorded at 593 nm. Ferrous sulfate solutions ($FeSO_4.7H_2O$) in the range of 125-750 µM were used as the standard for drawing a calibration curve. Vitamin E was used as the positive control. The test was performed in triplicate and the results were expressed as mmol $FeSO_4.7H_2O$ equivalent per gram of extract (mmol FSE/g).

Antibacterial activity

Bacterial strains

Potential antibacterial activity of the extracts and essential oils were individually evaluated against a set of 7 bacteria, including *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (PTCC 1188) and *Salmonella paratyphi* A (ATCC 5702). Bacterial strains were provided from Iranian Research Organization for Science and Technology (IROST) and were cultured in nutrient agar (NA) overnight at 37 °C.

Disk diffusion assay

Antibacterial activities of the essential oils and extracts were evaluated using agar disc diffusion method [20,21]. Extracts solutions were prepared at the concentration of 30 mg/mL using DMSO as the solvent and were filtered through 0.45 µm Millipore filters for sterilization. 100 µL of suspension containing 10^8 CFU/mL of bacteria was spread on nutrient agar (NA). The impregnated discs (6 mm in diameter) with 10 µL of the oils or extract solutions (300 µg/disc) and DMSO (as negative control) were placed on the inoculated agar. All plates were incubated at 37 °C for 24 h and the diameters of inhibition zones (mm) were measured. Gentamicin (10 µg/disc) and rifampin (5 µg/disc) were used as the positive controls and each assay was repeated twice. The antibacterial activity was categorized as weak, moderate, or strong depending on the diameter of the growth inhibition zone (<10, 10–15, and >15 mm, respectively).

Micro-well dilution assay

Minimal inhibition concentration (MIC) values of the samples were determined using micro-well dilution assay, in the case of bacterial strains sensitive to examined samples in disc diffusion assay [20,21]. The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts and oils samples were dissolved in 10% DMSO (500 µg/mL) and serial two-fold dilutions were made in a concentration range of 7.8-500 µg/mL in 10 mL

sterile test tubes containing brain heart infusion (BHI) broth. The 96-well plates were prepared by dispensing 95 µL of the cultures media and 5 µL of the inoculums into each well. A 100 µL aliquot from the stock solutions of the samples at the concentration of 500 µg/mL was added into the first well. 100 µL from their serial dilutions were then transferred into six consecutive wells. The last well containing 195 µL of the cultures media without the test materials was used as the negative control. The final volume in each well was 200 µL. Gentamicin and rifampin were also used as the positive controls. Contents of each well were mixed on the plate shaker at 300 rpm for 20 s and then incubated at appropriate temperature for 24 h. Bacterial growth was determined by the presence of a white pellet on the well bottom and was confirmed by plating 5 µL samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the plant samples required for inhibiting the growth of each microorganism. All tests were repeated twice.

Results and Discussion

Essential oil composition

The hydrodistillation of *D. ammoniacum* aerial parts gave yellowish oil with a yield of 0.2% (v/w), on dry weight basis. Thirty-four compounds representing 90.3% of the oil were identified as a result of GC and GC/MS analysis of the aerial parts essential oil (table 1). The results showed that the oil was rich in hydrocarbon sesquiterpenes (35.0%) and oxygenated non-terpenes (28.7%) with β-himachalene (9.3%) and β-chamigrene (8.7%) as the main compounds. Three diterpenes, namely neophytadiene (1.6%), neocembren (3.0%) and phytol (3.5%) were also characterized in aerial parts oil. An earlier study on *D. ammoniacum* leaves has reported β-himachalene and neophytadiene in the essential oil with the relative percentages of 1.9% and 3.7%, respectively. Gurjunene and ocimenone stereoisomers, were reported as the main compounds of the leaves and fruits oils, however, were not detected in the aerial parts oil [15,16].

Hydrodistillation of the roots afforded pale yellow oil (yield 0.3% (v/w)). GC and GC/MS analysis of the obtained oil led to the identification of thirty-five compounds, of which β-bisabolene (15.1%), hexadecanal (13.2%) and (E)-nerolidol (11.3%) were the most abundant components (table 2).

Table 1. Chemical composition of the essential oil of *D. ammoniacum* aerial parts

No.	Compounds[a]	RI[b]	%
1	α-pinene	955	0.5
2	methyl heptenone	989	1.5
4	cumene	1012	1.4
5	β-cymene	1015	0.6
6	γ-terpinolene	1057	0.4
7	linalool	1090	0.2
8	nonanol	1140	1.3
9	camphor	1146	0.2
10	(Z)-2-nonenal	1150	0.3
11	β-citronellol	1208	0.5
12	β-damascenone	1370	0.3
13	α-cedrene	1395	2.6
14	cedr-8[15]-ene	1419	4.5
15	thujopsene	1429	1.5
16	nerylacetone	1455	4.0
17	caryophyllene	1440	3.3
18	α-selinene	1475	1.9
19	β-chamigrene	1495	8.7
20	β-himachalene	1498	9.3
21	α-bisabolene	1504	3.2
22	nerolidol	1517	1.7
23	α-trans-sesquicyclogeraniol	1580	5.7
24	(Z,E)-farnesal	1706	3.0
25	pentadecanal	1715	6.9
26	Dodecyl methacrylate	1756	2.7
27	17-octadecenal	1818	4.2
28	13-tetradecenal	1823	2.5
29	neophytadiene	1830	1.6
30	neocembren	1918	3.0
31	n-hexadecanoic acid	1957	3.9
32	Phytol	2016	3.5
33	(Z,Z,Z)-9,12,15-Octadecatrien-1-ol	2056	3.7
34	oleic acid	2115	1.7
	Hydrocarbon monoterpenes		1.5
	Oxygenated monoterpenes		5.2
	Hydrocarbon sesquiterpenes		35.0
	Oxygenated sesquiterpenes		10.4
	Hydrocarbon diterpenes		4.6
	Oxygenated diterpenes		3.5
	Hydrocarbon non-terpenes		1.4
	Oxygenated non-terpenes		28.7
	Total identified		90.3

[a]Identified compounds listed in order of elution from HP-5MS column. [b]Relative retention indices to C_8-C_{24} *n*-alkanes on HP-5MS column.

The results showed that the essential oil was dominated by the presence of oxygenated non-terpenes (54.0%), mainly hexadecanal (13.2%), tetradecanal (10.5%) and 1,15-hexadecadiene (7.1%). Essential oil of *D. glabrum* roots has previously been reported to contain δ-cadinene (12.8%), β-bisabolene (7.5%) and α-fenchyl acetate (6.3%) as the main compounds [22]. Non-terpene compounds have been reported up to 12.1% in essential oil of *D. glabrum* roots, whereas this group of constituents were characterized at higher levels (62.1%) in the essential oil of *D. ammoniacum* roots [22]. Beside genetic diversity, some extrinsic factors such as climate, soil properties, insect and microorganisms stress, time of harvesting, plant preparation procedure and method of essential oil extraction, could have a role in chemical composition of the essential oils [23].

Table 2. Chemical composition of the essential oil of *D. ammoniacum* roots

No.	Compounds[a]	RI[b]	%
1	camphene	946	0.3
2	pseudocumol	976	0.4
4	p-cimene	1007	0.4
5	γ-terpinen	1051	0.2
6	menthol	1140	0.5
7	fenchyl acetate	1229	0.2
8	methyl carvacrol	1241	1.0
9	dodecanal	1388	0.5
10	p-cymene, 2,5-dimethoxy	1399	0.9
11	(E)-β-farnesene	1454	0.8
12	α-farnesene	1495	1.0
13	β-bisabolene	1505	15.1
16	(E)-nerolidol	1563	11.3
14	1,15-hexadecadiene	1581	7.1
15	(Z)-asarone	1599	2.5
17	tetradecanal	1618	10.5
20	(E)-2-tetradecen-1-ol	1670	7.5
21	1-heptadecene	1680	0.3
22	pentadecanal	1714	3.7
23	2-pentadecanone	1717	2.0
24	hexadecanal	1785	13.2
25	tetradecanoic acid	1794	5.1
26	13-tetradecenal	1823	2.3
28	hexadecyl-oxirane	1900	0.9
30	n- hexadecanoic acid	1954	1.7
31	methyl linoleate	2095	2.2
32	oleic acid methyl ester	2098	0.3
33	heneicosane	2100	0.3
34	linoleic acid	2132	1.3
35	oleic acid	2141	0.3
	Hydrocarbon monoterpenes		0.9
	Oxygenated monoterpenes		2.6
	Hydrocarbon sesquiterpenes		16.9
	Oxygenated sesquiterpenes		11.3
	Hydrocarbon non-terpenes		8.1
	Oxygenated non-terpenes		54.0
	Total identified		93.8

[a]Identified compounds listed in order of elution from HP-5MS column. [b]Relative retention indices to C_8-C_{24} n-alkanes on HP-5MS column.

Antioxidant activity

The results of DPPH free radical-scavenging and FRAP assays have been demonstrated in table 3. In DPPH assay, ethyl acetate and chloroform extracts of the roots along with the ethyl acetate extract of the aerial parts were found to have the highest free radical-scavenging activity with IC_{50} values of 21.3±2.7, 31.8±4.1 and 62.7±3.0 μg/mL, respectively, compared to vitamin E (IC_{50} 14.3±2.2 μg/ml). Ethyl acetate extracts of both plant samples also exhibited the highest ferric reducing power in FRAP assay with 112.7±8.1 and 54.0±6.2 mmol FSE/g, respectively, in comparison with vitamin E (127.8±6.3 mmol FSE/g). It has been previously reported that hydroalcoholic extract of *D. aitchisonii* and ethanolic extract of *D. aucheri* aerial parts have possessed a weak free radical-scavenging activity in DPPH assay with IC_{50} values of 488 and 200 μg/mL, respectively [24,25].

Table 3. Antioxidant activity of the extracts and essential oils of *D. ammoniacum* aerial parts and roots

Samples (Extracts and positive control)		DPPH assay (IC_{50} μg/ml)	FRAP assay (mmol $FeSO_4$S. $7H_2O$/g)
Aerial parts	*n*-Hexane	277.1±9.1	10.2±1.7
	Chloroform	134.5±6.2	35.4±2.8
	Ethyl acetate	62.7±3.0	54.0±6.2
	Methanol	146.3±3.8	12.5±3.1
	Essential oil	2134±17.6	0.2±0.08
Roots	*n*-Hexane	303.1±11.3	0.7±0.06
	Chloroform	31.8±4.1	16.5±2.3
	Ethyl acetate	21.3±2.7	112.7±8.1
	Methanol	106.1±5.3	20.73±4.2
	Essential oil	1887±11.8	0.3±0.02
Vitamin E		14.3±2.2	127.8±6.3

Although chemical constituents of the roots and aerial parts of this species have not been investigated yet, presence of phenolic compounds such as sesquiterpene coumarins/phenols, flavaonoids and phloroacetophenone glycosides reported from the other *Dorema* species might be associated with appearance of noticeable antioxidant activity of the mentioned extracts from *D. ammoniacum* aerial parts and roots [26-29]. Essential oils of the aerial parts and roots, however, exhibited the least antioxidant activity in both DPPH and FRAP assays (table 3). A previous report on free radical scavenging activity of the essential oil of *D. glabrum* roots has also reported the essential oil as a weak antioxidant in DPPH free radical-scavenging assay (IC_{50} 2.2 mg/mL).

Antibacterial activity

In antibacterial activity assay, as shown in table 4, ethyl acetate and chloroform extracts of the roots exhibited strong antibacterial activity toward *B. subtilis*, *P. aeruginosa* and *S. aureus*. Both ethyl acetate and chloroform extracts of the aerial parts inhibited the growth of *P. aeruginosa*, strongly. The former fractions also showed strong and moderate antibacterial activity against *B. subtilis*, respectively. Essential oil samples exhibited antibacterial activity against *S. dysenteriae*, and activity of the root oil was found higher than gentamicin (table 4). Previous studies about the antibacterial activity of *D. ammoniacum* gum resin have reported it to be a potent antimicrobial agent with a broad spectrum activity especially against some gram-positive bacteria, including *B. subtilis, B. cereus, B. pumilus, S. epidermidis, S. aureus, Micrococcus luteus* and *Streptococcus faecalis* [10,11].

Table 4. Antibacterial activity of the extracts and essential oils obtained from aerial parts and roots of *D. ammoniacum*

	Aerial parts					Roots					Antibiotics	
	Hex.[a]	Chl.[b]	Et.[c]	Met.[d]	E. oil[e]	Hex.	Chl.	Et.	Met.	E. oil	Rif[f]	Gen[g]
Gram-negatives												
S. paratyphi	-	-	-	-	-	-	-	-	-	-	-	21[h] (500)[i]
P. aeruginosa	-	19 (500)	19 (250)		-		18 (500)	20 (250)		-	-	23 (500)
E. coli	-	-	-	-	-	-	-	-	-	-	11 (500)	20 (500)
Gram-positives												
B. subtilis	-	14 (500)	16 (250)	-	-	10 (500)	16 (500)	18 (250)	-	-	13 (15.5)	21 (500)
S. dysenteriae	-	-	-	-	10 (500)	-	-	-	-	17 (250)	8 (250)	18 (500)
S. aureus	14 (500)	14 (500)	10 (500)	-	-	10 (500)	19 (500)	18 (500)	-	-	10 (250)	21 (500)
S. epidermidis	-	-	-	-	-	-	-	10 (500)	-	-	40 (250)	35 (500)

Notes: -: no antimicrobial activity. [a]*n*-hexane extract, [b]chloroform extract, [c]ethyl acetate extract, [d]methanol extract, [e]essential oil, [f]rifampin (5 µg/disc), [g]gentamicin (10 µg/disc), [h]inhibition zone in diameter (mm) around the impregnated discs including diameter of the disc (6 mm) [weak activity (<10 mm), moderate activity (10–15 mm), strong activity (>15 mm)], [i]minimal inhibition concentrations (MIC) (as µg /mL).

Moreover, the essential oil of *D. ammoniacum* fruits has been found to possess strong antimicrobial activity on *B. subtilis*, *S. epidermidis* and *S. aureus* with inhibition zones 23, 22, 17 mm and MIC values 3.75, 3.75 and 7.5 mg/mL, respectively [15]. Regarding the observed correlation between antioxidant and antibacterial activity of the tested extracts, it could be assumed that these bioactivities are exerted by the same groups of phytochemical substances. However, more studies need to determine the compounds involved in antibacterial activity of *D. ammoniacum*.

The results of the essential oil analysis of the aerial parts and roots of *D. ammoniacum* introduce them as sources of hydrocarbon sesquiterpenes and oxygenated non-terpenes, respectively. Considerable antioxidant and antibacterial activities of the aerial parts and roots of this species also suggest this medicinal species as an appropriate candidate for further and more in-depth phytochemical and toxico-pharmacological studies for identification of the antioxidant and antibacterial principles and evaluation of their potential for medicinal purposes or food industries as natural preservatives.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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