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Composition and *in vitro* antimicrobial activity of the essential oil of *Dorema ammoniacum* D. Don. fruit from Iran

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Abstract: The genus *Dorema* (Apiaceae) is represented in the flora of Iran with seven species of which two, *D. ammoniacum* D. Don. and *D. aucheri* Boiss. are endemic. Ripe fruits of *D. ammoniacum* collected just in the deciduous time were subjected to hydrodistillation to yield the essential oil, which was subsequently analyzed by GC and GC–MS. Twenty-nine compounds were identified and quantified, representing 95.1 % of the total oil. (*Z*)-Ocimenone (22.3 %) and (*E*)-ocimenone (18.1 %) were the main components of the oil. *In vitro* antimicrobial activity of the oil was evaluated against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The results of the antimicrobial assay of the oil by the disc diffusion method and the MIC *values* indicated that the oil exhibited moderate to high antimicrobial activity, especially against *B. subtilis and S. epidermidis* with *MIC* value of 3.75 mg ml^{-1}.

Keywords: *Dorema ammoniacum*; Apiaceae; essential oil composition; antimicrobial activity; ocimenone; (*Z*)-ocimenone; (*E*)-ocimenone.

INTRODUCTION

Essential oils obtained from many plants have recently gained in popularity and scientific interest. Many plants are used for different purposes, such as food, drugs and perfumes. Researchers are interested in biologically active compounds isolated from plant species for the eradication of pathogenic microorganisms because of the resistance that microorganisms have acquired against antibiotics.[1]

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The genus *Dorema* D. Don. (Apiaceae) is represented in the flora of Iran by seven species, among which two are endemic, *D. ammoniacum* D. Don. and *D. aucheri* Boiss.[2,3] *D. ammoniacum*, a vulnerable species, grows to a height of about 1–2 m and in spring and early summer contains a milky juice. It is one of the most important endemic medicinal plants in many arid and semi-arid regions of Iran, such as the Yazd, Isfahan and Semnan provinces, which are known by the local Persian names of Kandal, Vasha and Koma-kandal.[2–4] *D. ammoniacum* produces a medicinal gum resin, commonly known as ammoniacum gum, which is found in cavities in stems, roots, and petioles.[5] The resin exudes from punctures in the stem, which can occur from insect attack. The resin serves as a carminative, diaphoretic, mild diuretic, expectorant, poultice, stimulant, antimicrobial, and vasodilator agent.[6] It is still used in Indian and Western Medicine and is listed in the British pharmacopoeia[4] as an antispasmodic and expectorant. It is occasionally used for chronic bronchitis and persistent coughs.[5] The antimicrobial activity of the dichloromethane–methanol (1:1) extract of the plant gum was previously reported.[7] A literature survey revealed that the essential oil compositions of *D. aucheri* aerial parts[8] and *D. ammoniacum* leaves[9] have already been reported. However, the chemical composition and antimicrobial activity of the essential oil of *D. ammoniacum* fruit has not hitherto been investigated, and hence the present study was focused on the possible uses of this oil in pharmacy and pathogenic systems.

EXPERIMENTAL

Plant material

Ripe fruits of *D. ammoniacum* were collected just in deciduous time from the Semnan Road toward Firoozkuh after Bashm Defile (35° 46’ 11” N, 52° 52’ 38” E and altitude of 1300 m), Semnan Province, Iran. A voucher specimen (AS-85406) was deposited at the Herbarium of Ecology and Systematic Department, Research Institute of Applied Science, Shahid Beheshti University, Tehran, Iran.

Isolation of the essential oil

Dried fruits of the plant (500 g) were hydrodistilled using a Clevenger type apparatus for 3 h according to the method recommended in the British Pharmacopoeia (1993).[10] The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until analyzed and tested.

GC and GC–MS analyses

The GC–FID analysis of the oil was conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml min^{-1}. The split ratio was 1.50. The oven temperature was increased from 60 to 250 °C at a rate of 5 °C min^{-1}. The injector and detector (FID) temperatures were maintained at 250 and 280 °C, respectively. The GC–MS analysis was performed on a Thermoquest-Finnigan Trace GC–MS instrument equipped with the same column and temperature programming as given for the GC analysis. The transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of

1.1 ml min^{-1} with a split ratio of 1/50. The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C6–C24) and the oil on a DB-5 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or of authentic compounds and confirmed by comparison of their retention indices with those of authentic compounds or with those reported in the literature.[11] Semi-quantitative data was obtained from the FID area percentages without the use of correction factors.

Microbial strains

Ten microbial strains were used which included; *Bacillus subtilis* (ATCC 465), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 85327), *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763).

Antimicrobial screening

The antimicrobial activity of essential oil was determined by the disk diffusion method.[12] Briefly, 0.1 ml of a suspension of the test microorganism (10^8 cells ml^{-1}) was spread on Mueller–Hinton Agar plates for bacteria and Sabouraud Dextrose Agar for the fungi. Sterile 6 mm disks, each containing 10 µl of essential oil were placed on the microbial lawns. The plates were incubated at 37 °C for 24 h for the bacteria and at 30 °C for 48 h for the fungi. The diameters of the zones of inhibition were measured and are reported in mm. Triplicate tests were performed in all experiments.

*Determination of minimum inhibitory concentration (*MIC*)*

The *MIC* values were determined by the broth microdilution assay.[13] Serial two-fold dilutions of the essential oil were made in Mueller–Hinton Broth containing 0.5 % Tween 80 for the bacteria and Sabouraud Dextrose Broth with 0.5 % Tween 80 for the fungi in 96-well micro titer plates. Fresh microbial suspensions prepared from overnight-grown cultures in the same media were added to give a final concentration of 5×10^5 organisms ml^{-1}. Controls of medium with microorganisms or the essential oil alone were included. The microplates were incubated at 37 °C for 24 h for the bacteria and 30 °C for 48 h for the fungi. The first dilution with no microbial growth was recorded as the *MIC*.

RESULTS AND DISCUSSION

Essential oil analysis

The hydrodistillation of *D. ammoniacum* fruits gave a yellow oil in 0.09 % (w/w) yield, based on the dry weight of the fruit. Twenty-nine components were identified representing 95.1 % of the total oil. The qualitative and quantitative essential oil compositions are presented in Table I, in which the compounds are listed in order of their elution on the DB-5 column. The major constituents of the oil were (*Z*)-ocimenone (22.3 %), (*E*)-ocimenone (18.1 %) and *β*-cyclocitral (9.9 %). (*Z*)- and (*E*)-ocimenone were previously reported as the main compounds of the essential oil of *Ferula latisecta* (Apiaceae).[14] The classification of the identified compounds based on the major groups is summarized at the end of Table I and shows that oxygenated monoterpenes (58.4 %) were the main group of com-

pounds. In an earlier investigation on the essential oil composition of *D. ammoniacum* leaves,[9] α-gurjunene (49.5 %), β-gurjunene (19.0 %) and α-selinene (4.6 %) were found to be the main constituents while, in the present study, (*Z*)- and (*E*)-ocimenone, β-cyclocitral and *ar*-curcumene were characterized as the major components, which could be attributed to their ecological variability or plant part. Masoudi *et al*. reported α-eudesmol (31.2 %) and δ-cadinene (10.9 %) as the main components of the essential oil of *D. aucheri* aerial part.[8]

TABLE I. Essential oil composition of *D. ammoniacum* fruit (*RI*: retention indices relative to C6–C24 *n*-alkanes on a DB-5 column)

No.	Compound	*RI*	Content, %
1	1,3,8-*p*-Menthatriene	1119	0.5
2	(*E*)-Tagetone	1126	2.2
3	(*Z*)-Tagetone	1133	3.2
4	(*E*)-5-Undecen-3-yne	1163	0.7
5	*trans*-2-Caren-4-ol	1178	2.2
6	β-Cyclocitral	1189	9.9
7	(*Z*)-Ocimenone	1213	22.3
8	(*E*)-Ocimenone	1220	18.1
9	*p*-Mentha-1,8-diene	1246	0.5
10	Piperitenone oxide	1293	0.5
11	α-Cubebene	1356	0.4
12	α-Copaene	1385	3.2
13	β-Bourbonene	1395	4.1
14	Italicene	1414	1.0
15	Di-epi-α-Cedrene	1427	2.9
16	α-Longipinene	1430	0.9
17	β-Cedrene	1433	0.5
18	β-Barbatene	1457	3.0
19	α-Humulene	1462	1.1
20	*ar*-Curcumene	1475	6.4
21	(*Z*)-(*E*)-Farnesene	1481	0.6
22	Germacrene D	1487	3.0
23	Bicyclogermacrene	1502	0.9
24	Cuparene	1506	2.9
25	δ-Cadinene	1522	0.8
26	Spathulenol	1576	1.2
27	Caryophyllene oxide	1583	0.7
28	Heptadecanoic acid	2069	1.4
Monoterpene hydrocarbons		–	1
Oxygenated monoterpenes		–	58.4
Sesquiterpene hydrocarbons		–	31.7
Oxygenated sesquiterpenes		–	1.9
Others		–	2.1
Total identified		–	95.1

Antimicrobial activity

The essential oil of *D. ammoniacum* was tested against four Gram-positive and three Gram-negative bacteria, as well as three fungi. The results of antimicrobial activity of the essential oil according to the disc diffusion method and *MIC* values indicated that the essential oil has moderate to high inhibitory activity against the tested bacteria, except for two microorganisms, *P. aeruginosa* and *A. niger* (Table II). The most sensitive microorganisms were *B. subtilis*, *S. epidermidis* and *S. aureus* with inhibition zones of 23, 22, 17 mm and *MIC* values of 3.75, 3.75 and 7.5 mg ml^{-1}, respectively. Five microbial strains, *E. coli*, *E. faecalis*, *K. pneumoniae*, *S. cerevisiae* and *C. albicans* were found to be less sensitive to the oil. The obtained results showed that Gram-negative bacteria and fungi are more tolerant to the antimicrobial activity of the essential oil than Gram-positive bacteria.

TABLE II. Antimicrobial activity of the essential oil of *D. ammoniacum* fruit (values are given as means ± standard deviation)

Microorganism	Essential oil		Standard antibiotics					
			Tetracycline (30 μg disc^{-1})		Gentamicin (10 μg disc^{-1})		Nystatine (30 μg disc^{-1})	
	IZ[a]	*MIC*[b]	*IZ*	*MIC*	*IZ*	*MIC*	*IZ*	*MIC*
B. subtilis	23±0.2	3.75	21±0.8	3.2	–	nt	nt	nt
E. faecalis	13±0.4	15	9±0.4	6.4	–	nt	nt	nt
S. aureus	17±0.3	7.5	20±0.4	3.2	–	nt	nt	nt
S. epidermidis	22±0.8	3.75	34±0.8	1.6	–	nt	nt	nt
E. coli	14±0.2	15	–	nt	23±0.8	3.2	nt	nt
K. pneumoniae	12±0.4	15	–	nt	20±0.8	3.2	nt	nt
P. aeruginosa	–	nt	–	nt	12±0.4	6.4	nt	nt
A. niger	–	nt	nt	nt	nt	nt	16±0.4	6.4
C. albicans	10±0.4	>10	nt	nt	nt	nt	18±0.4	3.2
S. cerevisiae	11±0.2	>10	nt	nt	nt	nt	18±0.8	1.6

[a]Zone of inhibition (in mm) includes diameter of the disc (6 mm); [b]minimum inhibitory concentration values as mg ml^{-1}. (–) inactive; (7–13) moderately active; (> 14) highly active; nt, not tested

CONCLUSIONS

Chemical characterization and antimicrobial screening studies on plant-based essential oils could lead to the discovery of new natural antimicrobials. Although the antimicrobial activity of the dichloromethane–methanol (1:1) extract of the plant gum was previously studied,[7] the present study is the first report on the antimicrobial activity of the essential oil from the fruits of *D. ammoniacum*. The oil showed promising antimicrobial activity against *B. subtilis*, *S. epidermidis* and *S. aureus*. Rajani *et al*. reported that ammoniacum gum extract has excellent antimicrobial activity.[7] The present results also indicate that the essential oil of *D. ammoniacum* has antimicrobial activity; hence a scientific basis is

provided for the traditional use of *D. ammoniacum* for bronchitis, respiratory infections, fever, cold and flu.[7]

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И З В О Д

САСТАВ И АНТИМИКРОБНА АКТИВНОСТ ЕТАРСКОГ УЉА ПЛОДА *Dorema ammoniacum* D. DON. ИЗ ИРАНА

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Род *Dorema* (Apiaceae) у Ирану представља седам врста биљака, од којих су две ендемске: *D. ammoniacum* D. Don. и *D. aucheri* Boiss. Зрели плодови *D. ammoniacum* су подвргнути дестилацији воденом паром ради добијања етарског уља које је анализирано методама GC и GC–MS. Идентификовано је двадесет девет састојака који су чинили 95,1 % уља. (*Z*)-оцименон (22,3 %) и (*E*)-оцименон (18,1 %) су били главни састојци. *In vitro* антимикробна активност уља је тестирана спрам седам Грам-позитивних и Грам-негативних бактерија (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* и *Klebsiella pneumoniae*) и три гљиве (*Candida albicans*, *Saccharomyces cerevisiae* и *Aspergillus niger*). Метода дифузије на диску и *MIC* вредности су показали да уље има средњу до велику антимикробну активност, посебно спрам *B. subtilis* и *S. epidermidis*, уз *MIC* вредност од 3,75 mg ml^{-1}.

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REFERENCES

1. B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, *Food Chem*. **90** (2005) 333
2. V. Mozaffarian, *A Dictionary of Iranian Plant Names*, Farhang Moaser, Tehran, Iran, 1996, p. 190
3. V. Mozaffarian, *Bot. Zhurn. (Leningrad)* **88** (2003) 88
4. K. H. Rechinger, *Flora Iranica*. *Umbelliferae*, Akademische Druck- und Verlagsanstalt, Graz, Austria, 1980, p. 380
5. J. H. Langenheim, *Plant resins: chemistry, evolution, ecology, and ethnobotany*, Timber Press, Portland, OR, USA, 2003, p. 412
6. N. Irvani, M. Solouki, M. Omidi, A. Z. Zare, S. Shahnazi, *Plant Cell Tiss. Organ Cult.* **100** (2010) 293
7. M. Rajani, N. Saxena, M. N. Ravishankara, N. Desai, H. Padh, *Pharm. Biol*. **40** (2002) 534
8. S. Masoudi, A. Esmaeili, M. A. Khalilzadeh, A. Rustaiyan, N. Moazami, M. R. Akhgar, M. Varavipoor, *Flavour Fragr. J.* **21** (2006) 801
9. S. E. Sajjadi, N. Ghassemi, P. Mohammadzamani, *Revue des régions Arides* **1** (2007) 194
10. British pharmacopoeia, HMSO, London, UK, 1993

11. R. P. Adams, *Identification of essential oils components by gas chromatogramphy/quadrupole mass spectroscopy*, Allured Publishing Co., Carol Stream, IL, USA, 2001
12. National Committee for Clinical Laboratory Standards (NCCLS), *Performance standards for antimicrobial disk susceptibility test*, 6th ed., Approved Standard, M100-A6, Wayne, PA, USA, 1997
13. National Committee for Clinical Laboratory Standard (NCCLS), *Performance standards for antimicrobial susceptibility testing*, 9th International Supplement, M100-S9, Wayne, PA, USA, 1999
14. Z. Habibi, P. Salehi, M. Yousefi, Y. Hejazi, A. Laleh, V. Mozaffarian, S. Masoudi, A. Rustaiyan, *Chem. Nat. Compd*. **42** (2006) 689.