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Phytochemical investigation of the essential oils from the leaf and stem of *Dorema ammoniacum* D. Don. (Apiaceae) in Central Zagros, Iran

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ABSTRACT

Background & Aim: *Dorema ammoniacum* D. Don. (ammoniac plant) belongs to Apiaceae family and *Dorema* genus. It is one of the most important endemic medicinal plants in its family which grows in the arid and semi-arid areas in the regions of Iran and some of the Asian countries such as Turkmenistan, Afghanistan, Pakistan and India. This study was carried out with aim of the extraction and identification of constituents of *D. ammoniacum* D. Don. in the western areas of Isfahan province, Iran.
Experimental: For this purpose, after the plant collecting in the mentioned area its target organs namely stem and leaf were separated, air dried, milled and weighted in the certain amount. Then the essential oil of the plant was isolated by hydrodistillation method and analyzed by GC/MS device.
Results: The results indicated that major components were Limonene (49.18%), Neophytadiene (10.21%), beta Caryophyllene (3.54%), Phytol (2.61%) and Neryl acetone (1.46%).
Recommended applications/industries: Several therapeutic cases have been reported in traditional medicine and currently in contemporary medicine for *Dorema ammoniacum* D. Don. that some of them are include the treatment of cough, vasodilator, anti-flatulence and anti-microbial. Also, the mentioned plant has varied applications in the fields of foods, cosmetic and detergent industries.

1. Introduction

Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. They have a widespread range of application from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments (Worwood, 1991; Farhang *et al*., 2015). The Apiaceae family has been distributed with 300 genera and 3000 plant species in the world approximately. The genus *Dorema* D. Don (Apiaceae) is represented in the flora of Iran by seven species, among which both *D. ammoniacum* D. Don. and *D. aucheri* Boiss. are endemic (Mozaffarian, 1996). *Dorema ammoniacum* D. Don. (ammoniac plant) is a perennial monocarpic plant grows to height about 1–3 m and in spring and early summer contains a milky juice. It is one of the most important endemic medicinal plants

in its family which grows in the arid and semi-arid areas in the regions of Iran and some of the Asian countries such as Turkmenistan, Afghanistan, Pakistan and India (Rechinger, 1980). The mentioned species in the Iranian traditional medicinal has numerous names. Some of the most prevalent names include ushaq, vasha, kandal, koma-kandal and also as Persian ammuniacum in Greek and medicinal literatures (Amin, 1991; Mozaffarian, 1996; Rechinger, 1980; Howes, 1950). *Dorema ammoniacum* D. Don. produces a medicinal gum resin, commonly known as ammoniacum gum, which can be found in the holes of stems, roots, and petioles (Langenheim, 2003). Overall, the plants extract constituents and their secondary metabolisms are provided use to efficacious on human and animal health that can be used to cure maladies (Evans, 1996). The gum resin of *Dorema ammoniacum* D. Don. in the Iranian traditional medicine has been considered beneficial in treatment of spastic pains, gastric disorders, intestinal parasitic infections and skin inflammations and as analgesic, stimulant, expectorant and laxative (Amin, 1991; Parsa, 1959; Zarshenas *et al*., 2013). In addition the existence resin in the stem of *Dorema ammoniacum* D. Don. are used as antimicrobial, vasodilator, a carminative, diaphoretic, mildly diuretic, expectorant and poultice (Irvani *et al*., 2010). *Dorema ammoniacum* D. Don. in the British pharmacopeia as the plant with various therapeutic properties has been recognized that some of the most important of them are as follow: antispasmodic, sputum containing horny sputum, cough irritant, sudorific, the regulation of menstrual, reducer of blood fats, prevention of diabetes, hepatoprotective mechanism, sexual stimulus and sedative (British herbal pharmacopoeia, 1983; British pharmacopoeia, 1993). Several studies have been conducted in relation to identification of chemical compounds of the gum and volatile oil of different parts of *Dorema ammoniacum* D. Don. in some mentioned countries (Yassa *et al*., 2014; Yousefzadi *et al*., 2011a; Yousefzadi *et al*., 2011b; Sajjadi *et al*., 2007; Appendino *et al*., 1991; and Arnone *et al*., 1991). In all these studies miscellaneous chemical compositions were identified and the amount of the yields of essential oils was varied. The mentioned plant species in France are utilized in the factories producing of glues and cosmetic industry. Moreover, this plant has other varied uses such as food industry, confectionary products, paint industry, manufacturing, and production of the detergents and especially in production of special glues for jewelry (Bown, 1995; Grieve, 1971; Chevallier, 2001). Plants, with their wide variety of chemical constituents, offer a promising source of new antimicrobial agents with general as well as specific antimicrobial activity (Evans, 1996). In the previous studies the antimicrobial activity of the extract of *Dorema ammoniacum* D. Don. had been reported (Rajani, 2002). The purpose of the current study was to identify all chemical compositions of the essential oil of *Dorema ammoniacum* D. Don. in the western areas of Isfahan province which is situated in the section of the Central Zagros area, Iran; for the first time.

2. Materials and Methods

2.1. Plant materials

Dorema ammoniacum D. Don. (Apiaceae) is perennial endemic native herb plant that grows as high as three meters (around one to three meters) in Iran, between the months of March and June. Samples of *Dorema ammoniacum* D. Don. aerial parts consisted of the leaves and stems collected in the middle of March 2015 (in its natural habitat situated in the Bardasiab zone of the Fereydun Shahr county which is part of Central Zagros area, Iran), during the flowering period. The taxonomic identity of the plant was confirmed by comparing the collected voucher specimen with that of the known identity available in the herbarium of the Department of Natural Resources, Isfahan University of Technology (IUT), Iran **(Fig. 1)**.

2.2. Experimental procedure

At first, 1000g of the aerial parts of plant specifically stems and leaves were dried in the standard position so that they were protected from light, humidity and infection; also, the samples of suspected to pest and immature organs were separated. All of the samples were powdered using a mill (Model PX–MFC90D) with the mesh size of 1mm for the following analysis. Meanwhile, some of the ecological factors of the sampling area are as follows: the climate of the area is cold, the mean annual rainfall and temperature were 549 mm and 10.1 ˚C respectively. The average of altitude of area is between 2676–2716 m and the soil texture of area is moderate, semi heavy to heavy.

2.3. Isolation of the essential oils

The air dried of the aerial parts of the plant (100 g) was separated for hydrodistillation using a Clevenger-type apparatus (European pharmacopeia, 1998) .The distillation was done for 4 hours. The essential oil obtained was kept in a dark glass sealed vial at 4˚C for further analysis. The yields of the oils were calculated based on dried weight of the plant material.

2.4. Gas chromatography/mass spectrometry (GC/MS) analysis

The oils were examined by GC/MS, using Agilent Technologies GC/MS 7890A (built on the industry-leading Agilent GC platform, USA) in the central laboratory of Isfahan University of Technology (IUT), Iran. The injector temperature was set at 220 ˚C for 5 minutes with a split ratio of 1:10. A 1 µl and a volume of 1000 ppm of the obtained oil solution in a GC grade (*n*-hexane) were injected. To isolate different oil components, a linear temperature program was adjusted as described here: first of all, the column was kept at 50 ˚C for 2 minutes, ramped at a rate of 10 ˚C/min to 150 ˚C, and held isothermal for 5 minutes; then the ramp of 20 ˚C/min was applied up to 220 ˚C and held isothermal for 10 minutes. The mass spectrometry detector was taken at 70 eV and the mass range was 40–400 *m*/z. The identification of components was carried out using their recorded spectra by utilizing the spectrometric electronics libraries provided by the instrument software (Wiley, 1994; Adams, 2001). Also, the comparative amounts of the essential oil constituents were stated and recorded as percentage obtained via retention time (R_t) and peak area (Area%).

3. Results and discussion

The light yellowish essential oil of *Dorema ammoniacum* D. Don. was obtained with the yield of 0.31 % (v/w) based on the dried weight of the aerial parts. The major compounds of the essence in dried organs were formed about 67% of the total oils so that mentioned identified compounds were Limonene (49.18%), Neophytadiene (10.21%), beta Caryophyllene (3.54%), Phytol (2.61%) and Neryl acetone (1.46%). In this study, 89 compounds belonged to seven main chemical groups consists of nitrogen containing compounds such as terpenes, hydrocarbons, alcohols, ketones and others compounds were identified in the essence of *Dorema ammoniacum* D. Don. Some of these groups were divided to more minor chemical functional groups for example: nitrogen containing compounds including (amines, alkaloids and amides), terpenoieds (monoterpens, sesquiterpens and diterpens), hydrocarbons (aliphatic, aromatic and cyclic hydrocarbons) and the others of remaining groups were assigned in the next levels. The quantitative amounts of mentioned compounds in order of priorities were as follows: nitrogen containing compounds including amines (73.5 %), alkaloids (17.2 %) and amides (9.3 %), terpenoieds consists of monoterpens (82.88 %), sesquiterpens (12.97 %) and diterpens (4.14 %), hydrocarbons involving aliphatic hydrocarbons (88.66 %), aromatic hydrocarbons (7.5 %), cyclic hydrocarbons (3.83 %), alcohols (2.76 %), ketones (3.95 %) and miscellaneous coumpounds (2.22 %). The specifications of the entire identified constituents of the essential oils of *Dorema ammoniacum* D. Don. along with their retention time and also GC/MS chromatogram of it are shown in Table 1 and figure 2 **(Table 1** and **Fig 2).**

Fig 1. *Dorema ammoniacum* D. Don.

In the current research, two major groups of constituents namely nitrogen containing compounds and terpens were reported more than the other compounds. Overall, nitrogen compounds play an important role in many aspects of life and commercial processes. Nitrogen is a molecule in every major drug class in pharmacology and medicine (Bussau, 1999; Tamras, 1995). On the other hand, terpenes are one of the large group of chemical compounds with more minor divisions which sometimes called isoprenoides. These compounds are used extensively for their aromatic

qualities and play a role in traditional herbal remedies (Connolly and Hill, 1996; Mann *et al.*, 1994). In this regard, a set of concise studies in relation to identification of constituents of *Dorema ammoniacum* D. Don. were carried out around the world. In most of which existence of different Terpenoieds compounds have been confirmed and some of the mentioned compounds were similar to the ones identified in this report (Mansouji Avval *et al.*, 2013; Yousefzadi *et al.*, 2011b; Yassa *et al*., 2014; Batooli *et al*., 2014). In spite of the reported constituents in the previous studies, there were a wide range of new identified compounds which were not reported in any of the previous research.

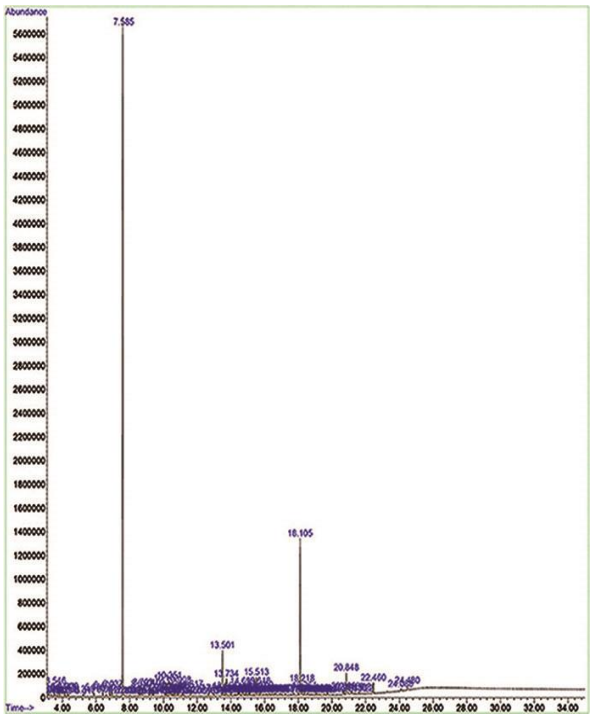

Fig 2. GC/MS chromatogram of *Dorema ammoniacum* D. Don.

The findings of the present research shows similarities and differences of essence constituents in mentioned organs of *Dorema ammoniacum* D. Don. compared to the previous conducted research. There are two significant reasons for such variances including, genetic characteristics (different genotypes of plants) and special environmental conditions (Omidbeigi, 2000).

Table 1. The specifications of the entire identified constituents of the essential oils of *Dorema ammoniacum* D. Don.

No.	Compounds[a]	Rt[b]	%
1	2,5-Dimethyltetrahydrofuran	3.162	0.25
2	Methylbenzene	3.222	0.10
3	Cyclohexane, 1,3-dimethyl, *trans*	3.351	0.3
4	Valeraldehyde	3.456	0.12
5	Octane	3.546	0.65
6	Imidazole-5-carboxylic acid, 2-amino	3.652	0.11
7	Butyl acetate	3.848	0.33
8	Cyclohexane, 1,2-dimethyl, *cis*	4.149	0.36
9	N,N-dimethyl cyclobutane-1,1-bis(methylamine)	4.194	0.06
10	Furfural	4.300	0.44
11	Deuteroacetone	4.353	0.18
12	Styrene	5.249	0.18
13	1-Methyldodecylamine	5.317	0.11
14	α-Pinene	5.882	0.32
15	Aziridine, 1-(2-buten-2-yl)	6.402	0.47
16	Sabinene	6.541	0.24
17	β-Myrcene	6.824	0.26
18	Decane	6.937	0.52
19	Acetamide, 2-chloro	7.209	0.08
20	**Limonene**	**7.585**	**49.18**
21	2-Butylamine	7.751	0.31
22	2-Pyridinepropanoic acid, α-methyl- β-oxo- ethyl ester	7.819	0.24
23	Phenyl Acetaldehyde	8.007	0.15
24	2-Amino-4-hydroxypteridine-6-carboxylic acid	8.520	0.07
25	Benzenamine, N-methyl-2-(2-propenyl)	8.572	0.17
26	Undecane	8.610	0.5
27	α-Terpinolene	8.723	0.61
28	Aramine	9.175	0.29
29	Limonene oxide	9.409	1.26
30	Isopropyl isocyanate	9.718	0.45
31	2-Hexanamine, 4-methyl	9.914	0.63
32	2,6-Dimethyl-4-pyrone	10.095	0.27
33	Dodecane	10.162	0.97
34	2,4-Hexadiene, 3-methyl	10.275	0.07
35	β-Fenchyl alcohol	10.351	1.27
36	Homarine	10.404	0.48
37	2-(N,N-dimethyl hydrazino) cyclohexane carbonitrile	10.494	0.19
38	Phenol, 4-(2-aminopropyl)	10.554	0.5
39	2-Oxo-3-methyl-cis-perhydro-1,3-be nzoxazine	10.607	0.35
40	*trans*-Carveol	10.697	0.58
41	Benzene, (2-fluoro-2-methoxycyclopropyl)	10.826	0.35
42	2(1H)-Naphthalenone, octahydro-8a-methyl, *trans*	10.908	1.14
43	p-Methoxyamphetamine	11.044	0.57
44	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)	11.127	0.32
45	2-Methyl amphetamine	11.255	0.13

46	Tridecane	11.617	0.55
47	1-(3,5-Dimethyl-1-adamantanoyl) semi carbazide	11.767	0.52
48	Benzenemethanol, alpha-(1-aminoethyl)	12.084	0.05
49	Naphthalene, 1,2-dihydro-1,1,6-trimethyl	12.672	0.25
50	Rimantadine	12.853	0.1
51	Heptadecane	12.981	0.36
52	**β-Caryophyllene**	**13.501**	**3.54**
53	2-Amino-1-(O-methoxyphenyl) propane	13.591	0.35
54	Calarene	13.651	0.43
55	**Neryl acetone**	**13.734**	**1.46**
56	2-Methoxy amphetamine	14.164	0.63
57	1-[a-(1-Adamantyl) benzylidene] thiosemicarbazide	14.405	0.23
58	Benzenemethanol, 3-hydroxy-alpha-[(methylamino) methyl]	14.465	0.2
59	delta-Cadinene	14.699	1.11
60	1 S-*cis*-Calamenene	14.797	0.16
61	Aptrol	14.857	0.35
62	Benzenemethanol, 4-hydroxy-alpha-[(methylamino) methyl]	14.925	0.31
63	Nerolidol	15.128	0.36
64	N-Methyl-propylamine	15.384	0.11
65	Pentadecane	15.475	0.32
66	Farnesol 2	15.513	1.37
67	Caryophyllene oxide	15.618	1.36
68	2-Hexanamine, 5-methyl	16.145	0.1
69	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl	16.274	0.22
70	2-Propanamine	16.703	0.1
71	2-Aminononadecane	16.922	0.27
72	Methanimidamide, N,N-dimethyl-N-phenyl	17.035	0.34
73	Methylpent-4-enylamine	17.713	0.7
74	Cyclobutanol	18.014	0.3
75	**Neophytadiene**	**18.105**	**10.21**
76	Hexahydro farnesyl acetone	18.218	0.86
77	n-Hexylmethylamine	18.293	0.06
78	Ethylene bromohydrin	18.361	0.46
79	Didodecyl phthalate	18.549	0.21
80	3-Propoxyamphetamine	18.836	0.09
81	Glycine, N-(N-acetyl-L-alanyl) butyl ester	19.069	0.2
82	2-Propenamide	19.250	0.13
83	2-Heptanol, 6-amino-2-methyl	19.348	0.05
84	Benzene ethanamine, 4-fluoro-beta,3-dihydroxy-N-methyl	19.484	0.08
85	1H-Indole-3-ethanamine, 6-fluoro-beta-methyl	19.529	0.05
86	Nonadecane	20.689	0.27
87	**Phytol**	**20.848**	**2.61**
88	Eicosane	22.460	0.83
89	Cyclotrisiloxane, hexamethyl	24.480	0.62
	Total identified		99.01

[a] Compounds listed in order of elution on the DB5 column.
[b] Retention time (as min)

Nevertheless, other factors such as phenology stages, the period of growing season and different organs of plants have been taken into consideration. It should be noted, our efforts have been focused on investigation and introduction of the new major chemical compounds which would be concisely described by the end of this study. The molecular structure of the major chemical compounds from the essential oil of *Dorema ammoniacum* D. Don. is shown in figure 3 **(Fig. 3)**.

Limonene is the first compound that is widely used in the process of manufacturing and production of various cosmetic products. Numerous applications are mentioned for this compound in literatures. Some of them are as follows: air care products, cleaning and furnishing care products, laundry and dishwashing products, personal care products and it has been considered as biofuel (Connolly and Hill, 1996; Mann *et al.*, 1994; Bauer, et al., 2001; Ming Lin *et al.*, 2015).

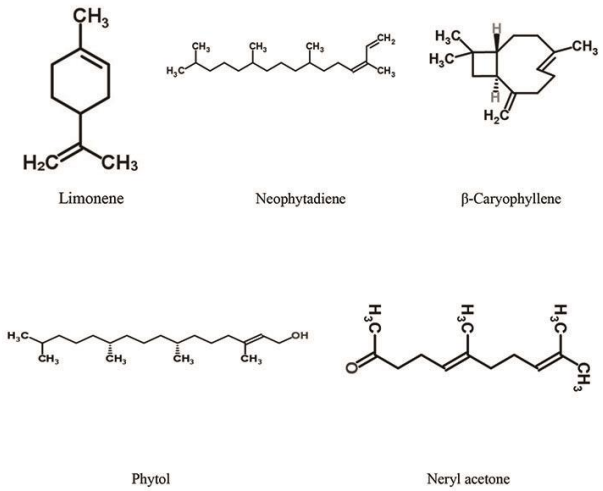

Fig 3. The molecular structure of the major chemical compounds from the essential oil of *Dorema ammoniacum* D. Don.

Neophytadiene is the second compound. It is one of the major compounds of *Nicotiana tobacum* L. that exists in leaves, stems and wastes of mentioned plant or is created by the hydration of phytol. It is a potent antimicrobial and anti-inflammatory compound and in some of literatures reviews it is identified as an antifungal terpenoid in the red alga, *Centroceras clavulatum* (C. Agardh) and Montagn. Other applications are as listed: antipyretic, analgesic and

vermifugic, which cures sores and inflammation (Stojanovic *et al.*, 2000; Mendiola *et al.*, 2008; Carretero *et al*., 2008). The third compound is beta Caryophyllene. This compound is extensively seen in the essential oils of various plants since it has several biomedical properties. The mentioned compounds contributes in the odor and flavor of the plants and also is used in lotions, creams and soaps (Skold *et al*., 2006). Moreover, several therapeutic features of this compound have been reported in literatures. Some of the most important of them are as follows: anti-anemic effect, anti-inflammatory agent and anti-mutagen, a protector of gastric injury and oxidative stress and finally anti-carcinogen (Legault and Pichette, 2007; Di Sotto *et al.*, 2008; Da Silva *et al*., 2007). Phytol is the fourth compound that like limonene are used in the fragrance industry and it could be used for manufacturing combined forms of the vitamins E and K_1 (Daines *et al*., 2003; Mc Ginty *et al.*, 2010). The latest major compound is beta Neryl acetone. This compound has been reported as one of the major combination in some angiosperm (flowering plant) families such as Rutaceae, Burceraceae, Poaceae, Lamiaceae, Lauraceae, Verbenaceae, Asteraceae, Cuppressaceae and Hypericaceae (Pengely, 1996). It is classified in the group of flavor and fragrance agent and participates in the manufacture of various solvents. Additionally, therapeutics effects of the mentioned compound in some studies related to medical field has been proven (Curtis and Williams, 2001; Curzons *et al.*, 1999; Huttenlocher *et al.*, 1971; Gilbert *et al*., 2000).

4. Conclusion

In this study, we have investigated existing constituents in the extract of *Dorema ammoniacum* D. Don. in one of its habitats in the Central Zagros, Iran. It led to discovery of some new constituents for the first time which were not reported in the previous studies. As discussed in the results section two major factors namely genetic features and environmental conditions have a significant contribution in shaping up and appearance of the various constituents. However, there are some habitats of the mentioned plant on earth that no phytochemical studies have been done on them and it needs to be further researched. *Dorema ammoniacum* D. Don. due to therapeutic properties is used in Iran folk medicine and it has various applications in cosmetics, detergent industries and food industry. Also, in the contemporary medicine the resin of mentioned plant has been utilized for the treatment of asthma and its case study is still ongoing. Finally, Iran due to its abundant species richness and particular climate conditions can be regarded as a unique country in the world in terms of medicinal species.

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