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Extraction and Essential Oils Profiling of Different *Dorema ammoniacum* D. Don. Organs and Evaluation of Antioxidant Capacity

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ABSTRACT

***Dorema ammoniacum* D. Don. is a perennial medicinal plant from Apiaceae family. There is some evidence in Iranian traditional medicine about the anticonvulsant and anthelmintic properties of *D. ammoniacum* gum resin exudating from its root and stem. In this study, *D. ammoniacum* was collected from southwestern part of Iran and their Essential Oils (EOs) content and compositions were investigated by gas chromatography techniques. The EO yield of *D. ammoniacum* root, stem, leaves, flower and gum were 0.4, 0.2, 0.24, 0.46, and 1.0 v/w%, respectively. Although there were some variations among different organs EOs, some similarities could be easily observed. The major compounds in the gum EO were (2*E*, 6*E*)-farnesol (12.2%), cuparene (11.5%), (2*Z*,6*Z*)-farnesol (8.7%), *β*-bisabolene (6.1%). The root oil had thymol (14.7%), heptacosane (12.8%), tridecanol (12.7%) and 4-methylene-5-hexenal (6.8 %) as the major compounds. Endo-Fenchyl acetate (13.1%), elemicin (10.5%), p-cymen-8-ol (6.8%) and thymol (5.6%) were identified in the stem oil. The major constituents of the leaves oil were *n*-hexadecanol (9.1%), cuparene (8.2%), Di-*n*-butyl phthalate (6.6%), *n*-eicosane (6.6%), bicyclogermacrene (5.4%) and *β*-bisabolene (5.2%). The major compounds in flower oil were tridecanol (13.2%), δ-elemene (11.2%), *n*-eicosane (8.2%), and heptadecanoic acid (7.8%). According to different applications, nine different extracts were also prepared from all organs and their Antioxidant Activity (AA), Total Phenol Content (TPC) and Total Flavonoid Content (TFC) were evaluated. Analysis of variance showed that there was a significant difference among all extracts of *D. ammoniacum* in AA, TPC and TFC (P≤ 0.01). Results showed that the highest radical scavenging activity was observed in M_2F (second Methanolic extract of Flower) and M_2L (second Methanolic extract of Leaves) samples with IC_{50} of 40.3 µg mL^{-1} and 43.6 µg mL^{-1} compared to BHT (26.0 µg mL^{-1}). The highest TPC in M_2F and M_2R (second Methanolic extract of Root) samples were 36.4 and 35.7 mg GAE g^{-1} DW of extracts, respectively. The highest TFC belonged to M_2L with 26.4 mg QE g^{-1} DW of extract. Results also showed that *D. ammoniacum* organs extracts contained moderate to high amounts of AA and TPC.**

Keywords: GC/MS, Bisabolene sesquiterpenes, *D. ammoniacum*, Sequential extraction, Volatile compounds.

INTRODUCTION

Medicinal plants consumption has been increased because of the widespread belief about their natural and healthy properties (Hosseini *et al*., 2018). The genus *Dorema* D. Don belongs to the Apiaceae family and comprises seven species in Iran, among them, *D. ammoniacum* D. Don. and *D. aucheri* Boiss. are endemic (Yousefzadi *et al*., 2011). *D. ammoniacum* is an important perennial medicinal plant that grows wild in central regions of Iran such as Yazd,

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Isfahan, Kerman and Semnan provinces, and its local names are Kandal, Vasha or Oshagh and Koma-kandal (Rechinger *et al*., 1987). As a medicinal use, gum resin of *D. ammoniacum* has been used as an anthelmintic for gastrointestinal disorders in Iranian traditional medicine (Amin, 2005). Moreover, antibacterial, vasodilatory and anticonvulsant protective effects of this herbal plant have also been reported (Ghasemi *et al*., 2018; Motevalian *et al*., 2017). *D. ammoniacum* gum resin has been recommended for treatment of seizures in Iranian traditional medicine (Khorasani, 2001; Tonkaboni, 2007). Generally, the gum resin is exudate from the root, stems and leaves (Rajani *et al*., 2001). Nowadays, some novel in *vivo* disease model like zebrafish has facilitated the drug screening with higher speed and lower (microgram scale) quantity of tested compounds. Zebrafish emerge as a robust disease model for evaluation of anticonvulsant activity of bioactive compounds and especially in natural products. Recently, Orellana-Paucar *et al*. (2013; 2012) showed that some bisabolene sesquiterpenoids in Essential Oils (EOs) of turmeric, inhibit PTZ-induced seizures in zebrafish and mice and had anticonvulsant activity. Identifying the other sources of these kinds of compounds could be of great interest. As *D. ammoniacum* has the potential in anticonvulsant activity; there are some reports about its chemical compositions as well. There have been a lot of differences in all reports about the EO composition of different organs (Masoudi and Kakavand, 2017). Studies of volatile oils from *D. ammoniacum* leaves have already been reported (Yousefzadi *et al*., 2011). The major constituents of the fruits oil of *D. ammoniacum*, collected from Birjand were (*Z*)- and (*E*)-ocimenone, *β*-cyclocitral and ar-curcumene in fruits and α-gurjunene (49.5 %), *β*-gurjunene (19.0 %) and *α*-selinene (4.6 %) in the leaves as the main components. Also, hexadecanal (11.1 %), *α*-cadinol (6.6 %), sesquicineol2- one (6.6 %), ethyl linoleate (6.3 %), ledol (5.1 %) and γ-eudesmol (4.4 %) were reported as the major constituents of the oil from stems and 2-pentadecanone (19.1 %), *β*-eudesmol (17.2 %), germacrene D (5.8 %), *α*-eudesmol (5.8 %) and spathulenoll (5.0 %) were the major components in EO of seeds (Hosseini *et al*., 2018). The oleogum resin contains a small amount of volatile oil (0.1–0.4%), resin and gum and the hydro distillation of *D. ammoniacum* stem, seed and fruit gave a yellow oil in 0.5, 0.3 and 0.09 (w/w%) yield, respectively (Hosseini *et al*., 2018; Rajani *et al*., 2002; Yousefzadi *et al*., 2011). Previous studies on EOs of *D. ammoniacum* from Shahroud, Semnan Province, showed three hydrocarbon monoterpenes (0.62%), five oxygenated monoterpenes (6.93%), 10 sesquiterpene hydrocarbons (14.34%), 13 oxygenated sesquiterpenes (37.89%), and 32 non terpenoid compounds (29.42%). *α*-Muurolol (13.68%) was the most abundant constituent followed by hexadecanoic acid (6.81%) and (*E*)-nerolidol (5.09%) (Masoudi and Kakavand, 2017). There are also some reports about some other bioactive compounds in the extracts of this plant. Salicylic acid, ammoresinol, ashamirone and some sesquiterpenes have been isolated and identified from *D. ammoniacum*. Phenolic compounds such as sesquiterpene coumarins, phenols, flavonoids and phloroacetophenone glycosides have been reported from the other *Dorema* species. In previous studies, a large variety of solvents including water, hexane, EtOAc, MeOH, and ethanol (Barbouchi *et al*., 2018; Prabakaran *et al*., 2018), acetone, MeOH, ethanol and water (El-Chaghaby *et al*., 2014; Li *et al*., 2017; Mohdaly *et al*., 2010), MeOH, chloroform, ethanol (Kumar *et a*l., 2013), and MeOH (Türkuçar *et al.*, 2021) were also used for extraction of phenolic compounds and antioxidants from plants. The various parts of the plant such as leaves, flowers, fruits and seeds have antioxidants like the flavonoids, tannins, coumarins, curcumanoids, xanthons, phenolics, lignans and terpenoids, which have caused great interest in separation of these natural antioxidants (Jeong *et al*., 2004). Many

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factors such as cultivation area, climatic conditions, genetic variations and others have caused the variation in the biological activity and phytochemicals of medicinal plants, which increase the study of available plants in different growing sites, countries and geographical zones (Norani *et al*., 2019).

In the present study, the EO compositions of *D. ammoniacum* leaves, stems, roots, flowers and gum from Kerman Province, Iran, were evaluated. The effect of different procedures of extraction according to different applications with different solvents (*n*-hexane, ethyl acetate, acetone, methanol, water and hydroalcoholic) on the Antioxidant Activity (AA), Total Phenol (TPC), and Total Flavonoid Content (TFC) of *D. ammoniacum* different organs was investigated.

MATERIALS AND METHODS

Plant Materials

The leaves, stems, roots, flowers and gum resin of *D. ammoniacum* were collected from Dalfard Rural District (57º 37′ 57″ N, 28º 58′ 26″ E), a rural district in Sarduiyeh District, Jiroft County, Kerman Province, Iran (Figure S1). All of the samples were collected during April of 2019 and air-dried in the shade. Plant identities were confirmed by Dr. A. Sonboli and a representative voucher specimen (MPH-2724) was placed in the Medicinal Plants and Drug Research Institute Herbarium (MPH) of the Shahid Beheshti University of Iran.

Isolation and Analysis of Essential Oils

Fifty grams of *D. ammoniacum* different organs were separately powdered and individually immersed in 500 mL of distilled water and the EO was isolated by hydrodistillation for 3 hours using a Clevenger type apparatus. The EOs were separated from the water and dried over anhydrous sodium sulfate and stored at 4°C until analysis. The EOs were analyzed quantitatively by an Agilent Technologies 7890B (Santa Clara, CA, USA) equipped with a flame ionization detector, and an HP-5 capillary Column (length 30 m, inner diameter 0.32 mm and film thickness 0.25 µm). The oven temperature was programmed from 60°C (2 min hold) to 280°C with 5°C min^{-1}. Helium with the flow of 1.1 mL/min was used as the carrier gas. The qualification of individual peaks was carried out by injecting the oil to a Thermoquest–Finnigan gas chromatograph, coupled with a trace mass spectrometer (GC/MS) 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 ionization voltage was set at 70eV and interface temperature and ion source were kept at 250 and 200°C, respectively. Finally, identification was confirmed by comparison

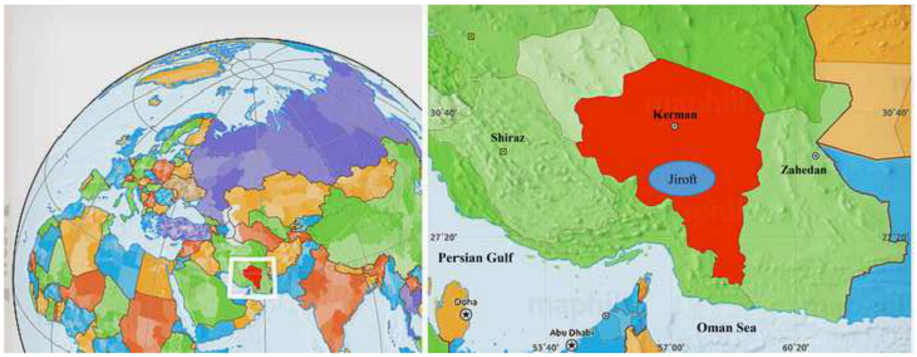

Figure S1. Map of collection site for *D. ammoniacum*.

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of each individual component's mass spectra with those of internal mass spectra library of the main library, Wiley 7.0 and Adams; further identification was based on comparison of peak retention indices by using a homologous series of normal alkanes (C8 to C24) recorded under the same operating conditions and the published data (Adams, 2001).

Preparation of Different Extracts

The protocol of extraction in our research group was standardized based on different applications. The first procedure was a sequential extraction using ultrasonication of 5 grams of plant materials in 50 mL of *n*-Hexane (Hx), followed by Ethyl Acetate (EA_2) and finally Methanol (M_2). This procedure led to separate different metabolites of plant material based on different polarities.

The second procedure was a sequential extraction starting with EtOAc (EA) and then a mixture of Ethanol/Water (50/50) (WE_2) to have a more general extract of plant material with EtOAc followed by more polar plant ingredients. The third procedure was just extracted with the MeOH (M) as a total extract, covering a diverse lipophilicity of chemical constituents. But MeOH is not a safe solvent for the formulation in the in *vivo* or clinical studies. The fourth procedure was performed using the mixture of EtOH/H2O (70:30) (WE), which can be used as an analogous of extraction protocol to infusion preparation, which is employed by traditional healers and/or general public. The fifth procedure used Acetone (A) solvent as a general solvent in extraction in phytochemical studies and can be prepared and dried more quickly than the other solvents. Despite of later procedures, the sixth procedure was completely a simulation of infusion which 5 grams of plant materials and 50 mL of boiled water were kept for 30 min and then filtered. The latest extraction is used in traditional medicine formulation of many medicinal plants around the world. The first to fifth procedures were prepared by ultrasonication assisted extraction (5 g in 50 mL of the aforementioned solvent, 30 minutes) and the sixth was an infusion (5 g in 50 mL of boiled water and 30 minutes). All the extracts were filtered through Whatman no.1 filter paper, then, concentrated in rotary evaporator at 40°C. The extracts were finally dried and stored at 4°C until analysis. The extracts containing water after concentration, were freeze-dried and were finally dried and powdered. A more concise protocol of extraction was used by Bremner *et al*. (2009). In fact, all the plant organs including Root (R), Stem (S), Leaves (L), and Flower (F) were introduced to all the above extraction procedures and, finally, 36 different extracts were prepared and abbreviated according to the combination of the above letters. Figure 1 summarizes the extraction protocols in this study.

Assessment of Antioxidant Activity against DPPH

The radical scavenging activity of all extracts against DPPH (2,2-Diphenyl-2 Picrylhydrazyl Hydrate) was determined by the previously described method of Bozin *et al*. (2007) using the IC_{50} to compare the antioxidant properties. The absorbance was recorded at 517 nm with ELISA reader (Epoch, BioTek instrument). The radical scavenging capacity (RSC) was calculated using the following formula:

In %= [(Ab-As)/Ab] ×100

Where, in is DPPH Inhibition, Ab is the Absorbance of the blank, and As is the Absorbance of the sample extract, or Butylated Hydroxytoluene (BHT) as a positive control. IC_{50} is the concentration of the sample whose inhibition percentage is 50%.

TPC and TFC Evaluation

The total phenolic content was determined

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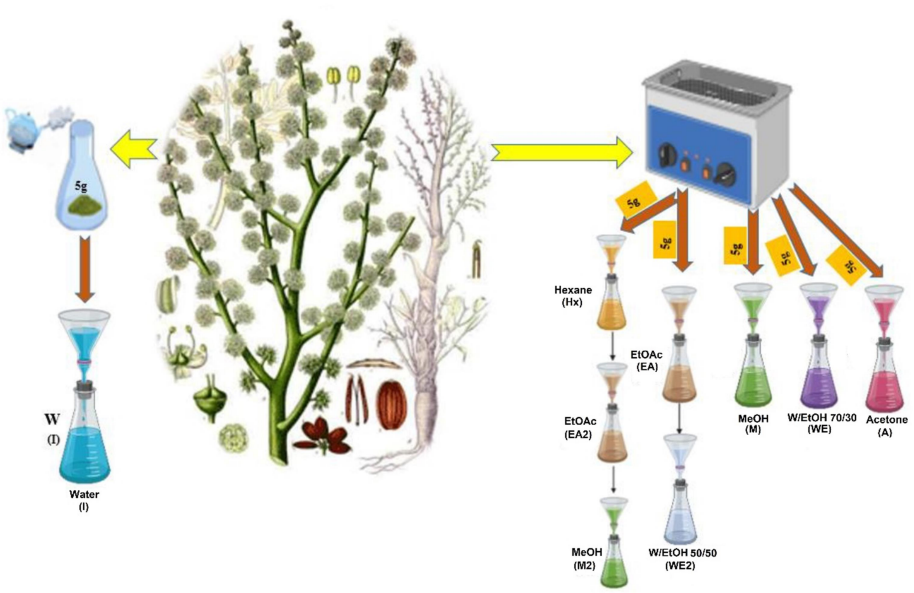

Figure 1. The protocol and solvents used for extraction from *D. ammoniacum.*

by using the Folin- Ciocalteu method (Slinkard and Singleton, 1977). A calibration curve was prepared using a series of methanolic gallic acid solutions (10, 30, 100, 250, 500, 1000 µg mL^{-1}), combined with 0.1 mL Folin- Ciocalteu reagent and, after 3 minutes, 0.3 mL sodium carbonate (7.5%). The absorbance of the mixture was measured after 2 hours at 765 nm using spectrophotometer (Smart spec plus, BIORAD). Twenty µL of all *D. ammoniacum* extracts with 0.05 g mL^{-1} concentration were combined with the above reagents, in three technical replications, to assess phenolic content. Gallic acid was used as the standard for a calibration curve, and the results were expressed as mg of Gallic Acid Equivalents per gram of Dry Weight of Extract (mg GAE g^{-1} DW Ext). The content of flavonoids was also measured by using colorimetric method of Ordonez *et al*. (2006). Briefly, extracts of *D. ammoniacum* were prepared at 0.5 g mL^{-1} in DMSO. The same amount of extract volume and aluminum chloride solution (2%, methanolic solution) was mixed in a test tube and absorbance was measured at 420 nm using the same 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 (5, 10, 50, 100, 250, 500 and 1000 µg mL^{-1}). The results were expressed as mg of Quercetin Equivalents dry per gram Dried Weight of Extract (mg QE g^{-1} DW Ext).

Statistical Analysis

All the data were analyzed according to analysis of variance based on a completely randomized design with three replications, using SAS Statistical Package Program and SPSS software. The significant differences between the group means were separated using the Least Significant Difference (LSD) test at 5% probability level.

RESULTS AND DISCUSSION

Essential Oil Composition

The EO yields from leaves, stems, roots, flowers and gum resin of *D. ammoniacum*

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were 0.4, 0.2, 0.24, 0.46 and 1.0 (v/w% relative to dry weight of plant), respectively. In comparison, Delnavazi *et al*. (2014) reported the EO content of areal part and root of *D. ammoniacum* as 0.2 and 0.3 v/w%, respectively. The previous studies showed that essential oil content was not the same in different plant organs such as the apiaceae family, including *Oliveria decumbens*, *Trachyspermum Ammi*, *Echinophora tenuifolia* and *Heracleum Persicum* (Hazrati *et al.*, 2020).

Generally, 67 compounds were identified by GC/MS analysis in all samples. Table 1 shows identified components of *D. ammoniacum* in different organs. In root oil, 32 components containing 90.0% of oil were identified. The major compounds in root oil were thymol (14.7%), heptacosane (12.8%), tridecanol (12.7%), 4-methylene-5-hexenal (6.8 %), (2*E*, 6*E*)-farnesol (3.7%) and *β*-bisabolene (3.1%). Fourty-three compounds comprising 89.2% of the stem oil were identified. Endo-fenchyl acetate (13.1%), elemicin (10.5%), p-Cymen-8-ol (6.8%), thymol (5.6%) and bicyclogermacrene (4.2%) were identified as the major components of the stem oil. Thirty-eight components were identified representing 95.7% of the leaves oil. The major constituents of the leaves oil were *n*-hexadecanol (9.1%), cuparene (8.2%), di-*n*-butyl phthalate (6.6%), *n*-eicosane (6.6%), bicyclogermacrene (5.4%) and *β*-bisabolene (5.2 %). In flower oil, 37 components containing 91.0% were identified. The major compounds in this oil were tridecanol (13.2 %), δ-elemene (11.2 %), *n*-eicosane (8.2%), heptadecanoic acid (7.8%), bicyclogermacrene (3.9%), and (*Z*)-caryophyllene (3.4%). Thirty-six compounds were identified in the gum EO and the percentage of identified compounds was 89.7 %. The major compounds detected in the EO of gum were (2*E*, *6E*)− farnesol (12.2%), cuparene (11.5%), (2*Z*,6*Z*)-farnesol, β-bisabolene (8.7%), bicyclogermacrene (4.3%), and *α*-elemene (3.2%).

The change in the essential oil components is also influenced by factors such as the age and the development stage of medicinal plants (Hazrati *et al*., 2020). *β*-Bisabolene, as the common major component, was in root, leaves, flower and gum oil of *D. ammoniacum*. Cuparene also was the most important common component identified in leaves, flower and gum oils. Figure 3 represents the common compounds and their quantity in different organs EOs. These compounds were available in all organs. Nevertheless, there was also a few monocyclic bisabolene skeleton type like α-bisabolol, Z-α-bisabolene, *β*-bisabolene, arcurcumene, ar-dihydro turmerone in the EOs of *D. ammoniacum* different organs. These compounds are more or less similar to ar-turmerone, *α*-turmerone, *β*- turmerone and atlantone, which were isolated from the

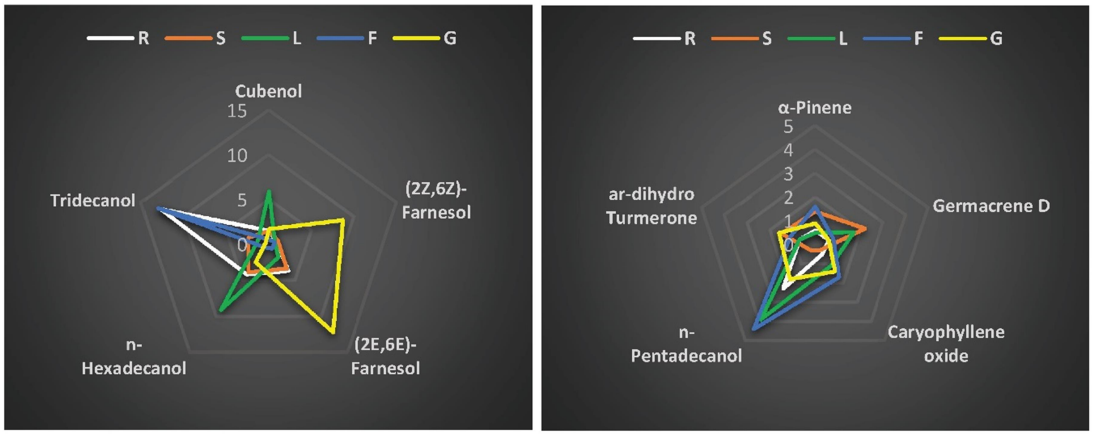

Figure 3. Comparison of compounds in all organs of *D. ammoniacum.*

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Table 1. Chemical composition (%) of root, stem, leaves, flower and gum essential oils of *D. ammoniacum* from Kerman Province.

No	RT	Components	Different organs of *D. ammoniacum*					RI[f]
			R[a]	S[b]	L[c]	F[d]	GA[e]	
1	4.4	4-methylene-5-Hexenal	6.8					893
2	7.8	*α*-Pinene	0.6	1.4	0.5	1.6	0.9	932
3	8.1	Sabinene		0.6		0.5		961
4	8.5	Myrcene	1.7					991
5	8.7	δ-3-Carene	4.7					1011
6	9.5	*p*-Cymene				1.6		1024
7	9.8	*β*-Ocimene	0.8	0.6			0.9	1032
8	10.1	(*Z*)-Sabinene hydrate	0.7				0.6	1065
9	11.1	€-Sabinene hydrate	0.6				0.8	1086
10	11.5	Isoamyl isovalerate	0.5			0.5		1103
11	11.8	(*E*)-2-Nonenal		0.4				1150
12	12.9	(*E*)-Pinocamphone	1.2					1158
13	13.1	Borneol	3.0					1163
14	13.4	*p*-Cymen-8-ol	0.5	6.8	1.1	0.7		1179
15	13.7	α-Terpineol	1.0					1186
16	13.9	*n*-Dodecane	0.8			1.1		1200
17	14.5	*endo*-Fenchyl acetate	1.2	13.1		0.4		1218
18	14.8	Thymol methyl ether		0.3		0.4		1232
19	15.1	Carvacrol methyl ether	0.55	1.2			2.2	1241
20	16.9	Thymol	14.7	5.6	6.3		2.3	1289
21	17.2	δ-Elemene		0.6	0.9	11.2		1335
22	19.2	α-Elemene	1.4	3.2	0.9		3.2	1389
23	19.6	*Z*-Caryophyllene	2.1	0.4	1.4	3.4	0.8	1408
24	19.9	2-Dodecanol		0.4	0.6			1410
25	20.4	*E*-Caryophyllene		2.0	1.1	2.7	0.9	1417
26	20.6	Dehydroaromadendrane		0.8	0.6		1.0	1460
27	20.7	*ar*-Curcumene		0.5			0.8	1475
28	20.8	α-Selinene			3.2			1476
29	21.0	γ-Muurolene	1.52	3.0	1.5		1.1	1478
30	21.4	(*Z*)-Farnesene		0.6	0.5	2.6	0.8	1481
31	21.6	Germacrene D	0.6	2.2	1.7	0.8	0.6	1484
32	22.0	(*E*)-*β*-Ionone			2.6	0.6	1.3	1490
33	22.2	Bicyclogermacrene		4.2	5.4	3.9	4.3	1502
34	22.3	α-Bisabolene			0.7			1504
35	22.4	β-Bisabolene	3.1	5.2	5.2		6.1	1505
36	22.7	(*Z*)-*α*-Bisabolene	1.1	1.3				1506
37	22.8	Cuparene			8.2	1.1	11.5	1506
38	22.9	Elemicin		10.5			1.1	1560
39	23.0	Spathulenol		1.3			3.2	1561
40	23.2	Caryophyllene oxide	0.5	0.3	1.1	1.7	1.4	1567
41	23.4	Tridecanol	12.7	2.4	1.3	13	0.4	1570
42	23.6	*ar*-dihydro Turmerone	0.7	1.5	0.7	1.1	1.6	1595
43	23.8	Cedrol			1.2			1600
44	23.9	Junenol		0.5	1.5	1.0	0.7	1618
45	24.1	γ-Eudesmol		0.3				1630
46	24.2	α-Muurolol		0.4	1.1	0.3	2.3	1644

Continued…

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Continued of Table 1. Chemical composition (%) of root, stem, leaves, flower and gum essential oils of *D. ammoniacum* from Kerman Province.

No	RT	Components	Different organs of *D. ammoniacum*					RI[f]
			R[a]	S[b]	L[c]	F[d]	GA[e]	
47	24.3	Cubenol	1.5	1.5	5.9	0.5	1.7	1645
48	24.7	(6*Z*)-Pentadecen-2-one		0.6	4.0	1.8	4	1667
49	25.2	Pentadecanal		0.9	0.9	0.8	1.8	1682
50	25.3	(2*Z*,6*Z*)-Farnesal		1.4	1.3	1.7	1.4	1684
51	25.6	*α*-Bisabolol				0.9		1685
52	25.7	(2*Z*,6*Z*)-Farnesol	0.9	1.1	0.7	0.7	8.7	1698
53	26.0	(2*E*,6*E*)-Farnesol	3.7	3.4	1.8	0.6	12.2	1742
54	26.3	*n*-Pentadecanol	2.3	0.3	3.9	4.4	1.8	1773
55	26.5	*n*-Hexadecanol	4.2	3.9	9.1	0.5	2.5	1874
56	26.7	di-*n*-butyl phthalate			6.6			1906
57	26.9	Hexadecanoic acid				4.9		1959
58	27.2	*n*-Eicosane		0.3	6.6	8.2		2000
59	27.3	Heptadecanoic acid				7.8		2069
60	28.2	*n*-Octadecanol					0.4	2077
61	27.4	*n*-Heneicosane				3.4		2100
62	28.7	Phytol		0.5	2.1	0.5		2122
63	28.9	(*E*)-Phytol acetate					3.3	2218
64	29.0	*n*-Tricosane		0.5	1.7	2.6		2300
65	30.2	*n*-Tetracosane	0.5	0.5	1.0	1.5		2400
66	32.0	*n*-Hexacosane					0.4	2560
67	41.6	*n*-Heptacosane	12.8	2.7	0.8			2700
Monoterpene hydrocarbons			9.1	2.6	0.5	3.7	3.2	
Oxygenated monoterpenes			22.65	27	7.4	2	4.5	
Sesquiterpene hydrocarbons			9.82	34.5	33.9	26.3	33.5	
Oxygenated sesquiterpenes			7.3	11.7	15.3	8.5	33.2	
Diterpenes				0.5	2.1	0.5	3.3	
Others			40.1	12.9	36.5	50	10.94	
Total compound			**88.97**	**89.2**	**95.7**	**91.0**	**89.7**	

[a] Root; [b] Stem; [c] Leaves; [d] Flower; [e] Gum. [f] Retention indices according to the normal alkanes between C8-C24.

turmeric oil and showed the antiepileptic properties (Orellana-Paucar *et al*., 2013; Orellana-Paucar *et al*., 2012). Figure 4 illustrates the structure of monocyclic bisabolene skeleton structures in the *D. ammoniacum* and turmeric oils. The antiepileptic properties of *D.ammoniacum* gum also might be attributed to those bisabolene skeleton compounds. The total percentages of bisabolene derivatives in root, stem, leaves, flower and gum were 4.9, 8.5, 6.6, 2.0 and 8.5%, respectively. However, in previous phytochemical studies on the EOs of *D. ammoniacum*, Takalloa *et al.* (2013) reported the major components in the flower oil as δ-cadinene (11.58%) and α− himachalene (7.71%), and in stem oil as δ-cadinene (16.24%), liguloxide (8.69%) and δ-amorphene (8.43%), and in root oil as 3-n-butyl phthalide (62.49%), benzyl butanoate (6.57%), and liguloxide (5.15%). Yousefzadi *et al.* (2011) found that (*Z*)-ocimenone (22.3%), (*E*)-ocimenone (18.1%) and β-cyclocitral (9.9%) were the major constituents in fruit oil of *D. ammoniacum*. Hosseini *et al*. (2014) reported 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%), and 2-pentadecanone (19.1%), *β*-eudesmol (17.2%), germacrene D (5.8%), α-eudesmol (5.8%) and spathulenoll (5.0%) were the major components in EO of seeds. The differences can be due to many factors such

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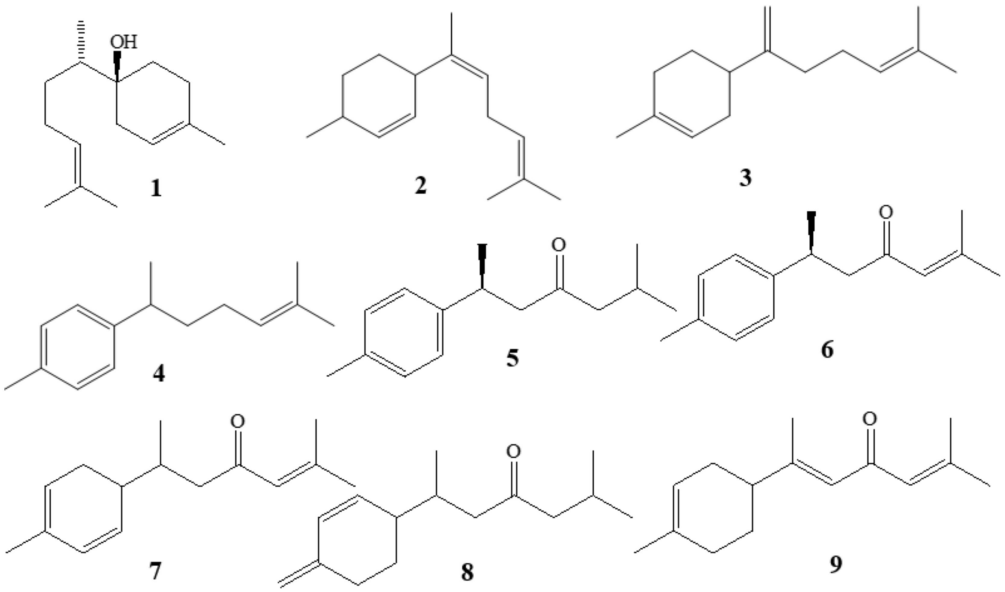

Figure 4. Structure of monocyclic bisabolene skeleton in *D. ammoniacum* (**1-5**) and turmeric oil. (**6-9**). **1:** α-Bisabolol; **2:** *Z*-α-Bisabolene; **3:** β-Bisabolene; **4:** *ar*-Curcumene; **5:** *ar*dihydro Turmerone **6:** *ar*-Turmerone; **7:** α-Turmerone; **8:** β- Turmerone; **9:** Atlantone

as genetic diversity, climate, soils, position and time of sampling, insect and microorganisms stress, method of EO extraction and other geological and environmental conditions (Dobravalskyte *et al*., 2013; Hüsnü and Buchbauer, 2015).

Plant Extraction

The yield of all extractions is presented in Figure 5. The diagram shows that the infusion extraction in most organs (except in flower) had the highest yield, while the lowest yield of extraction was in the hexane extract, except for the stem, in which the hydroalcoholic (70:30) had the minimum yield of extraction. Generally, the *D. ammoniacum* contained more polar compounds than the non-polar compounds.

Antioxidant Activity (AA)

Analysis of variance showed that there was significant difference ($P \leq 0.01$) between all extracts of *D. ammoniacum* in antioxidant activity (Table 2). The results of comparison of antioxidant activity are shown in Figure 6-A. In DPPH assay, the highest radical scavenging activity was observed in M_2F (methanolic extract of flower) and M_2L (methanolic extract of leaves) samples with IC_{50} 40.3 and 43.6 µg mL^{-1} compared to BHT (26.0 µg mL^{-1}), a synthetic industrial antioxidant. The reason for this result can be attributed to the ability of methanol to extract more polar compounds, where in this extract all the non-polar and, perhaps, the lower antioxidant compounds are separated by *n*-hexane and EtOAc extracts before it as well (Dube *et al*., 2017). The lowest activity was found in EW_2F (hydroalcoholic Extract of Flower) and EW_2S (hydroalcoholic Extract of Stem) with IC_{50} 126.2 and IC_{50} 117.4 µg mL^{-1}. Delnavazi *et al*. (2014) reported that ethyl acetate and chloroform extracts of the roots along with the EtOAc extract of the aerial parts had the highest free radical-scavenging activity with IC_{50} values of 21.3, 31.8 and 62.7 µg mL^{-1}, respectively, compared to vitamin E (IC_{50} 14.3 µg mL^{-1}). The previously studies of hydroalcoholic

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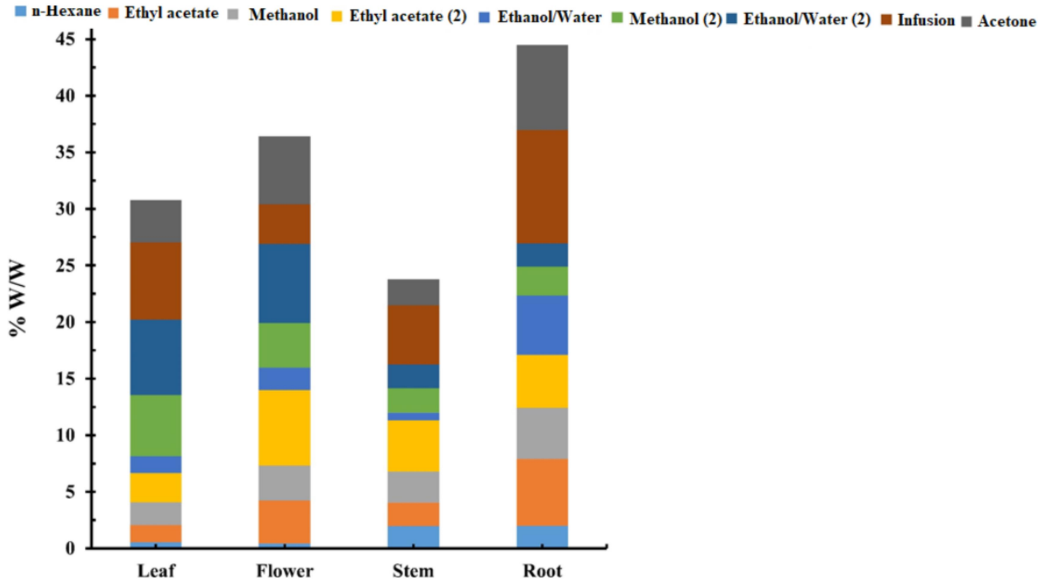

Figure 5. Extraction yield of different solvent from different organs of *D. ammoniacum.*

extracts from *D. aitchisonii* aerial parts and ethanoic extract of *D. aucheri* aerial parts showed a weak antioxidant activity in DPPH assay with IC_{50} values of 488 and 200 µg mL^{-1}, respectively (Khanahmadi *et al*., 2012; Nabavi *et al*., 2012). 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*. (2019) reported the highest radical scavenging activity was observed in *Smyrnium cordifolium* metanolic extract with 59.2 mg TE g^{-1} extract and *Smyrniopsis munzurdagensis* extract showed low antioxidant activity with 2.29 mg TE g^{-1}. Sarikurkcu *et al*. (2019) studied effects of different solvent extracts of Antioxidant activity of *Symphytum anatolicum* and they reported the antioxidant assays the MeOH extract had a highest and an interesting antioxidant activity with IC_{50} of 2.7 mg TE g^{-1}, while this value for water and EtOAc solvent extract were 5.5 and 10.5 mg TE g^{-1}. The observed differences between antioxidant activities can be related to the reaction time and the synergism between the various bioactive compounds in the different extracts.

Total Phenolic and Flavonoid Contents

Results showed a significant difference ($P \leq 0.01$) between all extracts of *D. ammoniacu*m in total phenolic and flavonoid content (Table 2). Results in Figure 6-B showed that the highest TPC in M_2F (second Methanolic extract of Flower) and M_2R (second Methanolic extract of Root) samples with 36.4 and 35.7 mg GAE g^{-1} DW. AF (Acetonic extract of Flower) had the lowest total phenolic content with 10 mg GAE g^{-1} DW. This is the first report about phenolic and flavonoid contents in *D. ammoniacum* and the effect of different solvents on the TPC, TFC and antioxidant activity in *D. ammoniacum*. Kuo *et al.* (2015) reported that, when using different solvents extract, MeOH extract showed the highest TPC. This could be due to the ability of methanol to extract phenolic compound (Airanthi *et al*., 2011; Lim *et al*., 2019). The highest TFC belongs to M_2L (second Methanolic extract of Leaves) with 26.4 mg QE g^{-1} (Figure 6-C). On the other hand, EW_2S (second hydroalcoholic Extract of Stem) exhibited the lowest levels of TFC (3.4 mg QE g^{-1} DW). Llorent-Martínez *et al.* (2020) found

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Table 2. Analysis of Variance of antioxidant activity (IC_{50}), total phenol and flavonoid of *D. ammoniacum.*

Mean Square				
Antioxidant activity (IC_{50})	Total Flavonoid (mg QE g^{-1} DW ext)	Total Phenol (mg GAE g^{-1} DW ext)	DF	Dependent Variable
1070.22^{**}	46.83^{**}	94.37^{ns}	8	Solvent
2370.68^{**}	26.06^{**}	178.86^{*}	3	Plant part
639.52^{**}	77.86^{**}	121.06^{*}	24	Solvent×Plant part
6.87	48.61	61.29	36	Error
3.5	7.34	27.85	-	CV

** Significant at 1% level of probability.

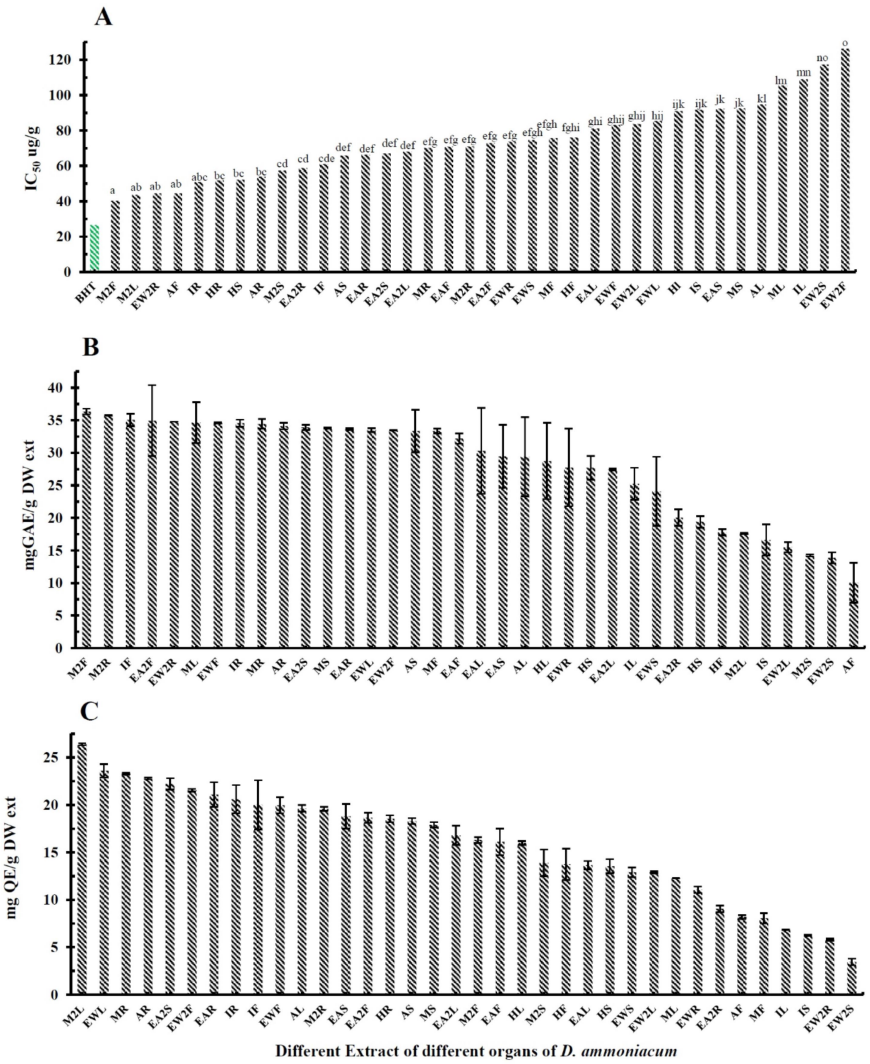

Figure 6. Comparison of antioxidant activity (A), total phenol content (B) and total flavonoid content (C) of different organs of *D. ammoniacum* based on different solvent for extraction M: First Metanolic extract, M_2: Second Metanolic extract, EW: Hydroalcoholic Extract 30:20 (v/v), EW2: Hydroalcoholic Extract 50:50 (v/v), A: Acetonic extract, I: Infusion, R: Root, S: Stem, L: Leaves, F: Flower.

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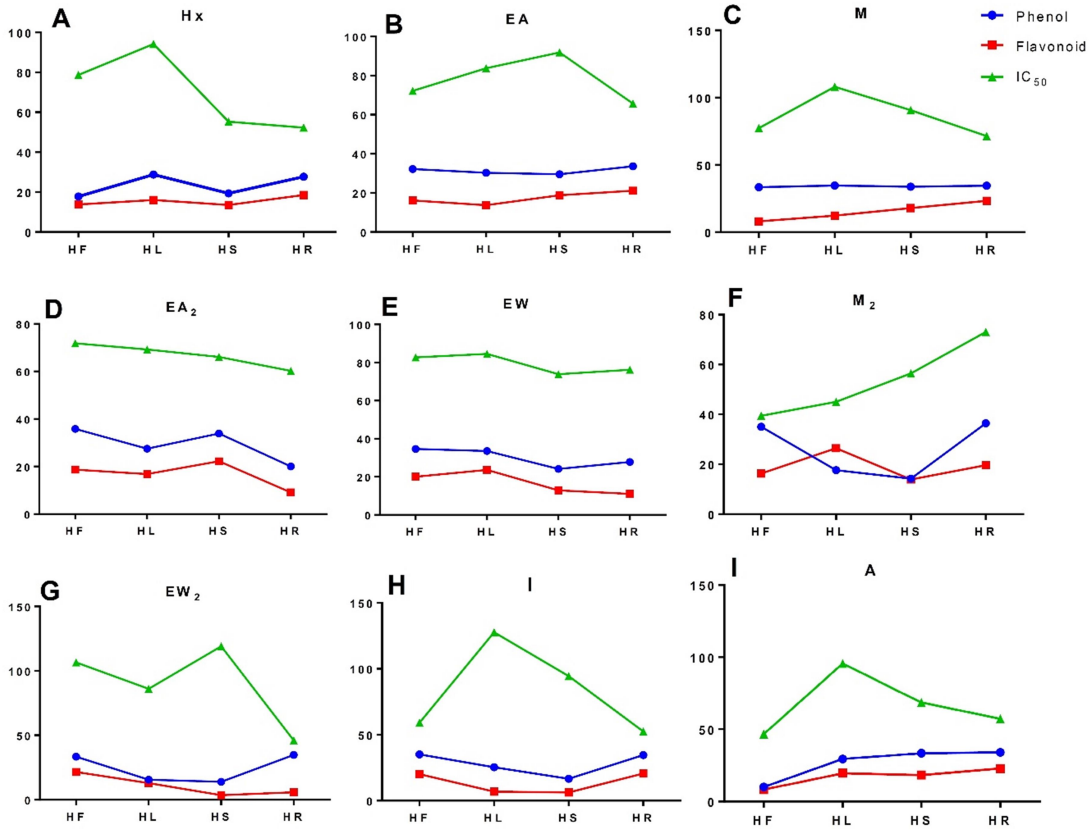

Figure 7. Correlation between antioxidant activity, TPC and TFC in different *D. ammoniacum* in different extraction solvent. A: Hexane extract; B: First EtOAc extract; C: First MeOH extract; D: Second EtOAc extract; E: First hydroalcoholic (70:30) extract; F: Second MeOH extract; G: Second hydroalcoholic (50:50) extract; H: Water infusion extract; I: Acetone extract.

that methanol extract of *Cirsium yildizianum* (46.78 mg RE g^{-1}) had the highest TFC and the lowest amount of TFC related to water extract. The differences in values of total phenol and flavonoid are probably related to the reaction time and the synergism between the disparate bioactive components in the various extracts (Sarikurkcu *et al*., 2019).

Correlation between AA, TPC and TFC

Correlation analyses were carried out by using a two-tailed Pearson's correlation test between total phenol and flavonoids and antioxidant activity in all extracts of solvents from *D. ammoniacum*. Although most of the results of studies represent a strong correlation between total phenolic and antioxidant activity (Figure 7: A-I), correlation was only significant and positive in ethyl acetate solvent ($r= 0.991$, $P\leq 0.01$) (Figure 7-B). Figure S2 also shows the correlation between the 3 parameters (AA, TPC and TFC) with a heat map diagram. In other solvents, no significant correlation was found between traits, while there was a relative correlation between TPC and TFC and antioxidant activity in all extracts. Phenolic compounds and flavonoids are the most abundant group of natural constituents found in various plants that are very important natural antioxidants and are able to adsorb and neutralize the free radicals and

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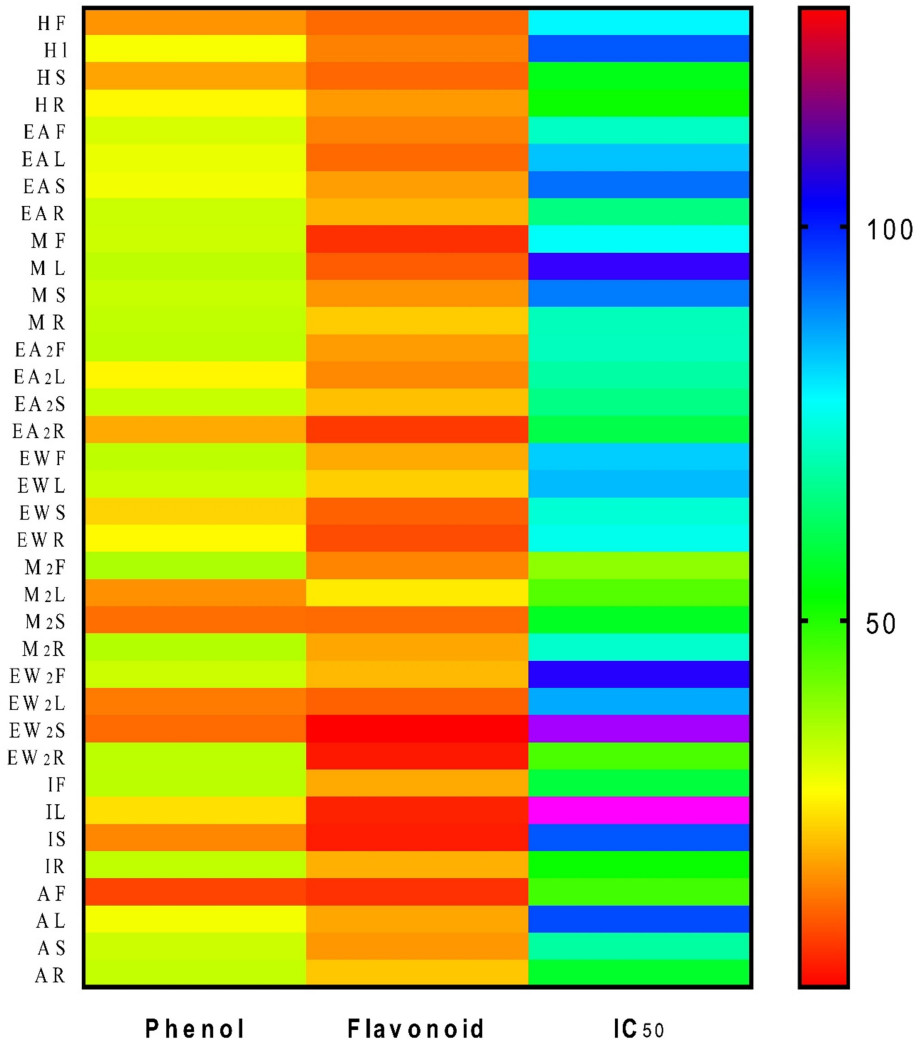

Figure S2. Investigation of correlation among antioxidant, TPC and TFC of different extracts from *D. ammoniacum*. M: First Metanolic extract; M_2: Second Metanolic extract; EW: Hydroalcoholic Extract 70:30 (v/v); EW_2: Second hydroalcoholic Extract 50:50 (v/v); A: Acetonic extract; I: Infusion. Different organs of D. ammoniacum, R: Root; S: Stem; L: Leaves.

exhibit antioxidant activity through radical scavenging (Dobravalskyte *et al*., 2013; Jimoh *et al*., 2011).

CONCLUSIONS

In conclusion, *D. ammoniacum* has relatively low yield of EO in its organs. However, the gum resin of *D. ammoniacum* had a significant amount of EO. The comprehensive EO evaluation has been carried out in one study and the comparison of the oils showed more variation than the similarities in different organs. However, the bisabolene sesquiterpene skeleton structure could be related to the antiepileptic activity of the ammoniacum gum resin. The present study provided also evaluation of different solvents extraction on the AA, TPC and TFC of *D. ammoniacum*. The results showed that the methanol extract had the highest

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content of phenol and flavonoid and antioxidant capacity of *D. ammoniacum*. Also, results showed that *D. ammoniacum* extracts had a moderate content of phenol and flavonoid and antioxidant capacity. Different solvent extractions based on different applications could be of interest according to the phytochemical studies of any plant material.

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شناسایی ترکیبات اسانس و بررسی ظرفیت آنتی‌اکسیدانی عصاره اندام‌های مختلف **Dorema ammoniacum D. Don.**

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چکیده

وشا (Dorema ammoniacum D. Don.) گیاه دارویی چندساله از خانواده چتریان می‌باشد. پراکنش وشا در مناطق مرکزی و شرق ایران شامل استان‌های یزد، اصفهان، کرمان، سمنان و منطقه خراسان و نام‌های محلی آن وشا، کندل و کما-کندل می‌باشد. در طب سنتی ایران شواهدی از اثرات ضد تشنج و ضد التهاب صمغ ترشح شده از ریشه و ساقه آن بیان شده است. در این تحقیق ریشه، ساقه، برگ، گل و صمغ وشا از منطقه دلفارد شهرستان جیرفت در استان کرمان جمع‌آوری گردید و پس از خشک شدن نمونه‌ها، اسانس آن به روش تقطیر با آب استخراج گردید. جهت شناسایی ترکیبات اسانس از دستگاه‌های گاز کروماتوگراف GC)) و گاز کروماتوگراف متصل شده به طیف‌سنج جرمی (GC/MS) استفاده گردید. بازده تولید اسانس برای ریشه، ساقه، برگ، گل و صمغ D.ammoniacum به ترتیب ۰/٤ %، ۰/۲ %، ۰/۲٤ %، ۰/٤٦ % و ۱ % بدست آمد. شباهت‌ها و تفاوت‌هایی در ترکیبات اسانس اندام‌های مختلف وشا مشاهده گردید. ترکیبات اصلی شناسایی شده در صمغ وشا (۲ (2/%12) E,6E)-Farnesol، (5/11 % Cuparene، 7/8) (2Z,6Z)-Farnesol %) و (1/6 β-Bisabolene %) بودند. در اسانس ریشه وشا (7/14 % Thymol، (8/12 % Heptacosane، Tridecanol (7/12 %) و (8/6-٤ methylene-5-hexenal %) به عنوان ترکیبات اصلی شناسایی شدند. Endo-Fenchyl acetate (1/13 %)، (5/10 % Elemicin، 8/6) Cymen-8-ol %) و (6/5 Thymol %) ترکیبات غالب شناسایی شده در اسانس ساقه وشا بودند. ترکیبات عمده شناسایی شده در اسانس برگ عبارت از (1/9 % n-Hexadecanol، Cuparene (2/8 %)، (6/6 % Di-n-butyl phthalate، (6/6 n-Eicosane %) و (2/5 β-Bisabolene %) بودند. در اسانس گل وشا (2/13 % Tridecanol)، (2/11 % δ-Elemene، 2/8) n-Eicosane %) و (8/7 Heptadecanoic acid %) به عنوان ترکیبات غالب بدست آمدند. عصاره‌های مختلف بدست آمده از اندام‌های مختلف وشا آماده گردید و خاصیت آنتی‌اکسیدانی، فنول کل و فلاونویید کل ارزیابی شدند. نتایج تجزیه واریانس نشان داد که صفات خاصیت آنتی‌اکسیدانی، فنول کل و فلاونویید کل در تمام عصاره‌ها معنی‌دار گردید ($p \leq 0.01$). نتایج نشان داد که بیشترین مهار رادیکال‌های آزاد در عصاره‌های M2F (عصاره متانولی گل وشا) و M2L (عصاره متانولی برگ وشا) با IC50 برابر با ٤۰/۳ و ٤۳/٦ میکروگرم بر میلی‌لیتر در مقایسه با BHT با IC50 برابر با ۲٦/۰ مشاهده شد. بیشترین فنول کل در عصاره‌های M2F (عصاره متانولی گل وشا) و M2R (عصاره متانولی ریشه وشا) با مقدار ۳٦/٤ و ۳۵/۷ میلی‌گرم گالیک اسید بر گرم عصاره خشک بدست آمد. بالاترین فلاونویید کل در عصاره M2L (عصاره متانولی برگ وشا) با مقدار ۲٦/٤ میلی‌گرم کوئرستین بر گرم عصاره خشک بدست آمد. همچنین نتایج نشان داد که گیاه وشا دارای فعالیت آنتی‌اکسیدانی و فنول کل به نسبت بالایی می باشد.

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