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Original Article

Secondary Metabolites and Antioxidant Activity in Different Iranian Accessions of *Dorema ammoniacum D. Don* and *Dorema aucheri* Boiss.

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ABSTRACT

This study was performed to evaluate different secondary metabolites (SMs) and antioxidant activity in 14 different genotypes from methanolic extracts of plant samples (leaves and stem) from *Dorema ammoniacum D. Don* (*D.am*) and *D. aucheri* Boiss. (*D.au*). The contents of SMs and antioxidant activity were measured using UV-visible spectrophotometer. The highest total phenolics content (34.3 mg/GAE DW) and antioxidant activity (92%) through DPPH method were detected in leaves samples of *D.au*9 genotype. Highest levels of total flavonoids (5.5 mg QE/g DW) and total flavonols (3.6 mg QE/g DW) were observed in leaves of *D.au*12 and *D.au*2, respectively. The highest content of anthocyanin (250 nano mol/g FW) and carotenoids (18 mg/g DW) were found in leaves samples of *D.au*7 and *D.au*3 genotypes. The results showed the superiority of *D. aucheri* for different SMs rather than *D. ammoniacum* Finally, leaves samples of *D. aucheri* and *D. ammoniacum* species showed higher contents for secondary metabolites which may be suggested for further appliances with medicinal and industrial aims.

INTRODUCTION

Secondary metabolites (SMs) in medicinal plants have been acknowledged to represent a vast capacity for producing different natural products such as essential oils, polyphenols and fatty acids that could be used as natural antioxidant in different industries as pharmaceutical applications [1,2], herbal beverages [3], cosmetics and perfumes [4] agrochemical [5] natural flavoring agents (Xu *et al.*, 2017) and insecticides [5]. Plant SMs also play an important biological and eco-physiological role which are crucial for plant defense against different environmental stresses [1,2]. Furthermore, SMs compounds with natural antioxidants properties (e.g., phenolic compounds, flavonoids, flavonols, alkaloids, steroids, anthocyanins and carotenoids) can function as free reactive oxygen species scavengers, and thus delay the lipid oxidation process in plant cell membranes under environmental stresses [1]. Biosynthesis and accumulation of SMs could be influenced by different factors as genetic, ontogenic, morphologic and environmental features [5,6]. Among environmental conditions, accumulation of SMs is strongly dependent on habitat characteristics, light intensity, temperature and soil characteristics [5,6]. Different types of SMs may synthesize through special regulatory path ways [7]. Subsequently a special transport route in certain cells, organs and tissues would be occurred in medicinal plants [7]. Therefore, tissue or organ specificity in plants is documented for biosynthesis and accumulation of SMs [5].

Dorema is an important genus of Apiaceae family which different prevalent species [8]. This genus includes perennial and herbaceous plants with elite medicinal properties [9]. Many species of *Dorema*, have been used in folk medicine for many decades for various human illnesses [8]. The six number of *Dorema* species are endemic to Iran which includes *D. ammoniacum* D. Don and *D. aucheri* Boiss. species. These two species are on the Red List of threatened Species [8]. *D. aucheri* Boiss. grows in west and southwest habitats of Iran [10]. In folk medicine of Iran, leaves of *D. aucheri* have been used as a vegetable for a local soup. This species is recommended for different medicinal properties as anti-allergic, antidiabtes, anti-inflammatory [10, 11]. The species of *D. ammoniacum* D. Don, as a perennial herb, grows globally in the arid and semi-arid climates of Afghanistan, Pakistan and Iran [9, 12]. *D. ammoniacum* as a monocarpous shrub, often grows in arid and semi-arid mountains of Iran [13]. It is known with local names of Kandal or Vasha [13]. The gum resin of *D. ammoniacum* which found in stems and roots of this species, have different medicinal and industrial properties as confectionery, dyeing, perfumery, detergent and jewelry adhesives [14,15]. Numerous chemical compounds including terpenes, coumarins and phenolic compounds have been isolated from *Dorema* species with a wide range of pharmacological activities [8]. *D. ammoniacum* is used in the treatment of different diseases such as bronchitis, respiratory allergies, anticonvulsant and spleen inflammation [12].

The study of chemical diversity in the natural population of *Dorema* species could provide new insights which may lead to the identification of superior genotypes with higher amounts of their main constituents and bioactive compounds for different applications in pharmaceutical, foods and cosmetic industries. Little studied on *D. aucheri* have been focused in evaluation of its SMs [16]. Most of the previous studies on *Dorema* species have been focused in evaluation of its medical properties such as toxicity and carcinogenicity [11, 15]. Also, little research has been done in terms of identifying different SMs of these species and comparing their chemical compositions of SMs from different plant organs [16,17]. Therefore, the present study was performed to screen different SMs including total phenolics, total flavonoids, total flavonols, anthocyanins, carotenoids, chlorophyll and also antioxidant activity from fourteen Iranian genotypes of these two species to provide new information for selecting superior genotypes with elite bioactive compounds.

MATERIALS AND METHODS

Plant Material Collection

The whole aerial plant samples were collected at the full flowering stage from different geographical regions of its natural habitats in Iran at 2019. Identification of the plant species was carried out by an expert of a plant botanist at Department of Natural Resources, in Isfahan University of Technology, Iran. The geographical characteristics of different natural habitats are presented in Table 1.

Chemicals and Reagents

The chemicals including folin-ciocalteau, sodium carbonate, aluminum chloride, potassium acetate, quercetin, acetone and methanol were purchased from Merck Company (Germany). The DPPH compound were purchased from Sigma- Aldrich Company (Germany).

Methanolic Extraction Assay

The whole plant samples were air dried at shade and room temperature (25 °C). The amounts of 100 mg of completely dried and ground plant samples (leaves and stem) were extracted with 3 ml of 80% methanol (20:80 distilled water: methanol) (Merck, Com.), then kept at cold temperature for 24 hours. The extracts were centrifuged (4000 rpm for 20 minutes) into completely sterile vials and prepared for further experiments.

Total Phenolics Content

By mixing an aliquot of methanolic extract (500 μL) with 0.1 N folin- ciocalteau reagent (1.5 mL) [18], the total phenolics content (TPC) was measured. After addition of 1.5 mL of 15% sodium carbonate solution, the mixture was maintained for 5 min and kept at room temperature for 90 min. For measuring the absorbance of the supernatant at 725 nm, a UV-Vis Spectrophotometer (Unico- UV 2100) was utilized. The concentration of TPC in the stem and leaves extract were represented as mg of gallic acid equivalent (GAE) per mg of extract dry weight.

Total Flavonoids Content

Leaves and stem extracts (0.5 mL), methanol 80% (1.5 mL), aluminum chloride 10% (0.1mL), distilled water (2.8 mL), and 1 M potassium acetate (0.1 mL) were mixed until overall volume of more than 5 mL and maintained at room temperature for 30 min. Such a reaction mixture absorbance was measure at 415 nm utilizing spectrophotometer.

Table 1 Characteristics of collection areas of genotypes of *D. ammoniacum* and *D. aucheri* species from Iran.

Species	Abbreviation	Collection area	Longitude	latitude	Height (m)	Average temperature (^{0}C)	Annual Precipitation (mm)
D. aucheri	*D.au*1	Pasargad, Fars	53° 01′ 24″	30° 07′ 20″	2180	12.5	348.1
D. aucheri	*D.au*2	Fereydunshahr, Isfahan	50° 03′00″	32° 51′ 19″	2550	9	393.5
D. aucheri	*D.au*3	Fereydunshahr, Isfahan	49° 48′51″	33° 01′07″	2500	9.2	390.7
D. aucheri	*D.au*4	Damchenar, Kohgiluyeh and Boyerahmad	51°11′ 33″	31°27′ 10″	2358	15.1	566.85
D. aucheri	*D.au*5	Shabliz, Kohgiluyeh and Boyerahmad	51° 26′	31°36′	2100	13.9	620.8
D. aucheri	*D.au*6	Dasht room, Kohgiluyeh and Boyer-Ahmad	51° 30′ 07″	30°29′50″	2280	11.1	826
D. aucheri	*D.au*7	Semirom, Isfahan	51°35′39″	31° 21′ 07″	2150	10.69	504.3
D. aucheri	*D.au*8	Sisakht, Kohgiluyeh and Boyerahmad	51°28′	30° 51′	2243	12.5	675.1
D. aucheri	*D.au*9	Margon, Kohgiluyeh and Boyerahmad	51°27′	30°48′	2145	14.3	639.16
D. aucheri	*D.au*10	Imam Qais, Chahar Mahal and Bakhtiari	51° 18′	31° 45′	2351	13.7	592.1
D. aucheri	*D.au*11	Sar Aghaaseyed, Chaharmahal and Bakhtiari	49° 54′	32° 42′	2185	9.2	1303
D. aucheri	*D.au*12	Ardal, Chaharmahal and Bakhtiari	50°40′ 24″	32° 01′ 28″	2500	9.1	588.8
D. ammoniacum	*D.am*1	Shahsavaran, Kashan, Isfahan	50° 00′35″	34° 03′53″	2300	17.8	236.1
D. ammoniacum	*D.am*2	Bard Asyab, Fereydunshahr, Isfahan	50° 5′ 23″	32°50′9″	2607	11.07	449

Table 2 Mean comparison for different studied traits in leaves and stem organs in *Dorema* species.

Traits Organs	TPC[¥] (mg/GAE DW)	TFD (mg/QE g DW)	TFL (mg/QE g DW)	Anthocyanins (nano/mol g FW)	TChl (mg/g DW)	Carotenoids (mg/g DW)	DPPH (%)
Leaves	20.41 ^{a}± 1.41	3.46 ^{a}± 0.19	1.85^a± 0.13	101.36 ^{a}± 10.08	81.27 ^{a}± 6.83	9.63 a± 0.97	80.80 a± 1.69
Stem	18.36 ^{b}± 1.18	2.1 ^{b}± 0.13	1.16^b ± 0.6	40.00 ^{b}± 3.29	41.11^b± 3.06	5.37 b ± 0.40	73.54 b± 1.57

[¥] TPC: total phenolics content; TFD: total flavonoids, TFL: total flavonols; TChl: total chlorophyll; DPPH: 2, 2-diphenyl-1-picrylhydrazyl. The same letters in each column are not significant at P<0.05 with LSD test.

The combined solution was utilized as a blank solution in the nonexistence of the extract. Total flavonoids (TFD) content was expressed based on quercetin equivalents (QE) mg per g of dried extract (mg QE/g DW) [19]. The concentrations of TFD and TFL were achieved based on calibration curve of quercetin (Y= 181.25 X+7.06; R^2= 0.98) (Fig. S1).

Total Flavonols Content

A mixture was prepared for total flavonols (TFL) content assay containing methanolic extract (0.25 mL), aluminum chloride ($AlCl_3$) solution (0.5 mL, 2% v/v), sodium acetate %5 (1.5 mL) and acid methanol (0.25 ml) [19]. After the maintenance of samples at room temperature for about 90 minutes, the absorbance was measured at 440 nm through spectrophotometer. The concentrations of total flavonols were shown as mg quercetin equivalence (QE) per gram of fresh extract.

Anthocyanin Content

At the first stage, acidified methanol (1% HCl) (3 mL) was used for homogenizing dried leaves (0.1 g) and stems (0.1 g) at room temperature [20]. After 24 hours, the total extract was centrifuged for 20 min at 4000 rpm. Acidified methanol solution was used as the blank solution. Then, the content of anthocyanin (Ant) was determined by spectrophotometer (Unico – UV/Vis 2100) at wave length of 550 nm based on the extinction coefficient of Raphanusin (33000 M^{-1} cm^{-1}).

Photosynthetic pigments assay

Extraction of total chlorophyll and carotenoids was done with acetone (80%) (Merck, Com.) [21] through a Spectrophotometer (Unico- UV/Vis 2100).

DPPH Radical Scavenging Activity

The diphenyl-2-picryl hydrazyl (DPPH) was used to estimate the Reactive Scavenging Ability (RSA). To measure RSA activity, 1 mL of DPPH solution in methanol (50 μM) was mixed with 20 μL of the methanolic extract from both the leaves and stem samples [22]. The mixtures were incubated in the dark condition for 20 min. The reduction of DPPH was measured at 515 nm through spectrophotometer (Unico- UV 2100). Ascorbic acid (Sigma, Inc.) was used as a positive control. The inhibition percentage (IP%) of DPPH free radicals was calculated as: IP (%) = [$(A_{blank} – A_{sample})$] / A_{blank}] ×100; Where, A_{sample} and A_{blank} are absorbance values plant organ extract samples and the control, respectively.

Statistical Analysis

The analysis of this experiment was performed as a factorial experiment in a completely randomized design with three replications. Analysis of variance (ANOVA) of the obtained data was performed using SAS software version 9.3 (SAS Institute, 2011), then the comparison of mean traits with the least significant Fisher test (LSD $_{5\%}$) was performed using SAS software. Hierarchical and PCA analyses was done by R- software (ver 3.4.3) [23].

RESULTS

The results of analysis of variance showed that the effects of plant organs (stem and leaves), genotype and the interaction effects of plant organs × genotype had significant effects on all measured traits (Table S1). Based on Table 2, mean comparison for different biochemical traits implied at higher means for different SMs and antioxidant activity in leaves explants rather than stem ones.

Phenolics Contents and Photosynthetic Pigments

Stem Samples

The mean comparison for phenolics in stem accessions of studied species demonstrated that the highest TPC (32.7 mg/GAE DW) and TFD (4 mg/QE g DW) were observed at *D.au*12 (Table 3). For stem samples, the highest TFL value were observed at *D.au*12 (1.6 mg/QE g DW) which was not significantly different with *D.au*9 and *D.au*11 genotypes (Table 3). The least TPC (7.6 mg/GAE DW) were observed at *D.au*6 genotypes (Table 3). The least TFD (1.1 QE/g DW) and TFL (0.7 QE/g DW) in stem samples were observed at *D.au*6 and *D.am*1 genotypes, respectively (Table 3). In stem samples, the highest (73.9 nano mol/g FW) and the lowest (10.7 nano mol/g FW) values for anthocyanin were determined in *D.au*12 and *D.au*10 genotypes, respectively (Table 3). In stem explants, the highest contents for TChl (71.7 mg/g DW) and Car (10.5 mg/g DW) were obtained in *D.au*12 and *D.au*3 genotypes, respectively (Table 3). The lowest content for chlorophyll (19.7 mg/g DW) and carotenoid (2.8 mg/g DW) were observed in genotypes of *D.au*1 and *D.am*2, respectively (Table 3).

Leaves Samples

The highest total phenolics content (34.3 mg/GAE DW) was observed at *D.au*12, but the lowest one (9.1 mg/GAE DW) were detected in genotypes of and *D.au*3 and *D.am*1 genotypes, respectively (Table 4).

Table 3 Comparison of mean stem biochemical traits of different genotypes of *D. ammoniacum* and *D. aucheri* species.

Genotype	Traits						
	TPC¥ (mg/GAE DW)	TFD (mg/QE g DW)	TFL (mg/QE g DW)	Ant (nano/mol g FW)	TChl (mg/g DW)	Carotenoids (mg/g-1 DW)	DPPH (%)
*D.au*1	8.0 ef±0.2	1.8 c-g±0.3	0.9 de±0.1	23.9 fg±1	19.7 e±3	4.7 b-d±0.3	71.1 de±1
*D.au*2	21.2 c±1	2.3 b-e±0.1	1.5 a±0.1	70.6 a±2	31.4 d±0.8	3.9 cd±0.4	80.2 bc±2
*D.au*3	16.6 cd±3	1.7 e-g±0.4	1.4 a±0.2	26.1 ef±0.8	62.8 ab±0.5	10.5 a±1	65.2 fg±0.6
*D.au*4	20.2 c±0.7	2.6 bc±0.2	1.4 a±0.1	46.1 c±1	31.8 d±0.6	3.9 cd±0.4	83.6 b±1
*D.au*5	20.7 c±0.3	1.7 d-g±0.2	0.9 b-e±0.2	46.1 c±1	54.4 bc±3	3.9 cd±0.4	69.9 d-f ±1
*D.au*6	7.6 f±0.3	1.1 g±0.1	1.3 a-d±0.1	23.2 fg±0.7	63.7 ab±6	4.7 b-d±1	70.2 d-f ±1
*D.au*7	8.8 ef±1	1.5 e-g±0.3	0.8 e±0.2	41.2 cd±1	29.2 de±4	5.3 bc±0.2	55.8 h±1
*D.au*8	20.2 c±0.4	2.1 c-f±0.1	0.71 e±0.2	15.8 gh±0.7	24.7 de±1	6.2 b±0.8	68.3 ef±2
*D.au*9	22 bc±4	2.6 b-d±0.5	1.4 a±0.3	71.2 a±7	70.8 a±3	8.9 a±1	77.1 c±3
*D.au*10	13.9 de±3	1.7 e-g±0.3	0.9 c-e±0.1	10.7 h±0.7	25.2 de±2	3.5 cd±0.5	59.7 gh±2
*D.au*11	28.1 ab±0.3	3.1 b ±0.3	1.5 a±2	55.7 b±7	20.0 e± 2	9.3 a±1	80.2 bc±3
*D.au*12	32.7 a±0.6	4.0 a±0.3	1.6 a ± 0.1	73.9 a±3	71.7 a±9	3.7 cd±2	94.3 a±1
*D.am*1	17.4 cd±1.1	1.5 fg±0.1	0.7 e ± 0.1	21.8 fg± 0.6	26.0 de±0.9	3.7 cd±0.2	75.4 cd±2
*D.am*2	19.6 cd±0.7	1.62 eg±0.4	1.3 a-c ±0.1	33.5 de±1.5	44.2 c±1.6	2.8 d±0.8	78.2 bc±1.9

¥ TPC: total phenolics content; TFD: total flavonoids, TFL: total flavonols; Ant: Anthocyanins; Tchl: total chlorophyll; DPPH: 2, 2-diphenyl-1-picrylhydrazyl. The same letters in each column are not significant at $P< 0.05$ with LSD test.

Table 4 Comparison of mean leaves biochemical traits of different genotypes of *D. ammoniacum* and *D. aucheri* species

Genotype	Traits						
	TPC ¥ (mg/GAE DW)	TFD (mg/QEg DW)	TFL (mg/QEg DW)	Ant (nano mol/g FW)	TChl (mg/g DW)	Carotenoids (mg/g DW)	DPPH (%)
*D.au*1	28 bc±0.9	4.4 b±0.3	1.1 d±0.01	77 c-e±2.1	67 bc±3	2.1 f±0.5	90 a±1
*D.au*2	26.5 b-e±1	3.2 d±0.5	3.6 a±0.1	158 b±3.2	85 b±8	7.4 fg±2	75 b-e±0.6
*D.au*3	9.2 g±0.2	1.4 f±0.04	1.7 cd±0.2	179 b±29.4	81 b±2	18 a±0.4	68 e±3
*D.au*4	10.8 g±1	3 de±0.6	1.7 cd±0.2	100 cd±3.8	68 bc±19	9.2 b-e±5.5	81 a-e±8
*D.au*5	27.5 b-d±1	3.4 cd±0.4	1.0 d±0.0	102 cd±8.2	149 a±9	6.5 c-f ±1	72 c-e±5
*D.au*6	9.2 g±0.5	3.1 d±0.1	1.5 cd±0.1	45 de±1.1	163 a±7	12 a-d ±1	85 a-d±3
*D.au*7	9.8 g±0.6	2.5 de±2	1.9 cd±0.3	250 a±64.6	86 b±19	12.1 a-d ±5	73.9 c-e±8
*D.au*8	25.9 c-e ±1	3.3 cd±0.4	1.1 d±0.2	72 c-e±5.2	23 f±1	1.5 f±0.2	71 de±1
*D.au*9	29.9 b±1	5.1 ab±0.1	1.4 cd±0.1	42.9 e±0.6	29 d-f ±4	6.1 d-f ±0.5	92 a±2
*D.au*10	16.7 f±0.2	2.6 de±0.1	1.6 cd±0.1	38 e±1.1	52 c-e±0.7	13.3 a-c±0.6	88 ab±2
*D.au*11	23.1 e±1	4.4 b±0.2	1.8 cd±0.3	72 c-e±2.2	31 ef±2	14.5 ab±1.1	86.7 a-c±2
*D.au*12	34.3 a±1	5.5 a±0.5	2.1 b-d±0.1	49.8 de±2.1	71 bc±0.7	17.2 a ±1	91.3 a±0.8
*D.am*1	9.1 g±0.3	2.1 ef±0.2	2.2 bc±0.8	126 bc±3.1	62 b-d ±3	3.9 ef ±1.2	71.8 de±10
*D.am*2	24.5 de±0.9	4.2 bc±0.3	2.9 ab±0.7	102 cd±10.3	150 a±5	12.9 a-c ±1.3	80.9 a-e±4

¥TPC: total phenolics content; TFD: total flavonoids, TFL: total flavonols; Ant: Anthocyanins; TChl: total chlorophyll, DPPH: 2, 2-diphenyl-1-picrylhydrazyl. The same letters in each column are not significant at $P< 0.05$ with LSD test.

Mean comparison demonstrated that the highest TFD (5.5 mg/QE g DW), and TFL (3.6 mg/QE g DW) were observed in *D.au*12 and *D.au*2 genotypes, respectively (Table 4), but the least values for TFD (1.4 mg/QE g DW) and TFL (1 mg/QE g DW) were observed at *D.au*3 and *D.au*5 genotypes (Table 4). In leaves samples, the highest content for anthocyanin (250 nano mol/g FW) was observed in *D.au*7 while the lowest (38 nano mol/g FW) was observed in *D.au*10 (Table 4). The highest content for leaves total chlorophyll (163 mg/gDW) was obtained in *D.au*6 genotype, but the lowest one (23 mg/g DW) was observed in *D.au*8 (Table 4). The highest (18 mg/gDW) and the least (1.5 mg/g DW) values for total carotenoids were identified in *D.au*3 and *D.au*8 genotypes, respectively (Table 4).

Antioxidant Activity

Higher free radical scavenging activity is distinguished with higher IP% values. For stem samples, the highest activity for DPPH (94.3%) were observed in stem *D.au*12 genotype but the lowest level of DPPH with (55.8%) was dedicated in *D.au*7 (Table 3). The highest (92%) and the lowest (68%) IP values were detected in *D.au*9 and *D.au*3 genotypes, respectively (Table 4) in leaves samples.

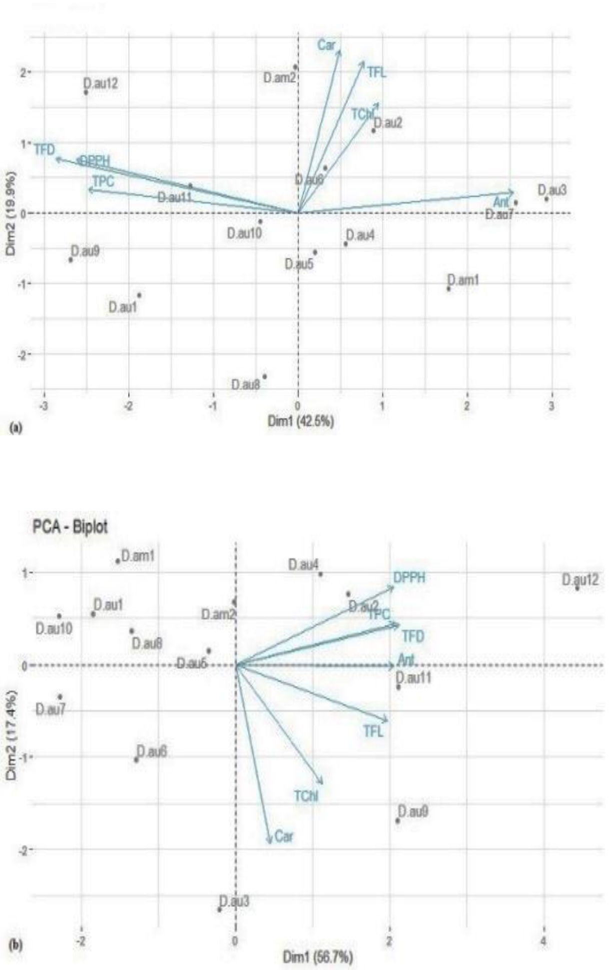

Fig. 1 PCA analysis to classification of 15 chemical constituents in EOs from leaves (a) and stem samples (b) in different *Dorema* species. Genotypes abbreviation: *D.au*1: *D. aucheri*1, *D.au*2: *D. aucheri*2, *D.au*3: *D. aucheri*3, *D.au*4: *D. aucheri*4, *D.au*5: *D. aucheri*5, *D.au*6: *D. aucheri*6, *D.au*7: *D. aucheri*7, *D.au*8: *D. aucheri*8, *D.au*9: *D. aucheri*9, *D.au*10: *D. aucheri*10, *D.au*11: *D. aucheri*11, *D.au*12: *D. aucheri*12, *D.am*1: *D. ammoniacum*, *D.am*2: *D. ammoniacum*.

Principle Component and Hierarchical Analysis

To evaluate relationships among *Dorema* genotypes, principle component analysis (PCA) was performed based on different studied traits. The scatter plot for different constituents and genotypes revealed phytochemical distances among the genotypes in the plot that reflects their genetic distances (Fig. 1a and b). According to PCA classification for stem samples, in about 74.1% of total variations were explained by the first two principle components (PC_1 and PC_2) (Fig. 1a). PC_1 and PC_2 justified a moderate share of percentage of phytochemical variance for total genotypes. The first PC shown the most positive correlation with TFD (0.44), TPC and Ant (0.43) and the least correlation with carotenoids (0.09) (Table S2). The second PC (PC_2) explained 17.4 % of the variation and shown the most positive correlation with carotenoids (0.73) and highest negative correlation with DPPH (-0.32). In stem samples, studied genotypes were divided into three groups. The genotypes of *D.au2*, *D.au*4, *D.au*11 and *D.au*12 formed separate groups with higher TPC, TFD, Ant and DPPH, which *D.au*12 had the highest values for TPC, TFD and DPPH (Fig. 1a). The genotypes of *D.au*3 and *D.au*9 genotypes formed second group, which including highest values for TChl and carotenoids. The two accessions of *D. ammoniacum* (*D.am*1 and *D.am*2) and other *D. aucheri* accessions formed the third group (Fig. 1a) which had relatively lower values for studied traits.

According to PCA classification for leaves samples, most of the variations (62.4%) were explained by the first two principle components (PC_1 and PC_2) (Fig. 1b). The first PC (PC_1) explained 42.5 % of the variation which shown the most positive correlation with TFD (0.52), and negative correlation with Ant (-0.46) (Table S3). The second PC (PC_2) explained 19.9 % of the variation and shown the most positive correlation with carotenoids (0.62) and least correlation with anthocyanin (0.07) (Table S3). According to PCA analysis the genotypes of *D.am* 2, *D.au*2, *D.au*4, *D.au*5, *D.au*6 and D.au 10 formed the first group (Fig. 1b). The genotypes of *D.au*3, *D.au*7 and *D.am*1 formed the second group, which *D.au*3 and *D.au* 7 had higher values for anthocyanins. The genotypes of *D.au* 1, *D.au* 9, *D.au* 11 and *D.au* 12 formed the third group, which the genotypes of *D.au* 9 and *D.au* 12 had higher values for TPC, TFD and DPPH.

Based on cluster analysis, the *Dorema* stem samples were classified into three groups which showed high similarity with findings from PCA analysis for and stem samples including: **1]** *D.au*1, *D.au*4, *D.au*11, *D.au*12 **2]** D.au3, *D.au*9 **3]** *D.am*1 and *D.am*2, *D.au*1, *D.au*5, *D.au*6, *D.au*7, *D.au*8, *D.au*10 (Fig. 2). Moreover, the leaves samples of all genotypes were categorized into three groups based on different studied traits including: **1]** *D.au*1, *D.au*9, *D.au*11, *D.au*12 **2]** *D.au*3, *D.au*7 **3]** *D.am*1 and *D.am*2, *D.au*2, *D.au*4, *D.au*5, *D.au*6, *D.au*8, *D.au*10 (Fig. 3). The grouping of the genotypes in hierarchical analysis confirms the PCA results in grouping.

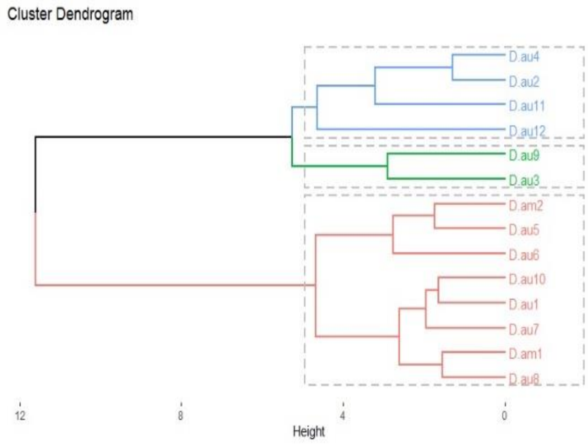

Fig. 2 Dendrogram of 14 *Dorema* accessions for stem samples using Ward clustering method based on different secondary metabolites and antioxidant activity. Genotypes abbreviation: *D.au*1: *D. aucheri*1, *D.au*2: *D. aucheri*2, *D.au*3: *D. aucheri*3, *D.au*4: *D. aucheri*4, *D.au*5: *D. aucheri*5, *D.au*6: *D. aucheri*6, *D.au*7: *D. aucheri*7, *D.au*8: *D. aucheri*8, *D.au*9: *D. aucheri*9, *D.au*10: *D. aucheri*10, *D.au*11: *D. aucheri*11, *D.au*12: *D. aucheri*12, *D.am*1: *D. ammoniacum*, *D.am*2: *D. ammoniacum*.

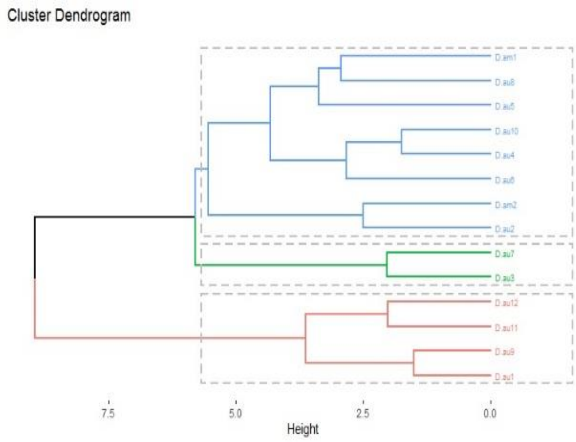

Fig. 3 Dendrogram of 14 *Dorema* accessions for leaves samples using Ward clustering method based on different secondary metabolites and antioxidant activity. Genotypes abbreviation: *D.au*1: *D. aucheri*1, *D.au*2: *D. aucheri*2, *D.au*3: *D. aucheri*3, *D.au*4: *D. aucheri*4, *D.au*5: *D. aucheri*5, *D.au*6: *D. aucheri*6, *D.au*7: *D. aucheri*7, *D.au*8: *D. aucheri*8, *D.au*9: *D. aucheri*9, *D.au*10: *D. aucheri*10, *D.au*11: *D. aucheri*11, *D.au*12: *D. aucheri*12, *D.am*1: *D. ammoniacum* 1, *D.am*2: *D. ammoniacum* 2.

Correlations Between Different Biochemical Traits

Simple correlations were calculated between different studied traits in two organs (leaves and stem) (Fig. 4 and 5). For stem samples, a significant positive correlation was observed between the amount of TFD and TPC (0.85**), which indicates a consistent increase in their amount (Fig. 4). Also a positive and significant correlation between the TFD with other traits as Ant (0.73**), and DPPH (0.76**) was observed. A positive and significant correlation between Ant with TFL (0.69**) was observed (Fig. 4). Also in stem samples, there was positive and significant correlation between DPPH activity with TPC (0.76**), TFD (0.76**) TFL (0.67**) and Ant (0.66*) (Fig. 4).

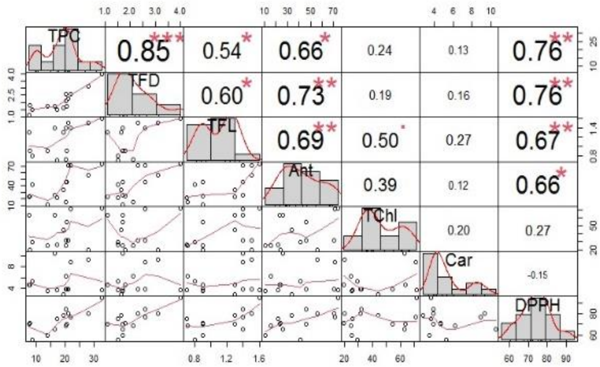

Fig. 4 Correlation coefficients between secondary metabolites and antioxidant activity on studied stem *Dorema* accession. TPC: total phenolics content; TFD: total flavonoids, TFL: total flavonols; Ant: Anthocyanin; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; TChl: total chlorophyll; Car; carotenoids; * and ** significant at $P< 0.05$ and $P< 0.01$, respectively.

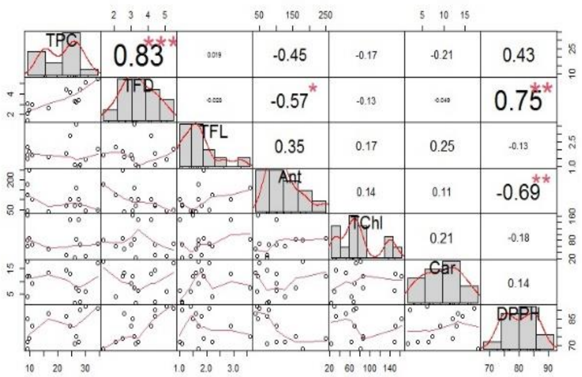

Fig. 5 Correlation coefficients between secondary metabolites and antioxidant activity on studied leaves *Dorema* accession. TPC: total phenolics content; TFD: total flavonoids; TFL: total flavonols; Ant: Anthocyanin; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; TChl: Total chlorophyll; Car; carotenoids; * and ** significant at $P< 0.05$ and $P< 0.01$, respectively.

For biochemical traits in leaves samples, a positive and significant correlation was observed between TPC and TFD (0.83**), indicating a direct relationship between TPC and TFD (Fig. 5). Also there was a significant positive correlation between

DPPH and TFD (0.75 **) (Fig. 5). Negative and significant correlation between TFD and Ant (-0.57*), and between antioxidant activity (DPPH) and anthocyanin content (-0.69 **) was obtained (Fig. 5).

DISCUSSION

This study was performed to evaluate 1different SMs and antioxidant activity in two different parts (leaves and stem) of two *Dorema* species (*D. ammoniacum* and *D. aucheri*). Literature review showed that no comprehensive study has done on evaluation of a wide range of SMs in different species of *Dorema* from different plant organs. The natural antioxidant potential has become one of the famous standards in most of industries, especially pharmaceutical sciences and nutraceutical as food preservatives and nutraceutical supplements [24]. Different phenolics such as flavonoids, flavonols and anthocyanins can act as free radical scavenger compounds with natural antioxidants roles [4, 25]. Also photosynthetic pigments as chlorophyll and carotenoids have major functions against reactive oxygen species in protective issues in plants [3]. Some previous research demonstrated at measurement of some phenolics (e.g. TPC, TFD and anthocyanin) in different *Dorema* species as D. *aucheri* [16, 17] but no study is reported in *D. ammoniacum*. The superiority of TPC, TFD, Car and Ant in leave samples rather than stem was in line with the reports of Mianabadi *et al*. [16] in *D. aucheri*. Visibly, significant differences exist among two plant organs for all the studied traits, implied at different extraction source on for delivery of different SMs in plants. In this study, differences in the plant source for extraction, eco-climatic differences, species and sub- specie variations have been retrieved to significantly influence the type and its quantity for different SMs, especially phenolic contents similar to previous reports on other medicinal plants [26-28]. Genetic (e.g. different chemotypes) and environmental (e.g. rainfall and temperature) factors have significant effects on the production of total phenolics in medicinal plants [20, 26]. Among different climatic factors, higher rainfall, relative humidity and elevations, in contrary to lower temperatures might be the main reason for higher content of total phenolics in *D.au*12 and *D.au*2 accessions, similar to the reports in other medicinal plants *Scrophularia striata* Boiss [27] and *D. aucheri* [17] for higher TPC production. The synthesis of certain products of the phenyl propanoid pathway including different favonoids (as favanones, favonols, anthocyanin) and chlorophyll are encouraged at higher elevations in response to UV in medicinal plants [29, 30, 31], similar to these findings. Literature review showed no reports on the measurement of flavonols in *Dorema* species. Total flavonols have a broad range of physiological effects in plants as natural antioxidants [25]. Superior genotypes such as *D.au*5 could be used as nutraceuticals in food industries or as a plant with special interest in medical researches.

Anthocyanins, with broad medical properties as antiviral and anti-inflammatory properties could reduce the risk of different human diseases [32]. So, stem samples of *D.au*9 and *D.au*12 genotypes, enriched with Ant, may be recommended as a natural source of non-enzymatic antioxidant for use in food and medical industries.

The current study revealed that both *Dorema* species possesses a powerful antioxidant activity in leaves and stem explants. The genotype of *D.au*12, the highest elevation, showed the most antioxidant activity among all of the accessions. It may be confirmed the positive relationship between the increase of antioxidant activity with altitude in in *Dorema* species. This finding was also reported in previous reports [27,29]. On the other hand, high antioxidant activity of these accessions due to their natural antioxidant properties can increase the benefits of these species as a complementary food and medical industries.

However, the correlations of the different phenolics and antioxidant activity of leaves and stem in *Dorema* species are scarcely explored. Some negative observed correlations between different traits, implied that production of these components in the studied organs s are inversely related to each other, in a way that by increasing the production of one of them the production of another one reduced and vice versa. However, the incidence of these correlations may depend on differences between two species and the growing conditions.

Interestingly, a positive correlation between DPPH activity and TFD in both samples (leaves and stem) demonstrated at high antioxidant activity of flavonoids compounds in these species in both organs. A positive correlation between DPPH activity with TFD, TFL and Ant in stems could be raised by the more various antioxidant compounds in stems, rather than leaves in these species. However, it could be suggested that the antioxidant activity of *Dorema* species on scavenging ability of DPPH are induced more by total flavonoids in both leaves and stem samples through their hydrogen donating capacity.

CONCLUSION

It seems that the evaluation of different geographical conditions, genetic diversity and different extraction source for biochemical compounds, is considered as significant factors for incidence of variation for different constituents phenolics, photosynthetic pigments and antioxidant activity in two *Dorema* species (*D. aucheri* and *D. ammoniacum*). The leaves showed the higher yield for different SMs and antioxidant activity. Therefor leaves of *Dorema* species may be more suitable for economic exploitation in different food industries and pharmaceutical aims when seeking the medicinal effects of the plant. However, it seems that more investigations are required to evaluate the safety and medical properties of the flavonoids compounds in both leaves and stem extracts and the effect of different edaphic factors on SMs variation in *D. aucheri* and D. *ammoniacum.*

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