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Evaluation of morphophysiological and phytochemical characteristics of *Datura stramonium* and *D. innoxia* from Iran under controlled conditions

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Abstract

Objective: *Datura* genus is an important annual or perennial medicinal plant, belonging to Solanaceae family. This study aimed to discover the diversity of compounds in the essential oils of *Datura stramonium* and *D. innoxia* focusing on morphophysiological and phytochemical traits.

Methods: Seeds of *D. stramonium* and *D. innoxia* were collected from the provinces of West Azarbaijan, Ardabil, and Yazd in Iran. They were sown in grow bags and placed in a greenhouse for three months at an air temperature of 25 °C. Air-dried areal parts were prepared and their essential oils were isolated by hydro-distillation. The essential oils' content and composition were measured, using gas chromatography.

Results: The comparison of morphophysiological, phytochemical, and volatile compounds of *Datura* showed a significant variation in all examined *Datura* populations. Camphor (17.5%), selin-11-en-4alpha-ol (8.5%), *n*-Dodecane (6.8%), and isobornyl acetate (6.3%) were major compounds in the oil of *D. stramonium*. Those of *D. innoxia* were mainly fatty alkanes, including n-dodecane (13.8%), n-tetradecane (12.0%), n-hexadecane (11.0%), and 3-dimethyl-2-pentanol (6.8%). The maximum values of leaf length, leaf width, and chlorophyll as important indicators among populations were identified in *D. stramonium* from Ardabil (P2) with 15.7 cm, 14.2 cm, and 6.06 mg/100 g FW, respectively. Genotype plus Genotype by Trait interaction (GGT) biplot analysis showed that the two first principal components (PC1 and PC2) acquired 87.6% of the total variation. Also, GGT biplot polygon was able to separate Turgor, a West Azarbaijan population (P3) with higher values of antioxidant activity traits, chlorophyll a (Chla), Chlb, ChlT, carotenoid, SPAD, flowering branches, seed weight, and altitude.

Conclusion: The extracts from the aerial parts of *Datura* have a relatively high level of antioxidants and total phenolic contents. Notably, significant differences were identified between *D. stramonium* and *D. innoxia*, regarding the studied

morphophysiological and phytochemical traits. Population P4 (*D. innoxia* grown in Abarkuh, Yazd), is recommended for its promising phytochemical properties.

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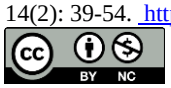

Abbreviations: AA: Antioxidant activity; BHT: Butylated hydroxytoluene; CAR: Carotenoid; Chla: Chlorophyll a; Chlb: Chlorophyll b; ChlT: Total chlorophyll; DW: Dry weight; FW: Fresh weight; GC: Gas chromatography; GC-MS: Gas chromatography-mass spectrometry; GGT biplot: Genotype plus Genotype by Trait interaction biplot; RSC: Radical scavenging capacity; TFC: Total flavonoid content; TPC: Total phenolic content.

Introduction

The genus *Datura* belongs to the Solanaceae family, and consists of 13 species, originating from America and found as an invasive plant in most subtropical regions of the world (Papagrigoriou *et al.* 2019). Among these, *Datura stramonium*, *D. innoxia*, and *D. metel* are the valuable species, also known as devil's trumpets (Partap *et al*. 2019). *Datura* genus comprises annual or perennial herbs, rarely shrubs or trees, with significant variability in morphology (Maslo and Šarić 2019). The phenotypic, physiologic, and genetic differences in medicinal crops lead to the variation of bioactive components within and between the species (Partap *et al*. 2019). *D. stramonium* and *D. innoxia* are important species of *Datura* genus with several uses in traditional and modern medicine (Batool *et al.* 2020; Al-Zharani *et al.* 2021). *D. stramonium* has traditionally been consumed for medicinal applications such as anti-inflammatory, analgesic, antidiarrhoeal, larvicidal, pesticidal toxicity, antifungal, and anticonvulsant. Furthermore, it is also used in the treatment of epilepsy and asthma (Aboluwodi *et al.* 2017; Mohammed *et al.* 2021). *D. stramonium* contains different types of alkaloids, including atropine, hyoscamine, scopolamine, tigloidin, aposcopolamine, apoatropine, and N-oxide (Batool *et al.* 2020). *D. innoxia* with big leaves and pale green is present as cultivated (as medicinal plant) and wild (Benabderrahim *et al.* 2019; Chamani *et al.* 2020). *Datura* has a significant effect on the eyes, nervous system, heart, blood, and body secretions due to its varied alkaloids (Ganjali *et al.* 2022). In addition to its alkaloids, saponins, tannins, steroids, flavonoids, phenols, and glycosides have been identified as major and important components of *D. stramonium* and *D. innoxia* (Al-Zharani *et al.* 2021; Avila *et al.* 2023).

There are a few studies on the chemical composition of *D. stramonium* essential oils (EOs), mostly of the leaves and seeds (Papagrigoriou *et al.* 2019). Studies of volatile oils from *D. stramonium* leaves have already been reported by Chandan *et al.* (2020). Citral (26.5%), 4,8-dimethyl-3,8-dien-2-one (11.2%), sesquirosefuran (11.1%), and geraniol (10.5%) were the major components in the EO of *D. stramonium* seeds (Aboluwodi *et al.* 2017). The major constituents of the leaves' oil, collected from Solan (India), were phytol acetate (10.76%), β-damascenone (9.67%), and β-eudesmol (7.2%) (Chandan *et al.* 2020). There are no reports about the volatile components of the *D. innoxia* as well as antioxidant activity, total phenol content, and total flavonoid of *D. innoxia* and *D. stramonium* from Iran. Also, a comprehensive study of their agro-morphological and phytochemical characteristics in the same ecological conditions has not been attempted yet. Investigating the diversity of plant species has been carried out with specific goals, including determining the contents and constituents of effective substances. Therefore, in the present study, seeds of *D. stramonium* and *D. innoxia* were collected from various regions across Iran to gain a deeper understanding of their chemical constituents and identify superior populations that could be introduced for industrial applications. This study can uncover intraspecific variations in the EO compounds of the two *Datura* species, focusing on morphophysiological and phytochemical traits.

Materials and Methods

Seed collection locations

Mature seeds were collected in late November 2021, from plants of *D. stramonium*, growing in the provinces of Ardabil, and West Azarbaijan, and from *D. innoxia* in the provinces of Yazd and West Azarbaijan of Iran (Table 1, Figure 1).

Plant materials and growing conditions

The collected seeds were planted in grow bags with 10 kg soil (sandy loam) and placed for three months in a greenhouse (25 °C) in the College of Agriculture, Tarbiat Modares University, Tehran, Iran. This study was conducted as a completely randomized design with three replications. All areal parts of both *D. stramonium* and *D. innoxia* plants, including stems, leaves, flowers, and seed-containing capsules, were collected and dried for the subsequent steps.

Essential oil extraction

A Clevenger-type apparatus was used to extract the EO from 50 g of shade-dried aerial parts (collected at the 50% flowering stage) of *D. stramonium* and *D. innoxia* by using hydro-distillation

Table 1. Information about the collection sites of Iranian endemic *Datura* populations.

Code	Species	Collection locations	Longitude (E)	Latitude (N)	Altitude (m)
P1	*D. stramonium*	Salmas, West Azarbaijan*	45°04'13"	37°42'18"	1330
P2	*D. stramonium*	Ardabil, Ardabil	48°15'34"	38°22'07"	1332
P3	*D. innoxia*	Turgor, West Azarbaijan	44°52'21"	37°12'11"	1800
P4	*D. innoxia*	Abarkuh, Yazd	53°26'20"	31°12'60"	1519

*The sample was collected from the Salmas City, West Azarbaijan Province, Iran.

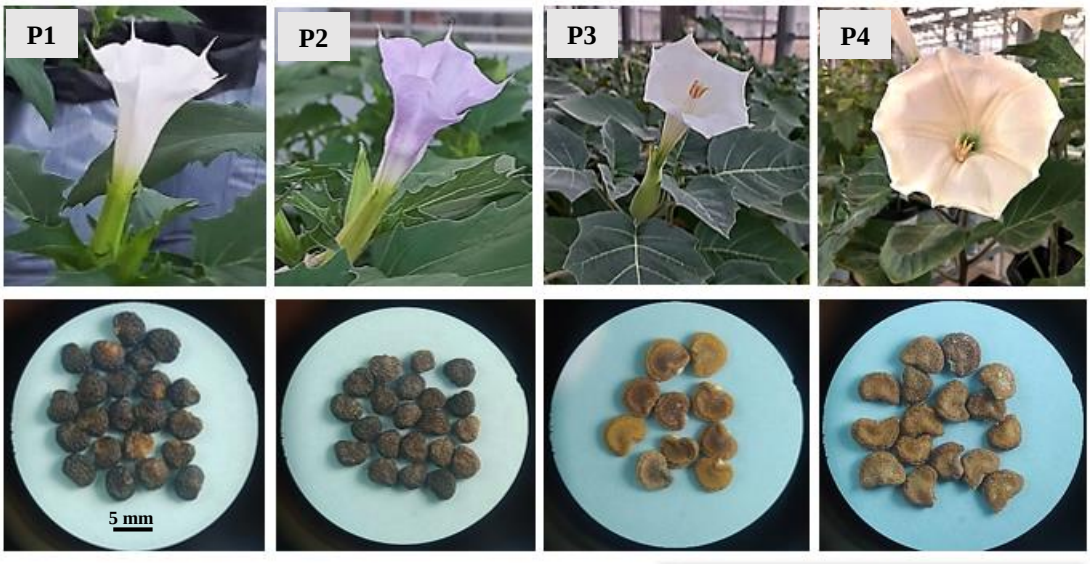

Figure 1. Flowers and seeds of *Datura stramonium* (P1, P2) and *D. innoxia* (P3, P4) populations.

for 3 h, as recommended in the British Pharmacopoeia (2009). The EOs were separated from the water and dried over anhydrous sodium sulfate and stored at 4 °C until analysis. The replications of each sample were mixed and only one EO sample was selected for analysis. The gas chromatography (GC) analysis was performed, using an Agilent Technologies 7890B (Santa Clara, CA, USA) with a flame ionization detector. The instrument was equipped with an HP-5 fused silica column (length 30 m, inner diameter 0.32 mm, and film thickness 0.25 μm) and helium was used as the carrier gas at a flow rate of 1.0 ml min^{-1}. The qualification of individual peaks was determined by injecting the oil to a Thermoquest-Finnigan gas chromatograph, coupled with a trace GC-MS with the same characteristic 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 70 eV. The ion source and interface temperatures were 200 °C and 250 °C, respectively. Identification was confirmed by the comparison of each component's mass spectra with those of the internal mass spectra library of the main library, Wiley 7.0 and Adams, and further identification was based on a comparison of peak

retention indices by using a homologous series (C8 to C24) recorded under the same operating conditions and the published data (Adams 2007).

Morphological and physiological features

Under the same conditions, four important morphological traits such as leaf length, leaf width, number of flowering branches, flowering branch length (cm), and seed weight (mg) were measured, using a ruler and caliper (Partap *et al*. 2019). Moreover, the SPAD CCM-200 Chlorophyll Meter (Porto Alegre, Brazil) was used to determine the chlorophyll content (Jiang *et al.* 2022). The extraction of pigments was carried out in stoppered tubes. Fresh leaves of samples were prepared with a laboratory homogenizer, using about 1 g of fresh material. Three extraction solutions were used for each sample of 90% (v/v) aqueous methanol solution. The homogenized mixture was separated by centrifugation at 1000 g for 15 min. The analytical determination was performed with a spectrophotometer (Smart spec plus, BIORAD, USA) at 645, 653, 662, and 664 nm, for chlorophyll a and b (according to each extraction solvent) and 470 nm for carotenoids (Costache *et al.* 2012).

Preparation of methanolic extract

The dried aerial parts of *D. stramonium* and *D. innoxia* were used in three replications to grind into powders for each sample. The extracts of samples were prepared by sonicating 5 g of dried plant material for 30 min in 50 ml of methanol. All the extracts were filtered through Whatman No.1 filter paper and then concentrated in a rotary evaporator at 40 °C. The extracts were finally dried and stored at 4 °C until analysis (Norani *et al.* 2023).

Measuring the total phenolic content and total flavonoid content

Total phenolic content (TPC) was determined, using the Folin-Ciocalteu method with three biological replications (Slinkard and Singleton 1977; Sivakumar 2021). The absorbance of the mixture was measured after 2 h at 765 nm, using a spectrophotometer (Smart Spec Plus, Bio-Rad, USA). Gallic acid was used as the standard for a calibration curve, and the results were expressed as mg of gallic acid equivalent dry weight of extract (mg GAE/g DW). The colorimetric method of Ordonez *et al.* (2006) was used to determine the total flavonoid content (TFC). The experiment for each extract was done in triplicate. A calibration curve was prepared, using a series of methanolic quercetin solutions (10, 50, 100, 250, 500, and 1000 $\mu g\ ml^{-1}$). The results were expressed as mg of quercetin equivalents dry per gram dried weight of extract (mg QE/g DW Ext).

Assessment of antioxidant activity against 2,2-diphenyl-2 picrylhydrazyl hydrate

The antioxidant activity (AA) of methanolic extracts of *Datura* populations was evaluated with 2,2-diphenyl-2 picrylhydrazyl hydrate (DPPH) radical scavenging activity based on the previously described method of Bozin *et al.* (2007), using IC_{50} to compare the antioxidant properties. The absorbance of the samples was measured at 517 nm with ELISA reader (Epoch, BioTek, USA).

The radical scavenging capacity (RSC) was calculated as:

$$\text{Inhibition} = [(Ab - As)/Ab] \times 100$$

Where "Ab" is the absorbance of the blank, and "As" is the absorbance of the sample extract, or butylated hydroxytoluene (BHT) is a positive control. IC_{50} values were also calculated.

Statistical analysis

The residuals were first tested for normality and subsequently, the analysis of variance (ANOVA) was performed based on a completely randomized design with three replications. Mean comparisons were performed, using the least significant difference (LSD). The analysis was conducted, using SAS (SAS Institute Inc. 2009) and SPSS software version 20 (Ganesh and Mohanraj 2022). Using GGEBiplot software, the GGT biplot method was used to analyze the effects of G (genotype) + GT (genotype on trait interaction).

Results

Essential oil composition

Figure 2 illustrates the GC profiles of the essential oils extracted from the areal parts of *D. stramonium* (P1) and *D. innoxia* (P4). The hydro-distillation of the areal parts of *D. stramonium*, and *D. innoxia* yielded EOs with a concentration of 0.02% (v/w) relative to the dry weight of the plants. In the sample P1, 41 compounds were identified, accounting for 98.3% of the oil composition (Table 2). The primary constituents of the essential oil from P1 included camphor (17.52%), selin-11-en-4alpha-ol (8.5%), n-dodecane (6.8%), and isobornyl acetate (6.3%). Other notable components in this oil were β-caryophyllene, n-hexadecane, and borneol. In contrast, sample P4 revealed 31 compounds that comprised 90.2% of the essential oil (Table 3). The major constituents identified in sample P4 included n-dodecane (13.83%), n-tetradecane (11.96%), n-hexadecane (10.97%), and 3-dimethyl-2-pentanol (6.79%). Additional significant components found in the oil of sample P4 were cyclopropaneoctanal, tetrahydro citronellene, n-octadecane, and n-nonane. The EO yields from the areal parts of samples P2 and P3 were 0.03% and 0.02% (v/w), respectively. In the areal parts of sample P2, 42 components were identified, accounting for 95.06% of the oil's composition. However,

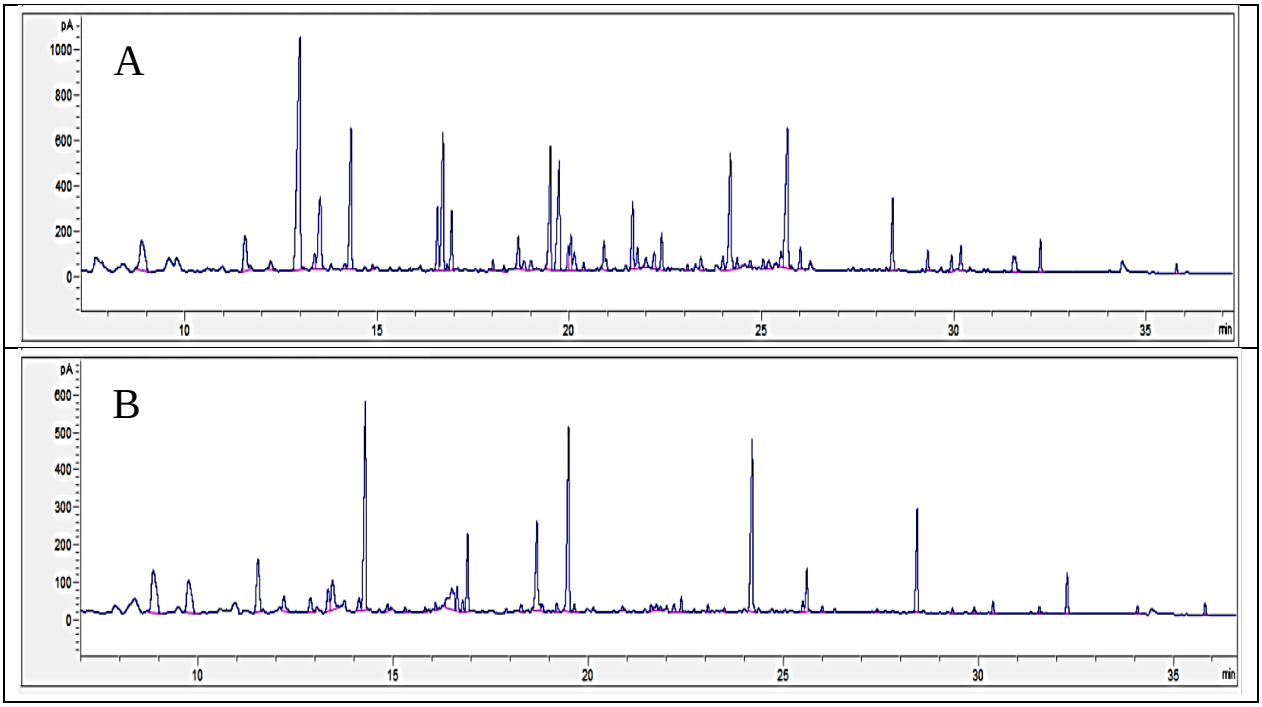

Figure 2. GC profile of essential oils from areal parts of *Datura stramonium* (P1; A) and *D. innoxia* (P4; B).

sample P3 contained 27 compounds that made up 78.9% of its EO. Key components found in the EO of P2 included camphor (29.9%), selin-11-en-4alpha-ol (13.0%), and borneol (10.4%). In sample P3, the major constituents were primarily fatty alkanes, with notable compounds such as n-decane (19.99%), 7-pentadecyne (11.78%), and linoleic acid (6.13%) (Morovati *et al.* 2023).

Morphological traits

The results of ANOVA for various morphological traits of the *Datura* populations are presented in Table 4. The maximum value of leaf length and leaf width among populations were observed in P2 with 15.7 cm and 14.2 cm, respectively (Figures 3A and 3B) while the minimum values of leaf length (10.3) were obtained from P3 and P4 populations. Seed weight varied from 5.9 (P2) to 14.6 (P3) (Figure 3C).

Physiological traits

ANOVA showed that all physiological traits were significantly ($p \leq 1\%$) influenced by populations (Table 5). A comparison of the amount of chlorophyll a (Chla) from *Datura* populations showed that the highest Chla content was detected in the leaf extract of P2 with 6.06 mg/100 g FW (Figure 4A). Also, the lowest amount of Chla belonged to P4 with 3.9 mg/100 g FW. The Chlb varied from 0.74 to 1.97 mg/100 g FW (Figure 4B). The P2 and P3 samples exhibited the highest ChlT of 7.97 mg/100 g FW, while the lowest levels were recorded in P4 at 4.80 mg/100 g FW, as well as in P1 (Figure

4C). The highest amount of carotenoids (CAR) was identified in P3 (2.59 mg/100 g FW; Figure 4D), also the samples P3 and P4 exhibited the highest SPAD (59.94 mg/100 g FW; Figure 4E), while the lowest values of CAR and SPAD were obtained in P4 (1.66 mg/100 g FW) and P1 (20.83 mg/100 g FW), respectively.

Table 2. Chemical composition (%) of areal parts' essential oils of *Datura stramonium* (P1).

No.	RT	Components	Molecular formula	%	RI
2	8.7	α-Pinene	$C_{10}H_{16}$	3.31	932
3	9.6	1,8-Cineole	$C_{10}H_{18}O$	2.7	1026
5	12.2	2-hydroxy-4-methyl Pentanoic acid	$C_6H_{11}O_3$	0.67	-
6	13.1	Camphor	$C_{10}H_{16}O$	17.52	1141
7	13.4	Levomenthol	$C_{10}H_{20}O$	0.81	1170
8	13.7	Borneol	$C_{10}H_{18}O$	4.83	1175
10	14.2	*n*-Dodecane	$C_{12}H_{26}$	6.76	1200
11	14.7	4,8-Dimethylnona-3,8-dien-2-one	$C_{11}H_{18}O$	0.17	1240
13	16.6	Geraniol	$C_{10}H_{18}O$	2.32	1267
14	16.8	Isobornyl acetate	$C_{12}H_{20}O$	6.3	1283
15	16.9	*n*-Tridecane	$C_{13}H_{28}$	2.35	1300
16	18.0	Nonanoic acid	$C_9H_{18}O_2$	0.4	1308
17	18.7	α-Copaene	$C_{15}H_{24}$	1.44	1374
18	18.8	Geranyl acetate	$C_{12}H_{20}O_2$	0.45	1387
19	19.0	2,3,3,4,5-pentaethyl 1,2,5-Oxadiborolane	$C_{10}H_{20}$	0.52	-
20	19.5	*n*-Tetradecane	$C_{14}H_{30}$	5.6	1400
21	19.7	β-caryophyllene	$C_{15}H_{24}$	6.01	1408
22	19.8	Isobornyl isobutanoate	$C_{14}H_{24}O_2$	0.96	1410
23	23.0	β-Damascenone	$C_{13}H_{18}O$	1.4	1417
24	20.1	(*E*)- Caryophyllene	$C_{15}H_{24}$	0.96	1441
25	20.4	(*E*)-β-Farnesene	$C_{15}H_{24}$	0.28	1454
26	20.9	Borane dimethyl	-	1.42	1482
27	21.7	Germacrene D	$C_{15}H_{24}$	2.72	1484
28	21.8	β-selinene	$C_{15}H_{24}$	0.83	1489
29	22.0	Bicyclogermacrene	$C_{15}H_{24}$	0.45	1500
30	22.2	Isobornyl isovalerate	$C_{15}H_{26}O_2$	0.7	1521
31	22.4	Isobornyl 2-methyl butanoate	$C_{15}H_{26}O_2$	1.41	1523
33	24.0	Caryophyllene oxide	$C_{15}H_{24}O$	0.56	1582
34	24.2	*n*-Hexadecane	$C_{16}H_{34}$	5.74	1600
35	24.5	α-Muurolol	$C_{15}H_{26}O$	0.44	1644
36	25.0	β-Eudesmol	$C_{15}H_{26}O$	0.41	1649
37	25.5	l,6,6-trimethyl-9-isopropenyl- 10-oxatricyclo d ec-Valerenal	-	0.71	-
31	25.8	Selin-11-en-4alpha-ol	$C_{15}H_{26}O$	8.53	1660
32	26.1	Valeranone	$C_{15}H_{26}O$	0.92	1674
33	28.4	Octadecene	$C_{18}H_{36}$	2.73	1789
34	29.6	6,10,14-Trimethylpentadecan-2-one	$C_{18}H_{36}O$	0.74	1842
35	30.0	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	0.52	1847
36	30.2	(*E*)-En-yn-dicycloether	$C_{13}H_{12}O_2$	1.05	1902
38	31.6	Hexadecanoic acid	$C_{16}H_{32}O$	1.26	1961
39	32.3	*n*-Eicosane	$C_{20}H_{42}$	1.15	2000
41	35.8	Docosane	$C_{22}H_{46}$	0.29	2200
		Total compounds	-	98.3	-

RT: Retention time = the time taken for a compound to move through the chromatography column to the detector after injection; %: Relative area of each compound in the essential oil sample; RI: Retention index according to the normal alkanes between C8-C24.

Table 3. Chemical composition (%) of areal parts' essential oils of *Datura innoxia* (P4).

No.	RT	Components	Molecular formula	%	RI
1	8.7	3,3-Dimethyl-2-pentanol	$C_7H_{16}O$	6.79	880
2	9.8	*n*-Nonane	C_9H_{20}	4.89	900
3	11.3	Tetrahydro citronellene	$C_{10}H_{22}O$	5.17	930
4	12.2	*n*-Decane	$C_{10}H_{22}$	1.29	1000
5	12.9	n-Undecane	$C_{11}H_{24}$	1.35	1100
6	13.3	α-Terpineol	$C_{10}H_{18}O$	1.65	1189
7	13.5	1-Heptynylbenzene	$C_{13}H18$	3.04	-
8	16.1	3-(2-furanyl)-2-Propenal	$C_7H_6O_2$	0.31	-
9	14.3	*n*-Dodecane	$C_{12}H_{26}$	13.83	1200
10	16.7	2-nitrophenyl azide	$C_6H_4N_4O_2$	1.43	-
11	16.9	*n*-Tridecane	$C_{13}H_{28}$	4.29	1300
12	18.7	Cyclopropaneoctanal	$C_{11}H_{20}O$	6.67	-
13	18.8	2,3,3,4,5-pentaethyl 1,2,5-Oxadiborolane	$C_{10}H_{20}$	0.42	-
14	19.5	*n*-Tetradecane	$C_{14}H_{30}$	11.96	1400
15	21.6	9,17-Octadecadienal	$C_{18}H_{32}O$	0.60	-
16	21.7	1,19-Eicosadiene	$C_{20}H_{38}$	0.47	-
17	21.9	Heneicosyl formate	$C_{22}H_{44}O_2$	0.11	-
18	22.21	Linoleic acid	$C_{18}H_{32}O_2$	0.54	-
19	22.4	8-Tetradecen-1-ol	$C_{16}H_{32}O_3$	0.81	-
20	23.5	2,10-dimethyl-9-Undecenal	$C_{13}H_{24}O$	0.21	-
21	24.2	*n*-Hexadecane	$C_{16}H_{34}$	10.97	1600
22	25.5	3,5-Dihydroxybenzoic acid	$C_7H_6O_4$	0.62	1617
23	25.6	7-Pentadecyne	$C_{15}H_{30}$	2.53	-
24	27.4	Monoelaidin	$C_{21}H_{40}O_4$	0.22	-
25	26.0	2-methyl-5-(1-methyl ethenyl)-Cyclohexanol	$C_{10}H_{18}O$	0.33	-
26	28.4	*n*-Octadecane	$C_{18}H_{38}$	5.90	1800
27	29.3	6,10,14-Trimethy-2-pentadecanone	$C_{18}H_{36}O$	0.25	1847
28	29.9	(2*E*,6*E*)-Farnesyl acetate	$C_{17}H_{28}O_2$	0.33	1854
29	31.6	*m*-Camphorene	$C_{20}H_{32}$	0.43	1960
30	32.3	Oleic acid	$C_{18}H_{34}O_2$	2.16	2171
31	35.8	Octadecanoic acid	$C_{18}H_{36}O$	0.63	2179
-	-	Total compounds		90.17	-

RT: Retention time = The time taken for a compound to move through the chromatography column to the detector after injection; %: Relative area of each compound in the essential oil sample; RI: Retention index according to the normal alkanes between C8-C24.

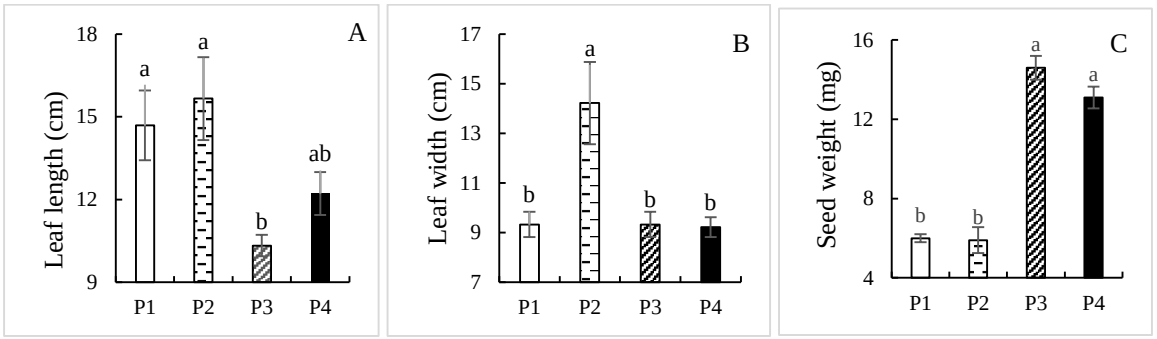

Figure 3. Means (± SE) of morphological characteristics of *Datura*. A) Leaf length, B) Leaf width, C) Seed weight; P1: *Datura stramonium* of Salmas-Urmia, P2: *D. stramonium* of Ardabil, P3: *D. innoxia* of Turgor, West Azarbaijan, P4: *D. innoxia* of Abarkuh, Yazd. Means with different letters are significantly different based on the LSD test.

Table 4. Analysis of variance of morphological characteristics of *Datura* populations.

S.O.V.	df	MS				
		Leaf length	**Leaf width**	**Plant height**	**Flowering branches**	**Seed weight**
Population	3	17.473*	18.187*	413.1	2.889	63.54**
Error	8	3.447	2.578	124.1	1.250	0.85
CV%		14.1	15.3	19. 9	14.9	9.3

*, ** Significants at ($p \leq 5\%$) and ($p \leq 1\%$), respectively.

Table 5. Analysis of variance of physiological characteristics of *Datura* populations.

S.O.V.	df	MS				
		Chla	**Chlb**	**ChlT**	**CAR**	**SPAD**
Population	3	3.6097**	1.30515**	8.7593**	0.5748**	974.42**
Error	8	0.0010	0.0006	0.0086	0.0001	63.33
CV%		0.63	1.79	1.46	0.53	17.75

Chla: Chlorophyll a; Chlb: Chlorophyll b; ChlT: Total chlorophyll; CAR: Carotenoids; SPAD; Chlorophyll index; ** Significant ($p \leq 1\%$).

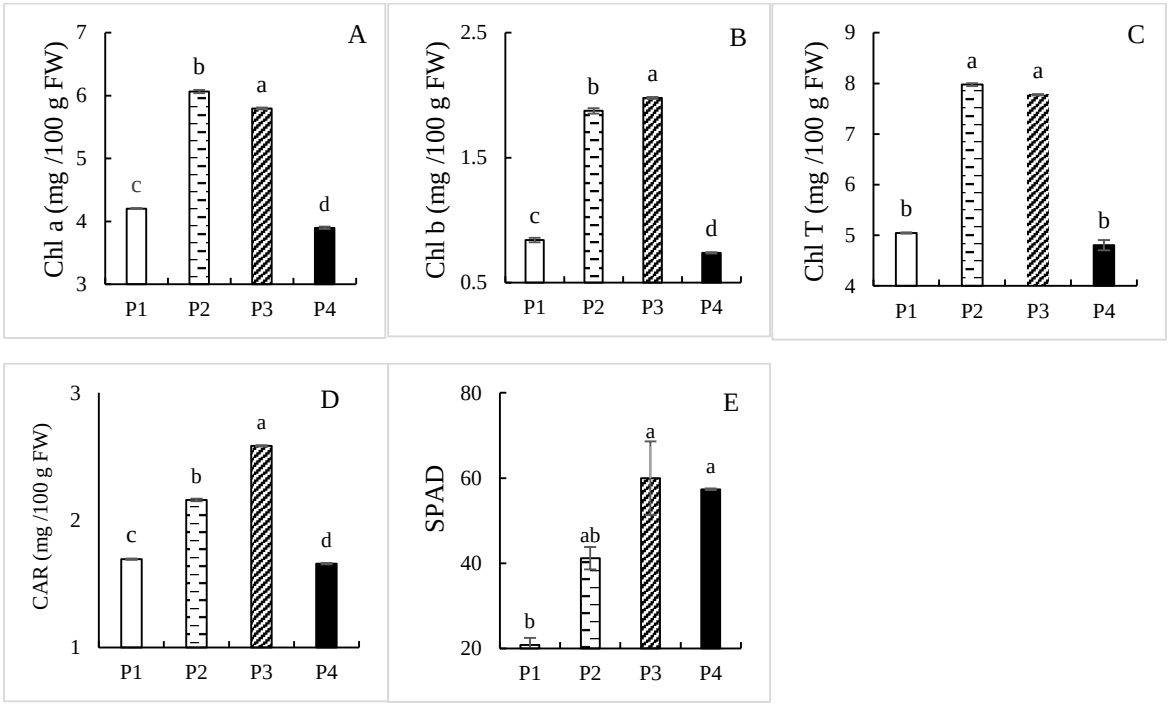

Figure 4. Means (± SE) of physiological characteristics of *Datura*. A) Chla: Chlorophyll a; B) Chlb: Chlorophyll b; C) ChlT: Total chlorophyll; D) CAR: Carotenoids; E) SPAD: Chlorophyll index; P1: *D. stramonium* of Salmas-West Azarbaijan, P2: *D. stramonium* of Ardabil, P3: *D. innoxia* of Turgor West Azarbaijan, and P4: *D. innoxia* of Abarkuh, Yazd. Means with different letters are significantly different based on the LSD test.

Total phenolic content and total flavonoid content

ANOVA showed that there was a significant difference ($p \leq 1\%$) between *Datura* populations for the TPC and TFC of aerial parts (Table 6). The extract from the P4 and P1 populations had the highest TPC with 4.35 and 3.66 mg GAE/g DW, respectively (Figure 5A). On the contrary, P3 had a low TPC with 1.45 mg GAE/g DW. Out of the test materials, the highest TFC was recorded for the P4 samples (2.39 mg QE/g; Figure 5B). On the other hand, P3 exhibited the lowest levels of TFC (0.61 mg QE/g DW). In the current report, the TPC and TFC of *Datura* populations from Iran are recorded for the first time.

Antioxidant activity

ANOVA showed that there was a significant difference ($p < 1\%$) among the populations in AA (Table 6). The population means for AA are demonstrated in Figure 5C. In the DPPH assay, the highest RSC (lowest IC_{50}) was distinguished in both P1 and P4 samples with an IC_{50} of 88.7 μg ml^{-1} and 106.4 μg ml^{-1}, respectively compared to BHT (33 μg ml^{-1}), a synthetic industrial antioxidant. The lowest AA (IC_{50} 386.5 μg ml^{-1}) was related to the P3 sample.

Table 6. Analysis of variance of phytochemical properties of *Datura* populations

S.O.V.	df	MS		
		Total phenolic content	**Total flavonoid content**	**Antioxidant activity**
Population	3	4.9078**	2.1257**	5686.7**
Error	8	0.07515	0.0011	137.5
CV%		9.11	2.58	6.35

** Significant at ($p \leq 1\%$).

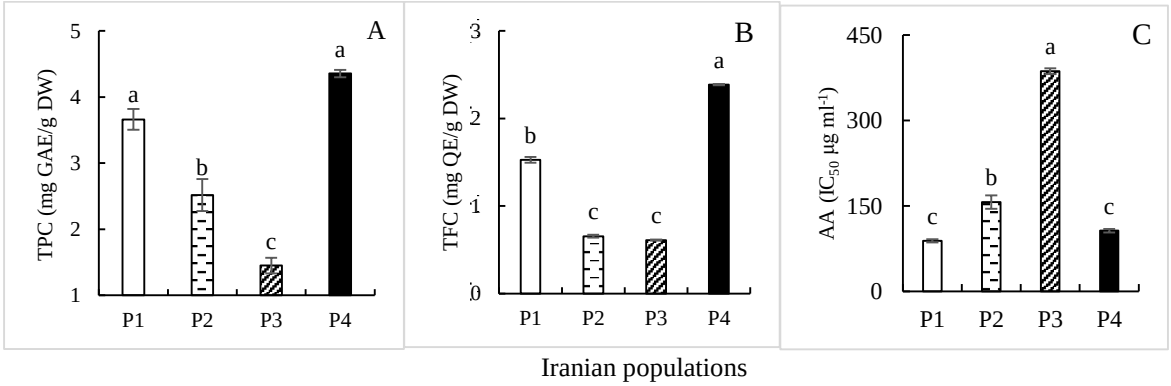

Figure 5. Means (± SE) of phytochemical characteristics of *Datura*. A) TPC: Total phenolic content; B) TFC: Total flavonoid content; C) AA: Antioxidant activity; 1) *D. stramonium* of Salmas, West Azarbaijan, 2) *D. stramonium* of Ardabil, 3) *D. innoxia* of Turgor, West Azarbaijan, 4) *D. innoxia* of Abarkuh, Yazd. Means with different letters are significantly different based on the LSD test.

GGT biplot for populations × trait relationship

The two principal components (PC1 and PC2) explained 87.6% of the total variation with a share of 50% and 37.6% of the total variation by PC1 and PC2, respectively. In the biplot vector representation of genotypes and traits, vectors are drawn from the origin of the biplot to the names of the traits for each trait. The angle between the vectors of two traits indicates the phenotypic relationship between them. As an example, seed weight and flowering branches had tangent vectors. Therefore, these two traits have a high relationship. On the other hand, seed weight and leaf length are in the same direction with a wide angle, which indicates a negative relationship between them. Also, the 90-degree angle between the vectors of seed weight and ChlT indicates the lack of a significant correlation between these traits (Figure 6A).

One of the most attractive features of a GGT biplot is its ability to show the which-won-where pattern of a population by environment dataset (Figure 6B). GGT biplot polygon view as depicted in Figure 6, can identify populations with the highest values for one or more characteristics. All populations, including P1, P2, P3, and P4 were in the vertex of the polygon. Most traits like AA, Chla, Chlb, ChlT, CAR, SPAD, flowering branches, seed weight, and altitude were located around the vertex P3.

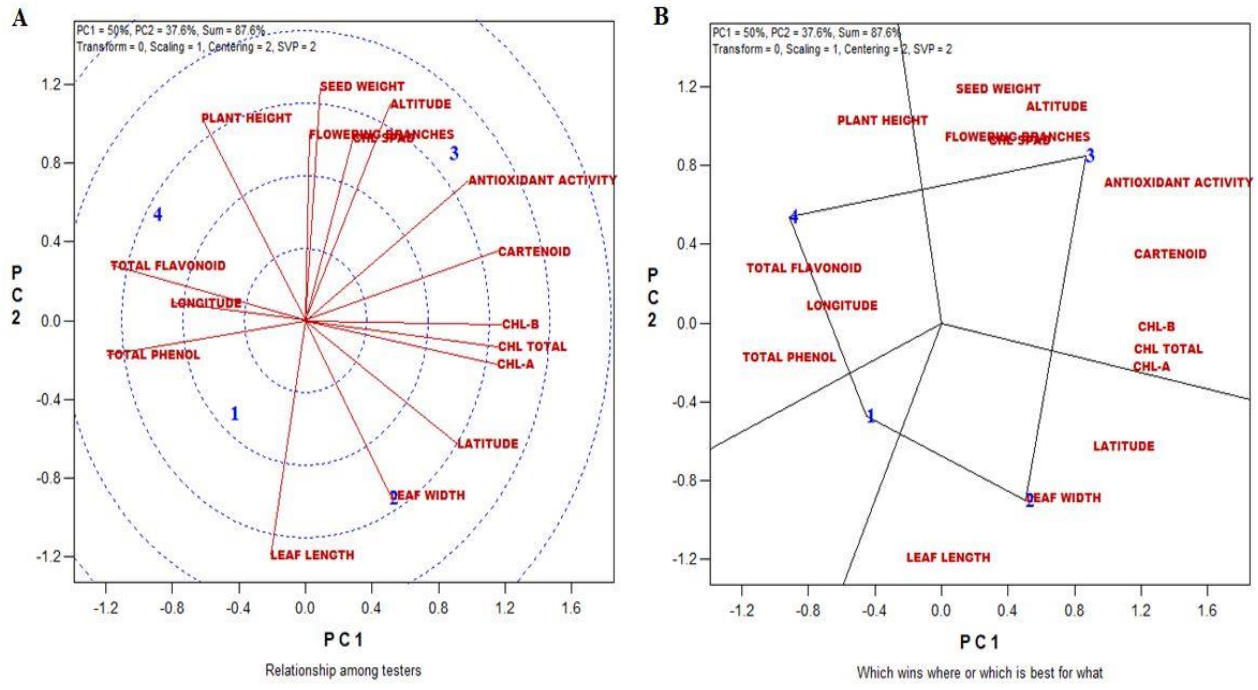

Figure 6. The GGT biplot to show the discriminating ability and representativeness of the traits with *Datura* populations (A); The "which-won-where" view of the GGT biplot based on the interactions of populations and observed characteristics showing which *Datura* population won the contribution of which characteristic (B).

Correlation

The results showed that AA (DPPH) had a significant correlation ($p \leq 5\%$) with TPC (0.86), TFC (- 0.66), leaf length (0.60), Chla (- 0.65), Chlb (- 0.77), ChlT (- 0.69), CAR (- 0.94), and SPAD (- 0.56). Leaf length showed a significant correlation with leaf width (0.71), SPAD (- 0.57), seed weight (- 0.74), and altitude (- 0.78). All physiological traits (except for SPAD) were negatively and significantly ($p \leq 5\%$) correlated with TPC. A significant correlation ($p \leq 5\%$) of altitude with CAR (0.67), SPAD (0.73), TPC (- 0.55), AA (- 0.87), and seed weight (0.90) was detected. Also, latitude showed a significant correlation ($p \leq 5\%$) with plant height (- 0.66), Chla (0.72), Chlb (0.67), ChlT (0.68), TPC (- 0.69), and TFC (- 0.89) (Table 7).

Table 7. Correlation among phytochemical properties, morphological characteristics, and physiological properties in *Datura* populations.

Trait	LL	LW	PH	FB	Chla	Chlb	ChlT	CAR	SPAD	TPC	TFC	AA	SW	Alt	Lat
LW	0.71**	1													
PH	-0.49	-0.43	1												
FB	-0.55	-0.51	0.56**	1											
Chla	0.02	0.56*	-0.46	-0.19	1										
Chlb	-0.10	0.46	-0.36	-0.07	0.98**	1									
ChlT	-0.03	0.52	-0.44	-0.17	0.99**	0.99**	1								
CAR	-0.36	0.17	-0.18	0.14	0.87**	0.94**	0.90**	1							
SPAD	-0.57*	-0.12	0.31	0.03	0.22	0.34	0.28	0.44	1						
TPC	0.31	-0.17	0.26	-0.14	-0.88**	-0.95**	-0.88**	-0.96**	-0.23	1					
TFC	-0.05	-0.44	0.52	0.07	-0.94*	-0.93**	-0.93**	-0.85**	0.01	0.91**	1				
AA	0.60*	0.12	-0.06	-0.37	-0.65*	-0.77**	-0.69*	-0.94**	0.56*	0.86**	0.66*	1			
SW	-0.74**	-0.49	0.62	0.41	-0.06	0.09	0.01	0.36	0.81**	-0.20	0.17	0.62*	1		
Alt	-0.78**	-0.41	0.40	0.48	0.24	0.40	0.30	0.67*	0.73**	-0.55*	-0.20	-0.87**	0.90**	1	
Lat	0.30	0.41	-0.66*	-0.09	0.72*	0.67*	0.68*	0.56	-0.42	-0.69*	-0.89**	-0.33	-0.53	-0.15	1
Long	0.06	0.05	0.37	-0.28	-0.47	-0.51	-0.45	-0.58*	0.31	0.70*	0.73**	0.55	0.16	-0.22	-0.84**

LL: Leaf length; LW: Leaf width; FB: Flowering branches; Chla: Chlorophyll a; Chlb: Chlorophyll b; ChlT: Total chlorophyll; CAR: Carotenoids; TPC: Total phenolic content; TFC: Total flavonoid content; AA: Antioxidant activity; SW; Seed weight; Alt: Altitude; Lat: Latitude; Long: Longitude
*, **Significant at ($p \leq 5\%$) and ($p \leq 1\%$), respectively.

Discussion

There are no reports about the volatile components of *Datura innoxia* as well as AA, TPC, and TFC of both *D. innoxia* and *D. stramonium* from Iran. Moreover, a comprehensive study of their agro-morphological and phytochemical characteristics in the same ecological conditions has not been done. The present study aimed to identify the diversity in the EO compounds of four *Datura* populations, focusing on morphophysiological and phytochemical traits. The yield of areal parts' EO in both studied species was 0.02% (v/w), respectively, which is in agreement with the previous reports of Aboluwodi *et al*. (2017) and Morovati *et al*. (2023).

Camphor, and selin-11-en-4alpha-ol were the major compounds in the oil of P1 in *D. stramonium*, which is consistent with the compounds obtained from the P2 study by Morovati *et al.* (2023). 2-hydroxy-4-methyl Pentanoic acid, Levomenthol, geraniol, *β*-caryophyllene, *β*-Damascenone, l,6,6-trimethyl-9-isopropenyl-10-oxatricyclod ec-Valerenal were found among the main compounds

detected in P1, but absent in P2. Our previous study indicated a difference in the chemical composition of *D. stramonium* essential oil which included dihydrocitronellol, *n*-undecane, citral, 2-nonen-4-one, spathulenol, *m*-camphorene, and (*E*)-phytyl acetate in the EO of population P2 compared to the EO of population P1. (Morovati *et al.* 2023). The major compounds detected in those of P4 in *D. innoxia* were mostly fatty alkanes, including n-dodecane, n-Hexadecane, and n-tetradecane, which did not agree with the compounds obtained from the P3 investigation by Morovati *et al.* (2023). Additionally, Morovati *et al.* (2023) showed significant differences in the EO of *D. innoxia* which included compounds n-undecane, 1-heptynylbenzene, 3-(2-furanyl)-2-propenal, and 2-methyl-5-(1-methyl ethenyl)-cyclohexanol in the EO of P4 compared to the EO of P3.

The result of GC-MS analysis of the current study exhibited remarkable differences between the components recognized in the oil profiles of *D. stramonium* and *D. innoxia*. The changes in the EOs components are probably influenced by factors such as age and the development stage of medicinal plants (Hazrati *et al.* 2020), genotype, climate, location, time of sampling, insect and microorganisms' stress, and other geological and environmental conditions (Başer and Buchbauer 2015; Norani *et al*, 2023). Moreover, in previous studies, camphor and borneol derivatives were reported to be the inhibitors of the influenza virus, filoviruses, and orthopoxviruses (Sokolova *et al.* 2021; Li *et al.* 2022). Phytochemical assessment is one of the substantial tools for quality evaluation which includes preliminary phytochemical screening and chemo-profiling (Partap *et al*. 2019). Generally, EOs of *D. stramonium* have been mostly reported as oxygenated monoterpenes and diterpenes (Aboluwodi *et al.* 2017). The comparison of the EO profile of *D. stramonium* and *D. innoxia* showed a considerable difference in their phytochemical constituents.

In the current report, the morphological investigation revealed that the maximum values for leaf length and width were in P2, measuring 15.7 cm and 14.2 cm, respectively. As the results show, there was a significant variation in some morphological characteristics including leaf length, leaf width, and seed weight of *D. stramonium* and *D. innoxia*. Similarly, morphological diversity in 12 Algerian populations of *D. stramonium* of different origins was previously reported (Morsli *et al.* 2011). Among the studied populations, P2 had bigger leaves and lower height, seed weight, and flowering branches than the other population of *D. stramonium* species (P1) and two populations of *D. innoxia* (P3 and P4). Many factors can cause differences in plant morphology, such as genetics, environmental conditions, and developmental processes (Li *et al.* 2020). Genes control various aspects of plant growth and development, such as leaf shape and size, stem length and thickness, and flower color and shape (Moradi *et al.* 2023). Different plant species or varieties can have different combinations of genes that result in distinct morphological features (Saletnik *et al.* 2022).

Results of the physiological characteristics of *Datura* showed significant diversity among the four examined populations. *D. stramonium* had the highest value for Chla and ChlT, while *D. innoxia* had the maximum amount for Chlb, CAR, and SPAD. *Datura* plants may have different amounts of chlorophyll types, depending on various factors, such as the species and the environmental conditions in which they were grown (Elisante *et al.* 2013).

DPPH is a stable radical that could define the free RSC of antioxidants (Molnar *et al.* 2017). In the DPPH method, the IC_{50} value is negatively related to the antioxidant capacity, as it expresses the quantity of antioxidants needed to decrease the radical concentration by 50% (Wang *et al.* 2008). The DPPH radical scavenging result of *Datura* methanolic extracts was from IC_{50} 88.7 to IC_{50} 384.5 (µg ml^{-1}). The lower the IC_{50} value, the higher the AA of the tested sample. TPC ranged from 1.4 to 4.4 mg GAE/g DW while TFC varied from 0.6 % to 2.4 mg QE/g. Other research also has determined that the *D. innoxia* leaf extract was a moderate antioxidant that contains a strong ability to scavenge DPPH free radicals with IC_{50} = 146.69 µg ml^{-1}, TPC with 70.2 mg GAE/g, and TFC with 34.2 mg QE/g (Bhardwaj *et al.* 2016; George and Mathur 2022). Phenolic and flavonoid compounds have been reported as possessing AA among other biological activities, scavenging free radicals or preventing their formation (Nasr *et al.*, 2014).

Understanding the relationships between different traits is critical for improving plant breeding programs and enhancing crop production and yield. A growing body of evidence suggests that various indicators have been developed to facilitate the selection of elite genotypes. Nevertheless, relying on a single agronomic trait without considering other beneficial characteristics has led to undesirable breeding outcomes. Therefore, plant breeding programs should focus on the interplay between multiple traits to achieve reliable results (Yadesa 2022). To support this, multivariate analysis, using GGE biplot analysis in this study identified critical traits for selecting top *Datura* populations.

Conclusion

Based on morphophysiological and phytochemical comparisons, four *Datura* populations consisting of P1, P2, P3, and P4 were compared to examine similarities and differences to identify a superior population. Based on the GGT biplot analysis, most of the traits like AA, Chla, Chlb, ChlT, CAR, SPAD, flowering branches, seed weight, and altitude were located around the vertex P3 (*Datura innoxia* grown in Turgor, West Azarbaijan). In the aerial parts' oil of *D. stramonium*, monoterpenes predominated over sesquiterpenes. The comparison of morphophysiological, phytochemical, and volatile compounds of *D. stramonium* and *D. innoxia* showed a significant difference among *Datura* populations for these characteristics.

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Conflict of Interest

The authors declare that they have no conflict of interest with other people or organizations.

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