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Morphological, Epidermal and Anatomical Properties of *Datura* Linn. Leaf in Sana'a City-Yemen and its Taxonomical Significance

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ABSTRACT

The morphological, epidermal and anatomical characters of two wild Datura taxa leaves grown in Sana'a city were investigated. Morphologically; the shape, apex, margin, base, texture, size and venation of lamina were studied. The epidermal characters including the properties of epidermal cells (shape, size & frequency), stomata properties (type of stomata complex, size, frequency, index and ratio) and type of trichomes were determined. Anatomically the characters of mesophyll and main midrib were investigated. The lamina morphological characters of the two studied Datura taxa leaves (shape, base, apex, margin, texture and type of venation) as well as epidermal characters including the properties of epidermal cells (length, size and frequency), stomata properties (size and frequency) & type of trichomes and the anatomical characters (thickness of Mesophyll and number of abaxial collenchyma layers in the midrib) shows a high significant in taxonomic value for the separation between the two studied Datura taxa.

Key words: Datura, Lamina, Stomata, Trichome, Mesophyll.

INTRODUCTION

The genus *Datura* Linn. comprises about 10 species distributed mainly in tropical and warm temperate regions, especially tropical American and Australia [1], only 3 species are known from Yemen, *Datura metel, D. innoxia & D. stramonium* [2] and only 2 species of *Datura* taxa so far recorded from Sana'a governorate, *D. innoxia & D. stramonium* [3, 4]

Few studies have been done on the morphological [1, 5, 6, 7], epidermal [8, 9] and anatomical [8] features of *Datura* taxa leaves.

However, no attempts seem to have been made to evaluate the taxonomical significance of those features. Therefore, the present study aims to investigate the morphological and anatomical features as well as epidermal features of *Datura* taxa leaves in Sana'a city and to evaluate their significance as key characters for differentiation.

MATERIALS AND METHODS

Fresh samples of *Datura* taxa were collected from different localities in Sana'a city during the May 2015 to August 2015 (Table 1) and identified according to Chaudhary [1], Wood [7] & Collenette [10] and compared with samples from the Herbarium of the Faculty of Science Sana'a University. For leaf morphology at least five to seven matured and well expanded leaves were investigated to record the leaf architecture characters of each species which were based on the terminology of (Approaches to identification of Angiosperm leaf remain) Dilcher [11]. The characters described were leaf petiole and lamina features (size, shape, apex, base, margin, texture & venation).

To study the epidermal characters fresh matured and well expanded leaves of the *Datura* taxa were cut at the median portion, the specimens were soaked in concentrated Nitric acid for 2 to10 hrs depending on the texture of the

leave. The appearance of the air bubbles indicated the readiness of epidermises to be separated. The samples were then transferred to Petri dish containing water and with the use of fine forceps and dissecting needle the upper (Adaixal) and lower (Abaxial) epidermis were separated. These were then cleaned with camel hair brush in water [12]. The two epidermal layers (Adaxial & Abaxial) were stripped and stained with Saffranin, Excess stain was rinsed off with clean water and mounted in glycerol on clean slides then covers by cover slide [13]. The slides were observed by using Leica (ATC 2000) microscope to determine lamina epidermal (adaxial & abaxial) characters of each species which were based on the terminology of Dilcher [11]. The characters determined were stomata complex features (stomata type, size & frequency); epidermal cell features (shape, size & frequency) and Trichomes features (Type & frequency). Photographs of lamina epidermis (Adaxial & Abaxial) characters were taken by Canon (IXUS255 HS) digital camera. The stomata frequency, epidermis cell frequency and trichome frequency were based on average obtained from observation of 10 microscope field with an area $625\mu m^2$ at x 200, the stomata index (SI) was calculated using the formula of Salisbury [14]; $SI = [S \setminus (S+E)] \times 100$, where S = No. of stomata in an area of $625\mu m^2 \& E = No.$ of epidermal cells in an area of $625\mu m^2$. The stomata ratio (SR) was helpful in defining the type of leaf. It is the ratio of the number of stomata on the abaxial epidermis to the number of stomata on adaxial epidermis, if SR>1 the leaves are classified as amphistomatic, if 0.1<SR<1 as hypoamphistomatic and if SR<0.1 as hypostomatic [15]. The stomata size (length x width); epidermal cell size (length x width) and guard cells area (length x width x Franc'os constant which is 0.78525) were based on average obtained from observation of 40 individual, by the help of ocular micrometer calibrated with stage micrometer (value of 400x locular small division $= 0.25 \,\mu$ m) and Image j program.

For the anatomical studies leaf lamina were cut to small samples each sample were fixed in formalin acetic acidalcohol solution for two days. After removing the fricative by distilled water, they were dehydrated with ethyl alcohol solution of 30%, 50%, 60%, 70%, 85%, 90%, and 100% before being embedded into paraffin and sectioned by using a rotary microtome. The sections were stained in a Saffranin O/Fast Green combination [16]. The anatomical characters (structure of the lamina, lamina epidermis and midrib) were examined by using Leica (ATC 2000) microscope and by utilizing the available anatomical literatures of Fahn [17] and Esau [18]. Photographs of the leaf sections were taken by Canon (IXUS255 HS) digital camera.

The thickness of the Mesophyll was measured by the help of ocular micrometer calibrated with stage micrometer (value of 400x locular small division = $0.25 \ \mu$ m) and Image j program, in addition to that the thickness of the Midrib was measured by the help of ocular micrometer calibrated with stage micrometer (value of 100 x locular small division = $1 \ \mu$ m) and Image j program.

Date	Location	Coordinates		Elevation	Таха	
		Longitude	Latitude	-		
May					Datura innoria	
2015	1	44°11'16.3"E	15°21'49.7"N	2271m asl.	&	
					Datura stramonium	
June	2	44°11'41.5"E	15°21'16.8"N	2266m asl.	Datura stramonium	
2015	3	44°11'13.9"E	15°22'07.8"N	2269m asl.	Datura innoxia	
July					Datura innoxia	
2015	4	44°11'42.8"E	15°20'38.2"N	2275m asl.	é.	
					Datura stramonium	
A					Datura innoxia	
August	5	44°11'20.3"E	15°21'55.1"N	2313m asl.	æ	
2015					Datura stramonium	
2015	6	44°12'23.3"E	15°20'1.44"N	2280m asl.	Datura stramonium	

Table 1:	Locality a	nd Date of	Collection	of the two	investigated	Datura taxa
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The taxonomical value of quantitative leaf morphological, epidermal and anatomical features were determined by T. test using Graph Pad Prism 6.01 program, if P- value P < 0.05 then the quantitative leaf features is significantly different.

RESULTS AND DISCUSSION

Morphological Analysis:

Table 2 &3 and Figure 1 demonstrate the main morphological properties of the studied Datura taxa leaves

The leaf architecture shows kind of difference aspects.

Lamina is, ovate – lanceolate, asymmetrical, coriaceous, grey green, pubescent, with a Campotodromous (Brochidodromous) venation (secondary veins joined together in a series of prominent arches, never terminating at the margin), up to 15.7×9.6 cm, acute at apex, repeand to sinuate at margin, oblique, asymmetrical at base in *Datura innoxia*, while it is ovate, asymmetrical, chartaceous, yellowish green, glabrescent, with a Craspedodromous venation (secondary veins terminating at the margin), up to 16.9×11.7 cm, acute to acumenate at apex, coarsely dentate to lobed at margin, oblique, asymmetrical at base in *Datura stramonium*, leaves of *Datura innoxia* and *Datura stramonium* are petiolate leaves with a normal petiole.

Identification key to the studied Datura taxa leaves based on their morphological characters:

+ Lamina ovate – lanceolate, coriaceous, Grey green, pubescent, Campotodromous venation, repand to sinuate at margin...... Datura innoxia.

- Lamina ovate, chartaceous, Yellowish green, glabrescent, Craspedodromous venation, coarsely dentate to lobed at margin...... *Datura stramonium*.

Epidermal Analysis:

Table 2 & 3 and Figure 2 shows the main epidermal characters of the studied *Datura* taxa leaves as clarified by light microscope.

Epidermis cell shape is rectangular with undulate cell wall in *Datura innoxia* and *D. stramonium* (Figure 2). The mean of epidermal cells size is largest in the adaxial surface of *D. stramonium* (22.9 μ m²) and the smallest mean of epidermal cell size (11 μ m²) was recorded in the abaxial surface of *D. innoxia* furthermore, the highest mean of epidermal cells density was found in the abaxial surface of *D. innoxia* (335 epidermal cell /625 μ m²) followed by 175 epidermal cell / 625 μ m² on the adaxial surface of *D. innoxia*; while the lowest mean of epidermal cells density (93 epidermal cell / 625 μ m²) was recorded on the adaxial surface of *D. stramonium* (Table 3).

Amonotetracytic (four cells enclosing guard cell in an irregular and variable pattern) and anisocytic (single ring of 3 cells -2 larger & 1 smaller- enclosing the guard cells) stomata complex types were occurred in the adaxial and abaxial surface of *D. innoxia* and *D. stramonium* (Table 2 & Figure 2) and this agrees with what Solereder [8] and Hameed & Hussain [9] recorded.



Figure 1: General Morphological Characteristic of Datura taxa Studied Leaf.

A-C: *Datura innoxia* leaf, A: Adaxial surface, B: Abaxial surface & C: Campotodromous (Brochidodromous) venation; D-F: *Datura stramonium* leaf: D: Adaxial surface, E: Abaxial surface & F: Craspedodromous venation.

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The largest mean of stomata size was in the abaxial surface of *D. stramonium* $(4.5\mu m^2)$ and the smallest mean of stomata size $(2.7 \ \mu m^2)$ was recorded in the abaxial surface of *D. innoxia* (Table 3) on the other hand the largest mean of guard cell area was found in the adaxial surface of *D. stramonium* $(1.6\mu m^2)$ and the smallest mean of guard cell area $(0.9\mu m^2)$ was recorded in the adaxial surface of *D. innoxia* (Table 3)

All the observed stomata are small size (Table 3) this is because, stomata whose guard cells less than 15 μ m long are designated -small while those are more than 38 μ m long are termed large **[19]**.

The Stomata ratio (SR) of *D. innoxia and D. stramonium* were 0.54 and 0.73 (0.1 < SR < 1) respectively this shows that leaves of the studied *Datura* taxa are hypoamphistomatic (leaves that have stomata on both surface, with more on the abaxial surface than the adaxial surface)



Figure 2: Types of Stomata in the Adaxial and Abaxial surface of Datura taxa leaves

A-B: *Datura innoxia* leaf, A: Adaxial surface, B: Abaxial surface; C-D: *D stramonium* Leaf, C: Adaxial surface, D: Abaxial surface; Am: Amonotetracytic stomata, An: Anisocytic stomata

The highest mean of stomata density was observed on the abaxial surface of *D. innoxia* (102 stomata complex /625 μ m²) whose mean of stomata index was 23.2 followed by 55 stomata complex /625 μ m² on the adaxial surface of *D. innoxia* whose mean of stomata index was 23.9; while the lowest mean of stomata density (30 stomata complex /625 μ m²) was recorded on the adaxial surface of *D. stramonium* whose stomata index was 24 (Table 3)

Two main types of trichomes were identified in the two *Datura* taxa studied leaves: glandular trichomes and nonglandular trichomes. According to observation about 7 and 3 morphological categories of glandular trichomes were recorded in *D. innoxia* and *D. stramonium* correspondingly. Furthermore; in *D. innoxia* 7 and 5 morphological categories of glandular trichomes were recorded on the adaxial and abaxial surface respectively; while in *D. stramonium* 1 and 3morphological categories of glandular trichomes were observed, the former on adaxial surface and the latter on abaxial surface (Table 2 & Figure 3).

On the other hand, the non-glandular trichomes were classified according to their surface in two types: smooth nonglandular trichomes and rough non-glandular trichomes, the former occurs in *D. innoxia* and the latter occurs in *D. stramonium*. Furthermore; the smooth non-glandular trichomes and rough non - glandular trichomes are subdivided structurally in to 4 and 6 morphological categories correspondingly (Table 2 & Figure 3). In addition to that; the 4 types of smooth non-glandular trichomes were only observed on the adaxial surface of *D*. *innoxia* leaf; while 4 types of rough non - glandular trichomes were recorded on the adaxial & abaxial surface of *D*. *stramonium* leaf respectively (Table 2).

Generally, the trichomes accrues on both surface of studied *Datura* taxa leaves with more density on the adaxial surface than the abaxial surface (Table 3), the highest mean of trichome density was recorded on the adaxial surface of *D. innoxia* (16 trichome/625 μ m²) followed by 8 trichome/625 μ m² on the abaxial surface of *D. innoxia*, while the lowest mean of trichome density (2 trichome /625 μ m²) was observed on the abaxial surface of *D. stramonium* (Table 3).

Identification key to the studied *Datura* taxa leaves based on their epidermal characters:

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	Changeton	Datur	D. Vala		
	Characters	D. innoxia	D. stramonium	P- value	
Ľ	Length cm Min (Mean ±SD) Max	4.1 (9.8±4.5) 15.7	6.9 (11.9±3.7)16.9	0.438	
Im.	Width cm Min (Mean ±SD) Max	2.768 (6.5±2.7) 9.6	4.3(8.8±2.8)11.7	0.2157	
na	Size μ m ² Min (Mean ±SD) Max	11.3 (73 ± 55.2) 150.3	30.1(112.8±62)198.4	0.3151	
	Epidermis cells Length µm Min (Mean ±SD) Max		1.3 (3.9 ± 1.3) 6.5	2.4 (5.5 ±1.8) 11	< 0.0001
			2.3 (3.9 ±0.8) 5.8	1.6 (5.1±2.1)10.2	0.0011
	Epidermis cells Width μm Min (Mean \pm SD) Max		1.3 (3±1.3)6.6	2.2(4.1 ±1.3) 8	0.0002
			$1.5(2.8\pm0.8)5.4$	1.8 (3.8 ±1.3)7.4	0.0001
	Epidermis cells Size µm ² Min (Mean ±SD) Max		$1.2~(12.5\pm9.5)~42.9$	6.2(22.9 ±10.6) 49.1	< 0.0001
			4.2(11 ±4.3) 22.6	2.9 (20.2 ± 12) 47.9	< 0.0001
	Frequency of Epidermis cells in an Area of $625 \ \mu m^2$ Min (Mean ±SD) Max		129 (175 ±41.4) 287	85(93 ± 6)104	< 0.0001
			300 (335 ± 21.6) 372	112(131 ±14.1)150	< 0.0001
		Ad	1.4 (1.9 ±0.4) 3	1.6 (2.6±0.5) 3.5	< 0.0001
	Guard cells Length $\mu m Min$ (Mean ±SD) Max		1.1(1.7±0.3) 2.2	1.5 (2.4 ±0.5) 3.4	< 0.0001
Epi	Guard cells Width μm Min (Mean ±SD) Max	Ad	0.4(0.6±0.1) 0.8	0.6(0.8±0.2) 1.2	< 0.0001
ideı		Ab	0.3 (0.6 ±0.2) 0.9	$0.4(0.7 \pm 0.2)1$	0.0076
mi	Guard cells Area $\mu m^{2}Min$ (Mean $\pm SD)$ Max	Ad	0.4 (0.9±0.3)1.7	0.8 (1.6±0.5) 3.2	< 0.0001
s		Ab	0.3(0.9±0.4)1.5	0.5(1.4±0.6) 2.6	< 0.0001
		Ad	1.5 (3 ± 1.1) 5.7	$2.7(5.2 \pm 1.4) 9.2$	< 0.0001
	Stomata complex Size $\mu m^2 Min$ (Mean ±SD) Max		1.3 (2.7 ±0.8) 4.2	1.8 (4.5±1.8) 8.4	< 0.0001
	Frequency of Stomata in an Area of 625 µm ² Min (Mean ±SD) Max		48 (55 ±9.2) 80	24 (30 ±3.5) 34	< 0.0001
			72 (102 ±14.4) 122	30(41 ±5.4) 48	< 0.0001
	Stomata ratio		0.54	0.73	-
	Stomata index Min (Mean ±SD) Max		21.8 (23.9 ±1.7) 27.5	20.3 (24 ±1.9) 26.2	0.9603
			18.7 (23.2±2.6) 25.9	19.6(23.6 ±2.1)27.7	0.7203
	Frequency of Trichome in an Area of $625 \ \mu m^2$ Min (Mean ±SD) Max		7 (16±5)25	3 (5±1.3) 7	< 0.0001
			1 (8 ±4.7) 15	1 (2±0.8) 4	0.0029
Ζ	Layers of Palisade parenchyma		1	1	-
esoph yll	Layers of Spongy parenchyma	-	$5(5.9\pm0.7)7$	5 (5.4 ±0.5) 6	0.2183
	Mesophyll thickness µm Min (Mean ±SD) Max	-	12 (17.2 ± 2.8)20	9.8(13.1 ± 2.2)15.5	< 0.0001
м	Number of Collegebourg Leaves Min (Massign) M	Ad	4 (4.4 ±0.5) 5	5(5.6 ±1.1) 8	0.0167
	Number of Collenchyma Layers Min (Mean \pm SD) Max		2(2.2 ±0.4)3	3(3.3±0.5)4	0.0001
id 1	Number of Paranchyma Layers Min (Mean ±SD) Max		5(6.2 ±1.1) 8	6 (8 ±1.7)10	0.0163
rib			6(7.2 ±0.7)8	7 (8 ±1.3)10	0.1348
	Midrib thickness Min (Mean ±SD) Max		48.2 (61±8.1)69.3	52.1(74.5±20.4)111.8	0.0449

Table 3: Quantitative Characteristics Datura taxa studied Leaves

Ad: Adaxial surface of the leaf, Ab: Abaxial surface of the leaf, SD: Stander Deviation. Significantly different (P < 0.05)

Ad: Adaxial surface of the leaf, Ab: Abaxial surface of the leaf

Type I: Glandular Trichomes (A-G):

- A- Unicellular head and unicellular stalk.
- B- Unicellular head with bicellular uniseriate stalk.
- C- Unicellular head with tricellular uniseriate stalk.
- D- Unicellular head with tetracellular uniseriate stalk.
- E- Bicellular head with unicellular stalk.
- F- Multicellular head with unicellular stalk.
- G- Multicellular head with bicellular uniseriate stalk.

Type II: Non-glandular Trichomes (H-Q):

- H- Smooth, bicellular uniserate with a round apical cell.
- I- Smooth, tricellular, uniserate with a round apical cell.
- J- Smooth, tricellular, uniserate with a long round apical cell.
- K- Smooth, tricellular, uniserate with an obtuse apical cell.
- L- Rough, simple unicellular. M- Rough, simple uniserate.
- M- Rough, simple uniserate.N- Rough, bicellular uniserate with a hooked apical cell.
- O- Rough, bicellular uniserate with a long acute apical cell.
- P- Rough, tricellular, uniserate with an acute apical cell.
- Q Rough, tetracellular, uniserate with a long hooked apical cell.

Figure 3: Types of Trichomes in Datura spp.



Anatomical Analysis:

Table 3 & Figure 4 Illustrate the main anatomical characters of the two *Datura* taxa studied leaves as clarified by light microscope.

The transverse section of the two Datura taxa studied leaves (Figure 4) shows the presence of cuticle on both adaxial and abaxial surface, the adaxial and the abaxial epidermis composed of uniseriate oval to rectangular cells.

Although the transverse section of the two *Datura* taxa studied leaves confirms that the leaves are from the type bifacial (Dorsiventral). The mesophyll in *D. innoxia* consist of one layer of elongate of Palisade parenchyma arranged like a row of stakes without air-space and 5-7 layers of irregular Spongy parenchyma with a few air-space; while in *D. stramonium* it composed of one layer of elongate Palisade parenchyma arranged like a row of stakes with a few air-space and 5-6 layers of irregular Spongy parenchyma with air-space.

According to observation the mesophyll in *D. innoxia* is thicker then the mesophyll *D. stramonium* with average thickness 17.2 μ m and 13.1 μ m correspondingly and this agrees with type of leaf texture in *D. innoxia* and *D. stramonium* (Table 2 & 3 and Figure 4).

On the other hand the midrib in the transverse section of the two *Datura* taxa studied leaves consist of cuticle on both adaxial and abaxial surface, adaxial and abaxial epidermis composed of uniseriate oval to rectangular cells followed by adaxial and abaxial collenchyma, in *D. innoxia* the adaxial layers and the abaxial layers of collenchyma consist of 4 -5 layers & 2-3 layers, while in *D. stramonium* there is 5-8 adaxial layers and 3- 4abaxial layers of collenchymas (Figure 4).

The main vascular bundle in the transverse section of the two *Datura* taxa studied leaves is surrounded by adaxial and abaxial layers of parenchyma, in *D. innoxia* there is 5-8 layers of adaxial paranchyma and 6-8 layers of abaxial parenchyma, while in *D. stramonium* there is 6-10 layers of adaxial paranchyma and 7 -10 layers of abaxial paranchyma.

According to observation the thickness of the *D. stramonium* midrib is much thicker than the midrib of *D. innoxia*, with average thickness 74.5 µm and 61 µm respectively (Table 3 & Figure 4).

Identification key to the studied Datura taxa leaves based on their Anatomical characters:

The results, proved that leaf architecture character are good taxonomic markers in plant identification and classification. The laminar shape, base, apex, margin and texture are the most useful morphological characters in separating the two studied species from each other. Also; this study shows that the type of venation is an important diagnostic character in distinguishing between the two species morphologically.

Epidermal characters have potential for taxonomic use as additional taxonomic characters [20]. The result of examining the epidermal cells shows that the length, size and frequency of epidermal cells vary from the leaf of one species to another among the genera.

The results shows that size of stomata is taxonomically important and can be used in designation between the two studied taxa, on the other hand stomata complex type in the two *Datura* taxa, was amonotetracytic and anisocytic; this is in agreement with observations recorded by Solereder, **[8]** and Hameed & Hussain **[9]**.



Figure 4: Transverse section of *Datura* taxa studied leaf blade.

A-D: Datura innoxia leaf blade, A: Midrid, B: Mesophyll, C: octahedral shaped crystals, D: crystal-sand E-H:Datura stramonium blade, E: Midrid, F: Mesophyll, G: octahedral shaped crystals, H: crystal-sand ep1: adaxial epidermis, ep2: abaxial epidermis, p1: adaxial parenchyma layers, p2: abaxial parenchyma , c1: adaxial collenchyma layers, , c2: abaxial collenchyma layers, pa: palisade parenchyma layer, s: spongy parenchyma layers, cy1: octahedral shaped crystals, cy2: crystal-sand

Stomata frequency shows a taxonomical significant because of its variation which is observed among the two taxa (Table 3). The stomata ratio in the two studied taxa clarify that the leaves are from the hypoamphistomatic type (Table 3) while stomata index is approximately the same; thus it does not provide an additional diagnostic feature in distinguishing between the two studied *Datura* species.

According to the result, Trichomes type presents a significant epidermal diagnostic character in the differentiation between the two studied *Datura* taxa (Table 2).

The anatomical study shows that there is a relationship between the thickness of mesophyll and type of leaf texture, *D. innoxia* leaves are Coriaceous, while *D. stramonium* are Chartaceous and the mesophyll in the transverse section of *D. innoxia* leaves is much thicker than the mesophyll in the transverse section of *D. stramonium*

On the other hand the midrib *D. stramonium* leaf is much thicker than the midrib of *D. innoxia* leaf and that is due to number of collenchyma and parenchyma layers.

CONCLUSION

According to the results the lamina morphological characters as well as epidermal characters and the anatomical characters of the two studied *Datura* taxa leaves shows a high significant in taxonomic value for the separation between the two studied *Datura* taxa.

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