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Leaf anatomy and venation of *Viola odorata* L

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Abstract

The present work comprises anatomical study of the species *Viola odorata* L. in Iraq. The epidermal characteristics of leaves were examined, leaf and petiole sections were studied also. It was clear that, the stomatal type of the species were found to be anisocytic type. Leaves were found to be amphistomatic, Spongy layer not fully differentiated and palisade has loosely arrangement. Glandular and aglandular trichomes were present and druses crystals. The venation was studied, Tertiary veins was random reticulate with straight and convex course, areoles was well development and the free ending veins unbranched and it shows an important characteristic for isolation and identification the species.

Keywords: *Viola odorata*, venation, anatomy, scientific progression

Introduction

The scientific progression in all fields of life and specially the uses of transmission and scanning electron microscopes has an important role to increase dependence of the anatomical characteristics beside to the morphological traits to isolation and identification of different taxonomic ranks, Stace (1989) ^[11] confirmed that the anatomical evidence are important because of it was the least affected by the environment compared with morphological traits, Attention increased to the anatomical characteristics that it become used for taxonomic subjects in the level of Genus, species and even varieties. Some researchers used the anatomical characteristics as an evidence to isolate the Genus as AL-Musawi (1979) ^[4] for the genus *Hyoscyamos* L. and AL-Bermani (1996) ^[1] for the Genus *Aeluropus* Trin. Some researchers used the quantitative anatomical characteristics as evidence for polyploidy as AL-Hussaini (1999) ^[3] for the Genus *Bromus* L., As well as AL-Shammary and Gornal (1994) ^[5] and Rao (1987) ^[10] were used the anatomical characteristics of trichomes to isolate some Genus and species for the families of Saxifragaceae and Sterculaceae.

There are several anatomical studies of *Viola odorata* as Metcalf and Chalk (1950) ^[8] which dealt with Violaceae and most of the dicotyledons families and the study of Watson and Dallwitz (2000). In Iraq there are an epidermal study for the vegetative organs of *Viola odorata* by Hasan and AL-Bermani (2008) ^[7], so this work aimed to study the anatomical characteristics of petiole and leaves in addition to the venation of *Viola odorata*.

Materials and Methods

Fresh specimen were collected from different regions in Baghdad city, the upper and lower surface of leaf and surface epidermis of leaves were prepared by using forcipes and then transferred to a slide containing a drop of safranin –glycerin, then covered with cover slide and examined under light microscope to study ordinary epidermal cells and stomata (AL-Dabbagh and Nasrullah, 2019) ^[2], Stomatal index was calculated according to the following formula: $(S/S + E)$, where S and E are the number of stomata and ordinary epidermal cells respectively in microscopic view field (Paul *et al.*, 2017) ^[9], the cross section of petiole and leaf were prepared by microtome and hand section and stained by toluidine blue or safranin and fast green (Demarco, 2017) ^[6], dimension were determinate by using an ocular micrometer

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Results and Discussion

1. Petiole (Figure 1, Table 1-2)

The cross section shape appeared as triangular with two lateral-adaxial wings and the third wing in the middle of it, the epidermis cells are isodimetric, Collenchyma consist of two layer and increase to three in the wings and druses crystals are present, the vascular bundle was crescent in the middle with two lateral adaxial accessory bundle in the wings. Metcalf and Chalk (1950) [8] confirmed that the single crescent shaped strand either alone or accompanied by smaller lateral ones in the limited number of *Viola* spp.

2. Leaf blade (Figure 2, Table 3-4)

The vertical section showed a curved in the abaxial of central region and slightly curved in the adaxial, the epidermis characterized by mucilageous internal walls and look like Transparent this agree with Metcalf and Chalk (1950) [8]. Spongy layer not fully differentiated and palisade has loosely arrangement, Metcalf and Chalk (1950) [8] refer that the Mesophyll including no clearly defined palisade tissue in certain species of *Viola*. The central vascular bundle has collateral type with collenchyma tissue in the adaxial side.

3. Stomata (Figure 3-4, Table 5-6): The leaf was amphistomatic that the stomata present in both adaxial and abaxial surface and it has anisocytic orientation, this agree

with the results of Hasan and AL-Bermani (2008) [7].

4. Trichome (Figure 5-9, Table 7)

Glandular hairs appear as clavate multicellular head-multicellular stalk in the teeth of the leaf margin that described by Metcalf and Chalk (1950) [8] as glandular shaggy hairs. Aglandular hairs present as unicellular cylindrical found in the abaxial surface and leaf margin with immersed base and surrounded by 6-8 cells in the abaxial surface, unicellular conical hairs found in the abaxial and adaxial surface and in the petiole surface.

5. Crystals (Figure 10)

The crystal found as druses type in the parenchyma tissue of the petiole and in the mesophyll of the leaf, Metcalf and Chalk (1950) [8] refer that the calcium oxalate present as solitary and clustered crystals in the mesophyll and petiolar ground tissue of *Violaceae* species.

6. Venation (Figure 11-15).

Primary veins was actinodromous which has 5 veins initiated from one point of leaf base while the secondary veins was festooned brochidodromous that have brochidodromous branches and looped in the leaf margin, the spaces between the secondary veins was irregular and absent intersecondaries, Tertiary veins was random reticulate with straight and convex course, Areoles was well development and the free ending veins unbranched.

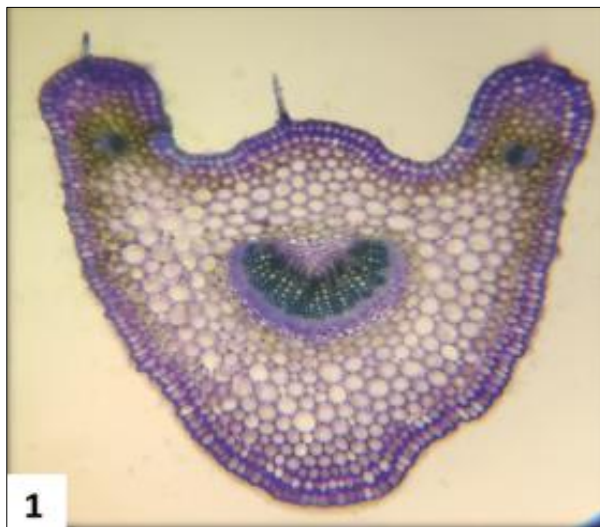


Fig 1: Show the petiole in 4X

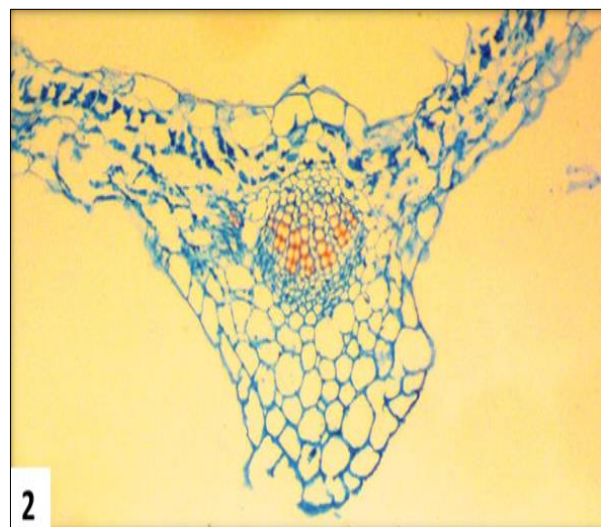


Fig 2: Show the lamina in 4X

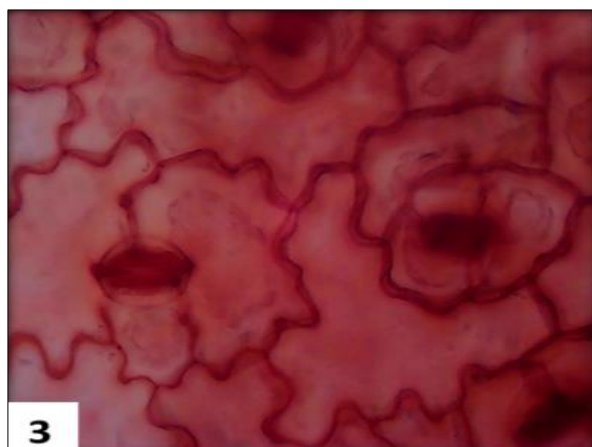


Fig 3: Show adaxial in 40X

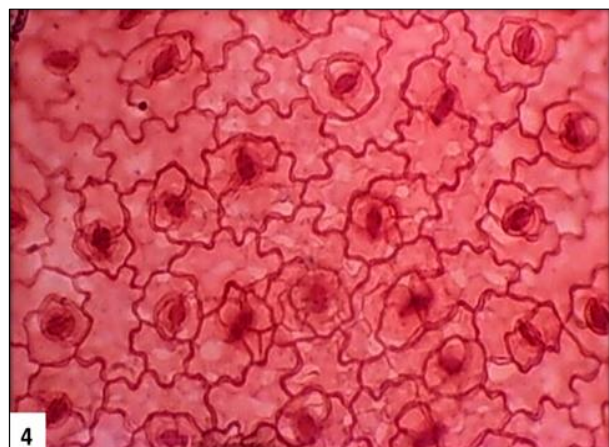


Fig 4: Show abaxial in 10X

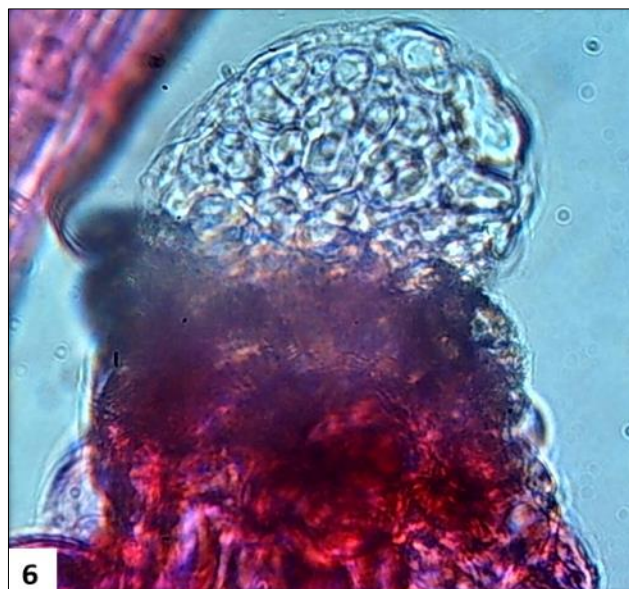


Fig 5 and 6: Show glandular hair in 10X and 40X.



Fig 7: Show unicellular conical hairs 40X

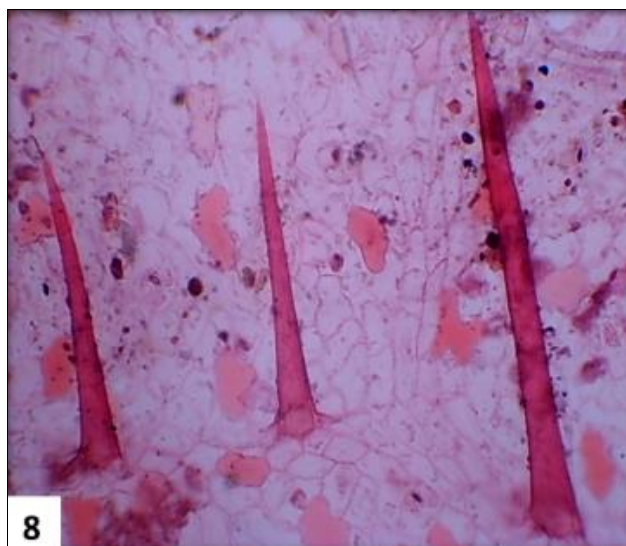


Fig 8: Show unicellular cylindrical hairs 40X

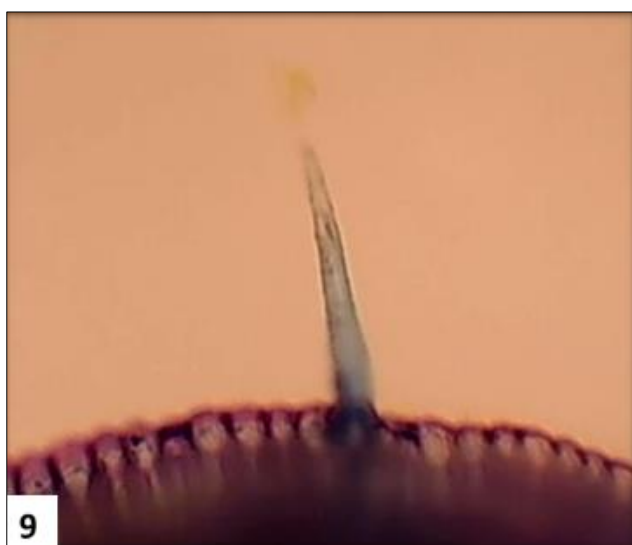


Fig 9: Show unicellular cylindrical hairs with immersed base in 40X

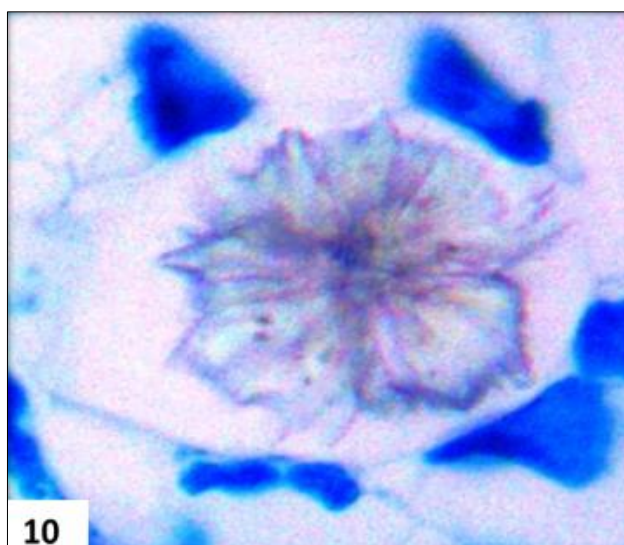


Fig 10: Show druses crystal in 100X



Fig 11: Show venation in the leaf of *Viola odorata*

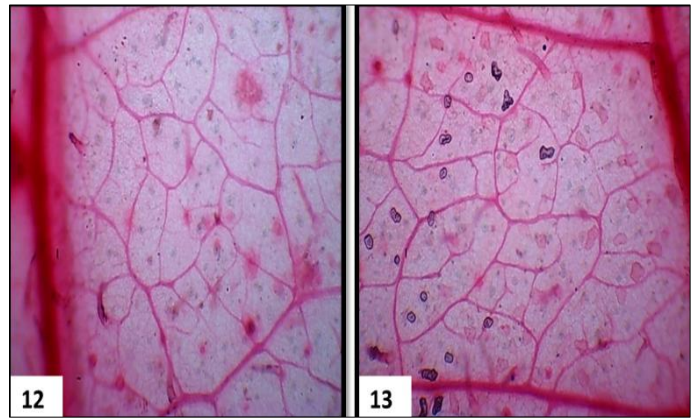


Fig 12-13: Show primary and secondary veins



Fig 14: Show tertiary veins



Fig 15: Show Areoles and the free ending veins

Table 1: Anatomical characters of petiole measured by micrometer (µm).

Species	Cuticle th.	Epidermis TH.	Collenchyma			Chlorenchyma		Parenchyma	
			TH.	Type	No.	TH.	No	Th.	No.
<i>Viola odorata</i>	7.5-10.3 (8.43)	20.6-33.5 (30.27)	65-85.8 (70.2)	Angular	1-3	78-130 (95.3)	3-6	208-260 (242.6)	7-10

Table 2: Vascular bundles characters of petiole measured by micrometer (µm).

Species	Dimension		Fiber	Phloem	Xylem	Fiber	X.C. No.	C.E. No.
	Length	Width						
<i>Viola odorata</i>	357-399 (371)	294-336 (304.5)	25.5-33.4 (30.9)	20.2-28.5 (25.7)	78.5-116 (103.5)	51.2-55.7 (52.9)	28-34	2-8

Table 3: Characters of vertical section in the (Lamina) measured by micrometers (µm).

Species	Blade							Mid vien			
	Cuticle		Epidermis		Palisade		Spongy		Collenchyma		Paren Thickness
	AD.	AB.	AD.	AB.	TH.	No.	TH.	No.	Thickness	Type	
<i>Viola odorata</i>	3.9-6.5 (4.9)	2.5-5.3(4.36)	26-33.8(30.85)	23.3-28.7(25.86)	36.4-41.6(38.86)	1	26-31.2(27.4)	1	20.8-31.2 (23.3)/ad 18.2-26 (20.6)/ab	angular/ad laminar/ab	117-169 (134)/ad. 156-181 (164)/ab.

Table 4: Characters of midrib region in the leaf measured by micrometers (µm)

Species	Shape	Central bundle							
		Dimension		Fibre Th.	Phloem Th.	Xylem Th.	Fibre Th.	X.C. No.	C.E. No.
		L.	W.						
<i>Viola odorata</i>	Sub-spherical	234-260 (243.3)	182-208 (199.4)	18.2-23.4 (21.6)	26.1-31.4 (29.56)	78-91 (87.7)	24.7-26.2 (25.7)	18-20	3-6

Table 5: Epidermis and stomata of abaxial leaf Surface measured by micrometer (µm).

Species	Stomatal cell					Ordinary cell			
	Dimension		L/W	Shape	Stom. Index	Stomatal complex	Dimension		Anticlinal wall
	L.	W.					L.	W.	
<i>Viola odorata</i>	28.6-31.2 (29.12)	18.2-23.4 (21.32)	1.36	Widely ellipsoide	30.7	Anisocytic	65-111.8 (87.88)	26-52 (39.52)	Curved-weekly undulate

Table 6: Epidermis and stomata of adaxial leaf surface measured by micrometer (μm).

Species	Stomatal cell					Ordinary cell			
	Dimension		L/W	Shape	Stom. index	Stomatal complex	Dimension		Anti. wall
	L.	W.					L.	W.	
<i>Viola odorata</i>	26.1-26.7(26.32)	20.8-23.4(21.66)	1.21	Widely ellipsoide	4	Anisocytic	54.6-91(70.12)	26-46.4(34.32)	Weekly undulate

Table 7: Aglandular Trichomes characters measured by micrometer (μm).

Trichome type	Trichome length	Trichome width	Base diameter	Trichome orientation
Unicellular conical	237-441 (385)	24.7-36.4 (32.2)	27.3-39 (35.8)	Oblique
Unicellular cylindrical	315-420 (393)	20.8-28.6 (26.8)	23.4-31.2 (28.4)	Straight
Unicellular cylindrical sunken	186-294 (265)	23.4-33.8 (30.3)	26-36.4 (21.6)	Straight

Conclusion

The use of transmission and scanning electron microscopes has significantly advanced the field of taxonomy by enhancing the reliance on anatomical characteristics alongside morphological traits for the isolation and identification of different taxonomic ranks. Stace (1989) emphasized the importance of anatomical evidence due to its minimal environmental influence compared to morphological traits. Consequently, anatomical characteristics are increasingly utilized in taxonomic studies at the genus, species, and even variety levels. This study aimed to investigate the anatomical features of the petiole, leaves, and venation of *Viola odorata*, contributing valuable insights into its taxonomic classification based on detailed microscopic analysis.

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