



Taxonomic Importance of Anatomical Features in a Certain Iraqi *Malva* L. (Malvaceae) Species

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Abstract

The microanatomical traits of seven *Malva* species were examined in this investigation. The species was gathered from various locales in Iraq during 2024. They are *M.neglecta* Wallr. , *M.nicaeensis* All. , *M.parviflora* L., *M.pusilla* Smth. , *M.sylvestris* L. ,and *M.verticillata* L. . We use these characters to examine the connections between the species under study. Two types of mesophylls have been recognized in *Malva* species. The findings demonstrated that the *M. sylvestris*, *M. parviflora* var. *cristata*, and *M.verticillata* species have dorsiventral (bifacial) leaf blades, while *M.parviflora* var.*microcarpa*, *M.neglecta*, *M. pusilla*,and *M.nicansis* were isobilateral. Petioles are semicircular in most species except *M.nicaeensis*, and *M.verticillata* was irregular. Most species have six separate vascular bundles (four large and two small), There were 7-9 in *M.parviflora* var.*cristata* and 3-6 in *M.sylvestris*.

The stem provides useful characteristics for species differentiation, including its thickness, size, and shape. The outline of the stem is irregularly ovate in *M.neglecta*, *M.nicaeensis*, and *M.parviflora* var.*macrocarpa*, while

it is semi-terete in *M.sylvestris*, *M.parviflora* var.*cristata*,and *M.verticillata*; however, it is irregular in *M.pusilla*. There are two to three layers of chlorenchyma cells in the cortex of every species under study. Then 5–14 layers of angular collenchyma tissue, and finally parenchyma tissue. Cortex thickness ranged from 653.33 µm in *M. neglecta* to 107.5 µm in *M. verticillata*.

Keywords: *Malva*. Anatomical characters, leaves, stems, Malvaceae family.

Introduction

The genus *Malva* L. contains annual or perennial plants that are indigenous to temperate climates, with over 100 species in subtropical and tropical climates. The United States, Africa, Europe, Mexico, the Mediterranean, China, South America, and South and Central Asia are all habitats of the genus (Abedin, 1979, Jedrzejczyk & Rewers, 2020, Naser *et al.*, 2022, Ray, 1995 and Zohary, 1987). Numerous significant microcharacters found in the anatomy of leaves and stems (Al-Saadi & Al-Taie 2014, Al-Saadi *et al.*, 2023, Al-Taie *et al.*, 2018, Al-Mousawi *et al.*, 2019, Metcalfe & Chalk, 1979) might serve as hints for taxonomic identification.

Traditional phytotherapy and medicine use all parts of the plant. In Turkey, Morocco, Egypt, and Iraq, *M. sylvestris* L., *M. neglecta* Wallr. and *M. parviflora* L., are

eaten as vegetables (Naser *et al.*, 2022). Mallow species are used in medicine to help with stomach, breathing and urinary problems; they have strong benefits for liver health, diabetes, ulcers, killing bacteria, and reducing inflammation (Keyrouz *et al.*, 2017). *Malva* species are rich in terpenoids, polysaccharides, vitamin A, mucilage, flavonoids, and malvin. Additionally, several mallow species are used in the phytoremediation (Keyrouz *et al.*, 2017 and Naser *et al.*, 2022). β -carotenes, glutathione, ascorbic acid, and α -tocopherols are all found in *M. parviflora*. Minerals like zinc, iron, copper, manganese, and phosphorus are also present (Alrashedi, *et al.*, 2021; Naser *et al.*, 2022 and Shadid *et al.*, 2021).

Numerous anatomical research studies on various species have focused on the (Shaheen *et al.*, 2010). Analysis of histochemistry

using methylene blue demonstrated the existence of the three species' mucilage (Güven and Duman, 2005 and Shahenn et al., 2009). Olotuah (2014) examined the petiole and leaf epidermis of two chosen hibiscus species to demonstrate their differences. Olotuah (2014) investigated the leaf's anatomy and petiole of *Hibiscus* species and showed differences between them. Naskar (2016) investigated the Malvaceae family's anatomical characteristics and created an identifying key for the delimitation of the genera. Said et al., (2018) also looked at the types of crystals, glandular hairs, cortical collenchyma, chlorenchyma, and mucilaginous cells in the cortex and pith, finding that the vascular bundle is arranged in a single ring and that the wide pith and leaf blade have a dorsiventral structure.

Karakish et al., (2020) study of 17 species of the Malvaceae family from Saudi Arabia and its systemic importance. Taia & Mahdy (2021) discovered that the macro- and micro-characteristics of leaves are helpful in distinguishing between species and elucidating the

evolutionary line within the species under study.

This work's goal is to identify the anatomical research related to seven *Malva* species in Iraq and elucidate the diversity within the *Malva* taxa and evaluate how anatomy contributes to establishing species relationships.

Materials and Methods

Seven taxa of *Malva* have been examined; fresh stems and leaves from these species were gathered during October 2023 to January 2024 from different regions of Iraq. The specimens were preserved in formalin, acetic acid, and alcoholic ethyl solution (F.A.A.) for 48 hours and then preserved in 70% alcohol.

Fresh leaf and stem material was kept in 70% alcohol for sectioning. Following a succession of ethyl alcohol dehydration, they were sliced with a rotating microtome, followed by safranin and rapid green staining, and painted in Balsam Canada (Johansen, 1940). An Olympus CH4 light microscope was used to view the sections, and a Digital Camera Type DCE-2 was used to take pictures. The anatomical definitions were referenced by

(Ditcher, 1974, Esau, 1965 and Radford, 1974).

Results and Discussion

lamina transverse sections

Transverse cross sections, the upper and lower epidermis cells, consist of uniseriate, oval, square-shaped, large circular, rectangular, and orbicular cells. The lamina's thickness is between 133.33 μm in *M. neglecta* and 244.10 μm in *M. parviflora* var. *macrocarpa*, and both types of epidermis cells a thin cuticle on top. The cuticle's thickness was found to be between 1.83 μm in *M. verticillata* and 3.85 μm in *M. neglecta* (Table 1).

Two different kinds of mesophylls have been acknowledged in *Malva* species. *M. parviflora* var. *crinata*, *M. sylvestris*, and *M. verticillata* species have dorsiventral (bifacial) leaf blades, while the *M. parviflora* var. *microcarpa*, *M. neglecta*, *M. pusilla*, and *M. nicaeensis* were isobilateral (Figure 1). Our results

agreed with Alcin & Ozbucak (2002) and Karakish et al, (2020), who reported that *Malva neglecta* leaves have dorsoventral, eglandular, and glandular hair. The palisade layer measures 50 μm in *M. neglecta* to 72.5 μm in *M. sylvestris*, while the spongy tissue measures 50.1 μm in *M. sylvestris* to 50 μm in *M. neglecta* (Table 1, Figure 1). All *Malva* species have druse crystals in their leaves, which concurs with (Metcalf & Chalk, 1950).

Most species have midribs that were curled or flattened on the upper side and typically grooved from the upper side of the midrib that was erect downward in *M. nicaeensis*, *M. pusilla* and *M. sylvestris* and All species have a single oval or circular bicollateral vascular strand. Furthermore, we noticed a large groove on the lower side of the midrib in *M. parviflora* var. *crinata*. Secretory cavities are very common in the taxa investigated; these leaf anatomical characteristics are in line with those of (Metcalf & Chalk, 1950).

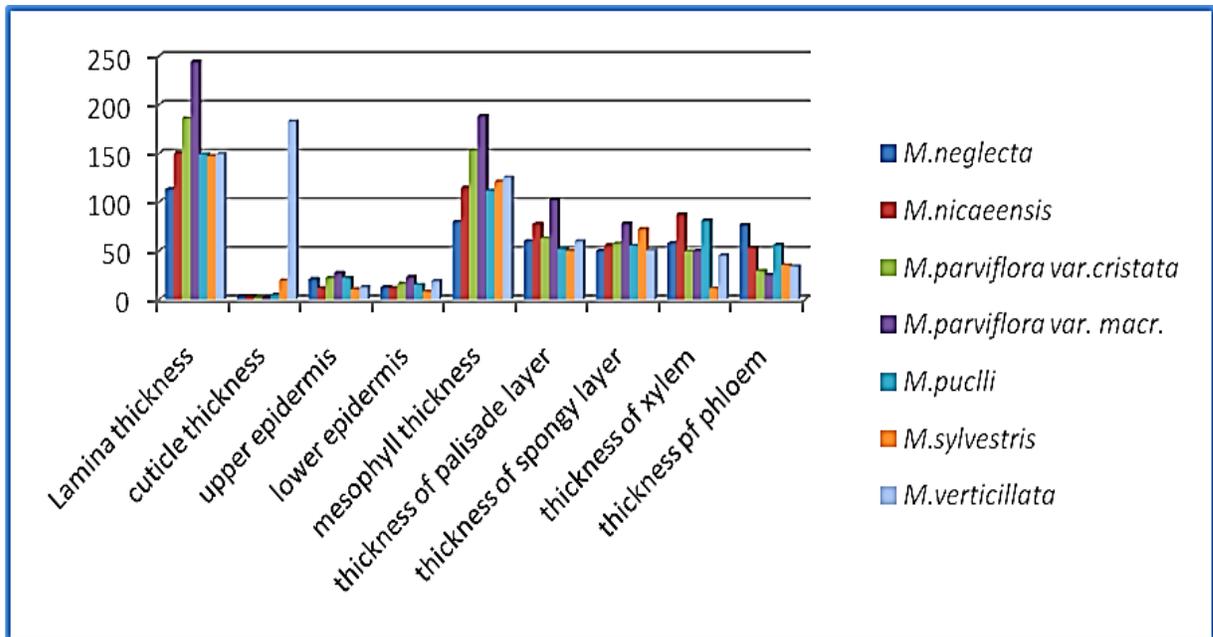


Figure (1): Leaf Measurement in *Malva* Species

Table (1):The anatomical features of leaves in *Malva* species (in micrometer).

Species	Lamina thickness	Cuticle thickness	epidermis		Thickness of palisadelay er	Thickness of spongy layer	Thickness of xylem	Thickness of phloem
			Upper epidermis	Lower epidermis				
<i>M.neglecta</i>	(115-112.5) 113.33	(3.75-3.25) 3.58	(22.5-20) 21.25	(-12.25) (13.75 12.83	(62.5-57.5) 60.25	(52.5-47.5) 50.00	(62.5-52.5) 58.33	(80-66) 76.87
<i>M.nicaeensis</i>	(157.5-144) 150.11	(2.75-2.5) 3.56	(12.5-10.78) 12.02	(12.5-10.34) 12.12	(80-75) 77.5	(50.6-55) 55.83	(90-85) 87.5	(55-57.5) 53.35
<i>M.parviflora</i> <i>var.cristata</i>	(190-175) 186.21	(2.25-2.12) 2.20	(23.75-21.25) 22.5	(21.25-12.5) 16.66	(70-55) 63.12	(60-55) 57.5	(50-47.5) 49.5	(32.5-23.75) 29.37
<i>M.parviflora</i> <i>var.microcarpa</i>	(250-209) 244.10	(2.25-2) 2.12	(37.5-25) 27.5	(25-21.25) 23.25	(105-100) 102.5	(82.5-75) 78.12	(55-45) 50.11	(30-23.75) 25.75
<i>M.pusilla</i>	(152.5-145) 149.04	(6.25-4.75) 5.31	(23.75-21.25) 22.5	(17.5-12.5) 15.22	(55-50) 51.87	(57.5-48.4) 55.62	(87.5-75) 81.12	(62.5-44) 56.25
<i>M.sylvestris</i>	(152-142.5) 147.5	(2-1.87) 1.95	(11.25-10) 10.83	(8.75-7.2) 8.25	(52.5-47.5) 50.01	(75-70) 72.5	(115-112.5) 113.25	(37.5-28.5) 35.41
<i>M.verticillata</i>	(165-137.5) 149.37	(2-1.75) 1.83	(13.75-12.25) 12.85	(25-12.5) 19.16	(62.5-57.5) 60	(52.2-48.75) 50.31	(55-44) 45.32	(40-26.4) 34.16

*Values outside of arches indicate the lowest and highest values, while values between arches indicate the mean.

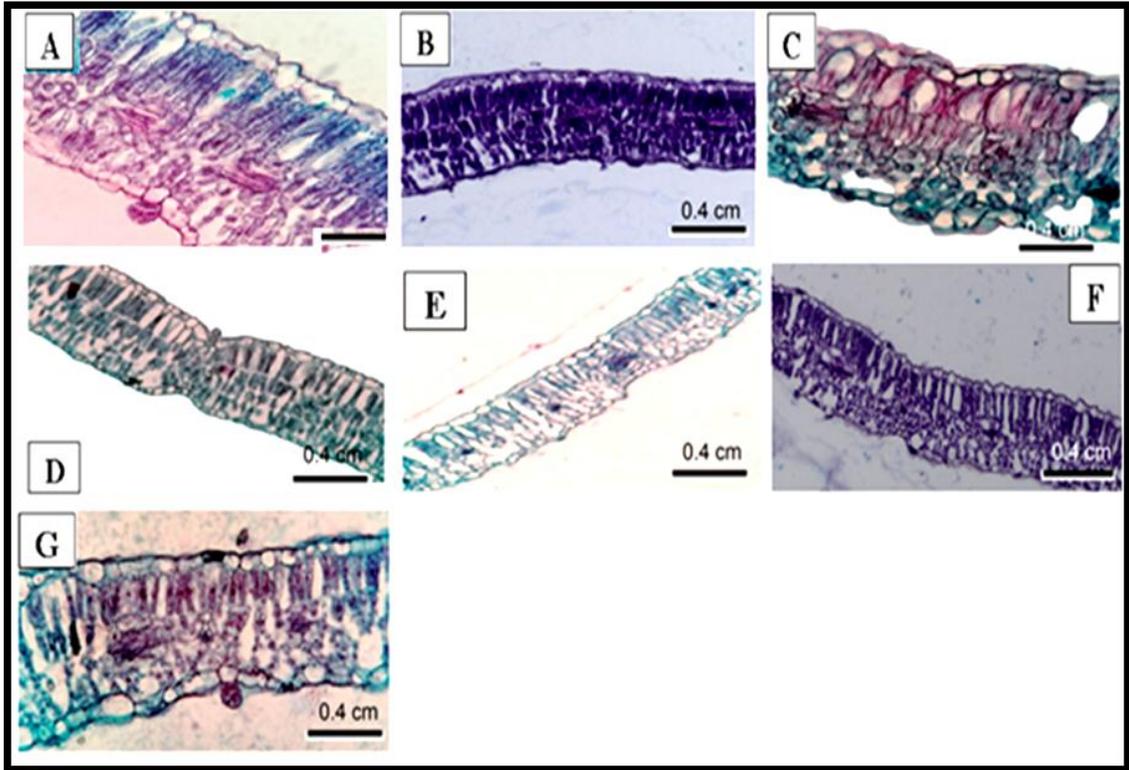


Figure (2): Transverse section of leaf lamina: (A) *M. neglecta* , (B) *M. nicaeensis*(C) *M. parviflora* var. *cristata* (D) *M. parviflora* var. *microcarpa*. (E) *M. pusilla* (F) *sylvestris* (G) *verticillata* (scale 50 μ m).

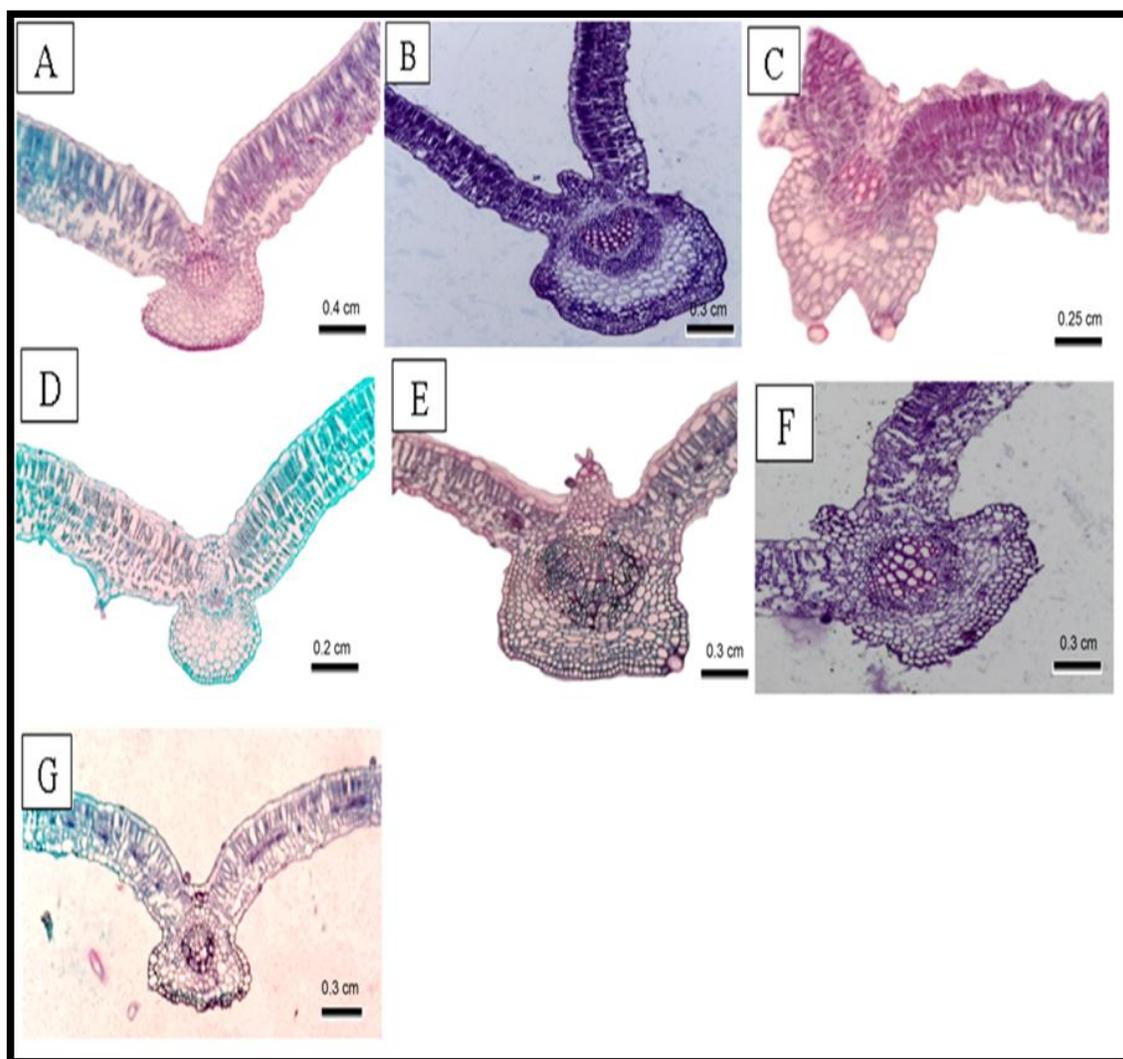


Figure (3): Transverse section of midrib: (A) *M. neglecta*, (B) *M. niceensis* (C) *M. parviflora* var. *cristata* (D) *M. parviflora* var. *microcarpa*. (E) *M. pusilla* (F) *M. sylvestris* (G) *M. verticillata* (scale 50 μm).

Transverse sections of petioles

Petioles are semicircular in most species except *M. nicaeensis* and *M. verticillata* which were irregular (Figure 4, 5). The thickness of petioles ranges from 679.50 μm in *M. verticillata* to 1343.75 μm in *parviflora* var. *microcarpa*. The epidermis is one layer, and the cell shape of the epidermis was cubic

or elongated in all species, surrounded by a cuticle layer. Thickness of epidermis between 7.37 μm in *M. nicaeensis* and 12.83 μm in *M. parviflora* var. *cristata*. However, the thickness of the cuticle varied between 1.91 μm in *parviflora* var. *microcarpa* and 2.62 μm in *M. verticillata*. Parenchyma tissue thickness was found to be between 111.25 μm in

M.verticillata and 280.62 μm in *M. sylvestris* (Table2).

Vascular bundles contain six separate vascular bundles (four large size bundles and two small) in most species, while it was 7-9 in *M.parviflora var.cristata* and 3-6 in *M.sylvestris*. Our results agreed with Karakish et al. (2020) who

found that six bundles exist in *M.verticillata*, while *M. parviflora* has seven to nine \pm equal vascular bundles. The thickness of xylem was variable, between 55.21 μm in *M.parviflora var.cristata* and 98.12 μm in *M.verticillata* whereas phloem was 28.11 μm in *M.verticillata* and 74.25 μm in *M.pusilla* (Table 2).

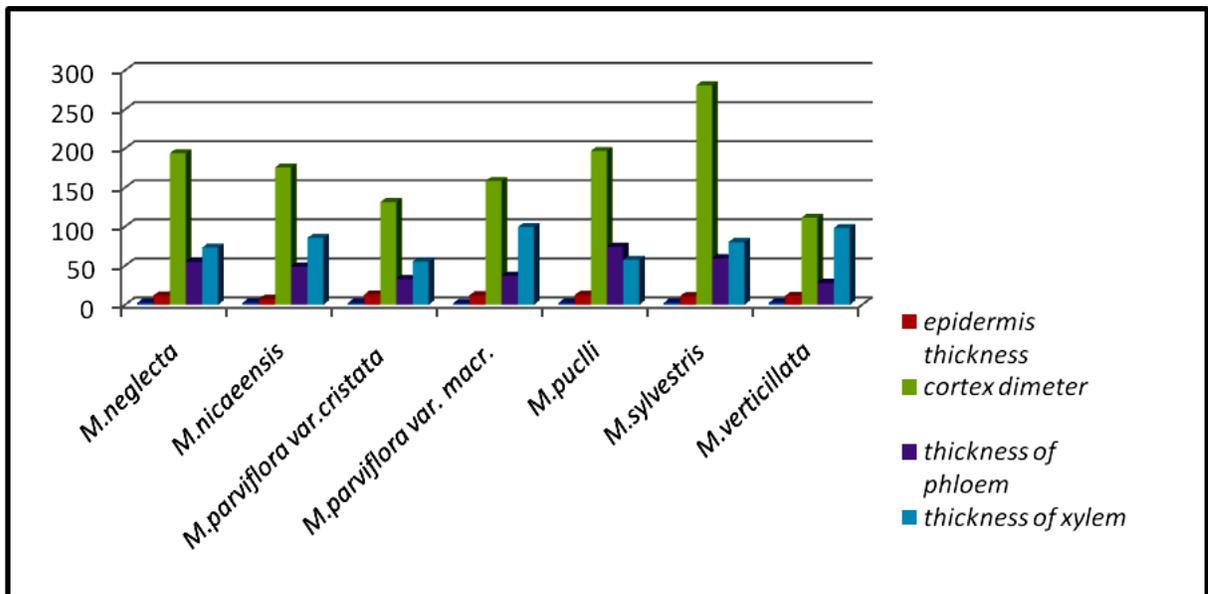


Figure (4): Measurement of petioles in some *Malva* species.

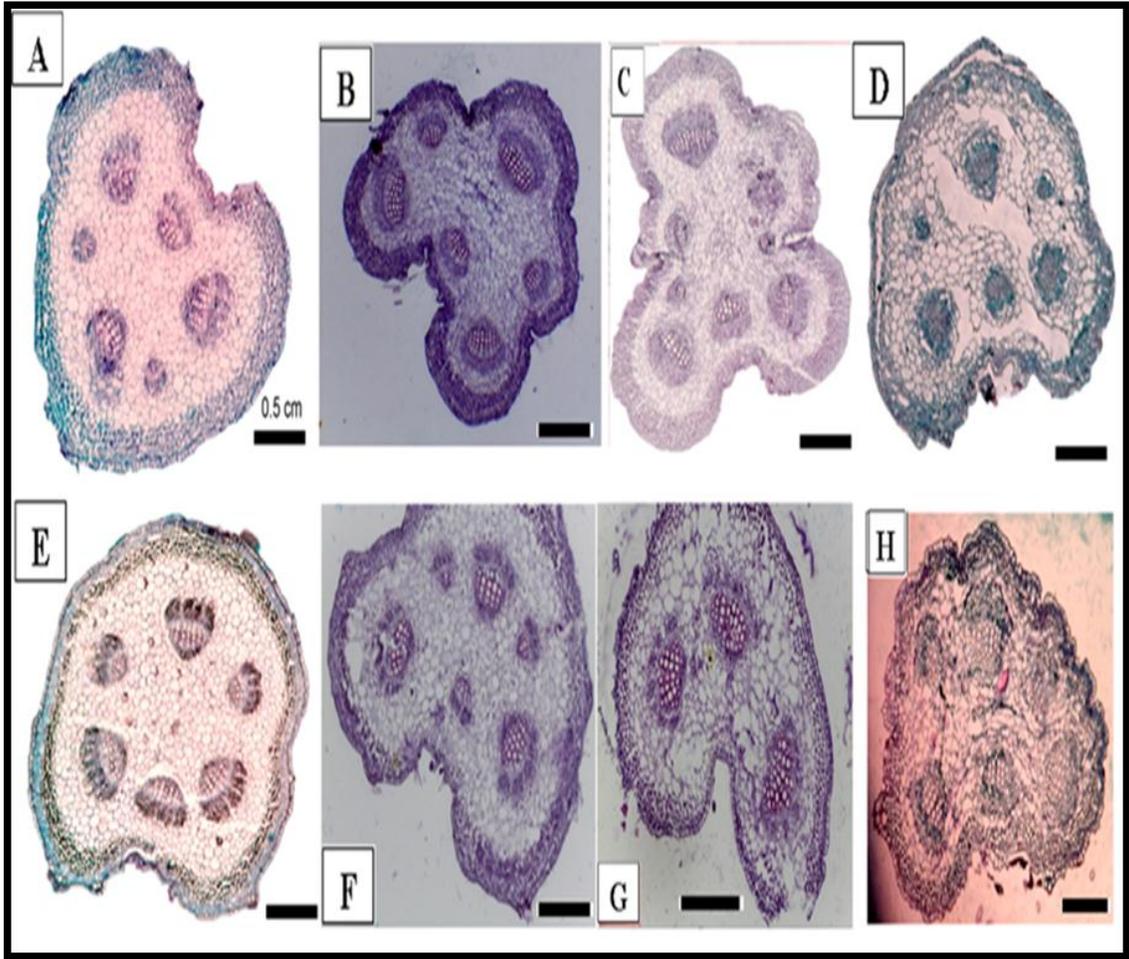


Figure (5): Transverse section of petioles : (A) *M. neglecta* , (B) *M. nicaeensis* ,(C) *M. parviflora* var. *crinata* (D) *M. parviflora* var. *microcarpa* (E) *M. pusilla* (F-G) *M. sylvestris* (H) *M. verticillata* (scale 50 μm).

Table (2):The anatomical features of petiolesin *Malva* species (in micrometers)

Species	Petioles diameter	Cuticle thickness	Epidermis Thickness	Cortex diameter	Phloem thickness layers	Xylem thickness μm	Number of vascular b.
<i>M.neglecta</i>	(1000-900) 950.74	(2.5-2.25) 2.41	(12.5-11.25) 11.75	(200-187.5) 193.75	(57.5-50) 55.11	(75-70) 73.12	6
<i>M.nicaeensis</i>	(1375-1250) 1331.25	(2.75-2.5) 2.61	(7.5-7) 7.37	(180-172.5) 175.62	(50-47.5) 48.75	(87.5-80) 85.33	6
<i>M.parviflora var.cristata</i>	(490.5-439.74) 465.07	(2.5-2.12) 2.29	(13.75-11.5) 12.83	(137.5-125) 131.25	(35-31.25) 32.91	(57.5-50) 55.21	7-9
<i>M.parviflora var.microcarpa</i>	(1400-1250) 1343.75	(2-1.87) 1.91	(12.5-11.25) 11.87	(162.5-157.5) 158.12	(40-35) 37.21	(105-95) 99.37	6
<i>M.pusilla</i>	(1275-1075) 1175.34	(2.5-2.12) 2.28	(12.5-11.25) 12.32	(205-187.5) 196.75	(75-70) 74.25	(62.5-55) 57.5	6
<i>M.sylvestris</i>	(1150-1100) 1124.25	(2.75-2.25) 2.43	(11.25-10) 10.68	(287.5-275) 280.62	(62.5-57.5) 59.37	(87.5-75) 80.14	6-4
<i>M.verticillata</i>	(750-650) 697.50	(2.75-2.5) 2.62	(12.75-10) 11.35	(112.5-110) 111.25	(30-25) 28.11	(100-95) 98.12	6

*The mean is represented by the values in between the arches, while the minimum and maximum values are represented by the values outside the arches.

Transverse sections of stems

The size, shape, and thickness of the stem provide excellent characteristics for species differentiation. The outline stem is irregular ovate in *M.neglecta*, *M.nicaeensis*, and *M.parviflora* var. *microcarpa* and semi-terete in *M.sylvestris*, *M.parviflora* var. *crispata*, and *M.verticillata*, while it was irregular in *M.pusilla* (Figure 6). These results agreed with Karakish *et al.* (2020) which reported that *Malva neglecta* was irregularly ovate to ovate. The thickness of stems ranged between 1456.25 µm in *M.verticillata* and 3708.33 µm in *M.neglecta*. The thickness of the cuticle was observed between 2.06 µm in *M.nicaeensis* and 4.25 µm in *M.neglecta* (Table 3, Figure 7). A layer of cuticle covers the uniseriate epidermis, which a multilayered cortex follows. that is rich in crystals. Thickness of the epidermis is between 7.56 µm in *M.nicaeensis* and 15.11 µm in *M.neglecta* (Table 3).

In all species studied, there are two to three layers of chlorenchyma cells in the cortex, followed by 5 to 14 angular collenchyma tissue; then, the

thickness of parenchyma tissue and cortex ranged from 107.5 µm in *M.verticillata* to 653.33 µm in *M.neglecta* (Figure 6,7).

In every species, the vascular system is an ongoing collateral cylinder. The phloem and xylem create a constant ring in *Malva* species. These findings align with the explanation provided by (Metcalf & Chalk, 1950). The xylem's thickness fluctuated from 79.37 µm in *M.nicaeensis* and 156.87 µm in *M.neglecta*; phloem was 51.84 µm in *M.sylvestris* and 100.62 µm in *M.neglecta* (Table 3). Pith is made up of thin-layered, polyhedral and isodiametric parenchymatous storage cells with intercellular spaces. Pith diameter is between 677.5 µm in *M.verticillata* and 2500.09 µm in *M.neglecta* (Table 3, Figure 6,7). Druses crystals were presented in the cortex layer and pith in all species.

Our results are consistent with those of Karakish *et al.*, (2020) who noted changes in the cortex structure and the separation of the vascular bundles in the stem of *M. parviflora*. Our findings in this study match those of other researchers who found that *Malva*

species contain druse crystals and mucilaginous cavities in the stem's cortex, phloem, and pith (Akbar et al., 2014; Guven & Duman, 2005; Romitelli & Martins, 2013; Shaheen et al., 2009 and Silva et al., 2023). Studies on *Althaea officinalis* L. and *Malva sylvestris* L. showed that *Althaea officinalis* and *M. sylvestris* contain a high

content of mucilage. As well as the stem shape of *Malva sylvestris* L. in cross-section, it has a circular shape (Mariana et al., 2019 and Özkan & Uzunhisarcık, 2009). According to several studies, ploidy level affects anatomical structure, stomata, and leaf morphology (Rakhimov et al., 2021 and Zhang et al., 2024).

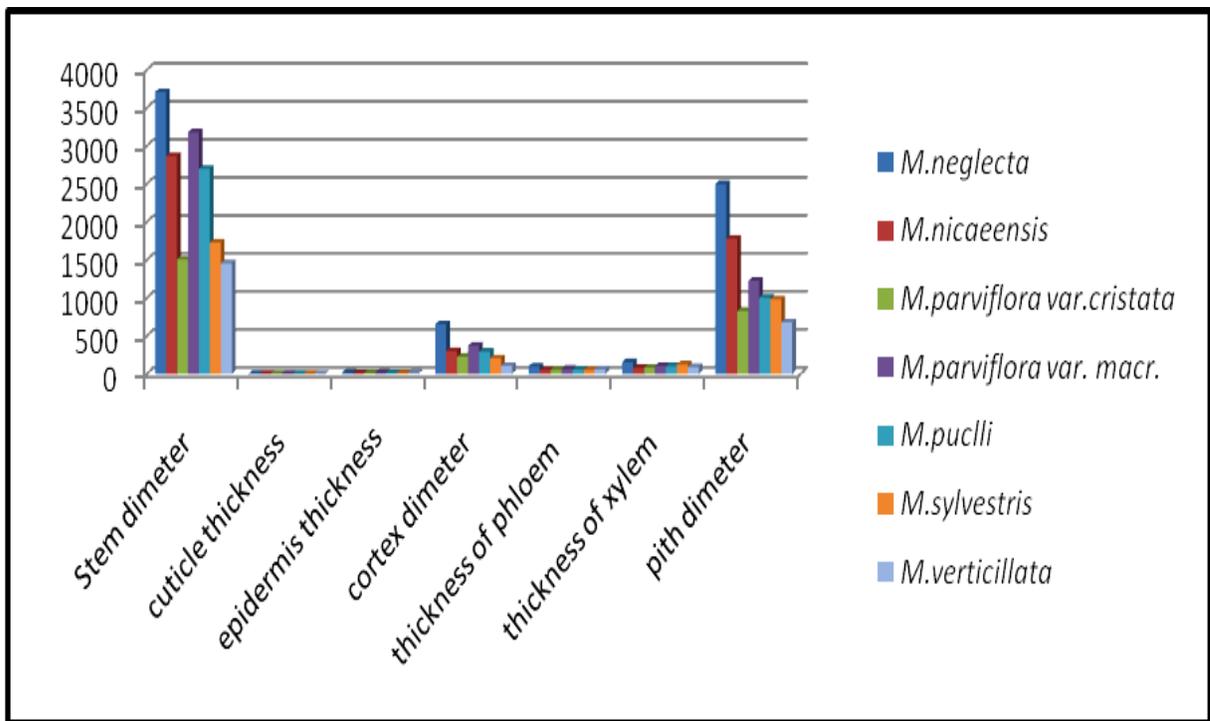


Figure (6): Measurement of stems in *Malva* species.

Table (3):The anatomical features of stem in *Malva* species (in micrometer).

Species	Stem dimeter	Cuticle thickness	Epidermis Thickness	Cortex dimeter	Phloem thickness layers	Xylem thickness layers	Pith dimeter
<i>M.neglecta</i>	(4625-4250) 4437.5	(5-3.75) 4.25	-13.75) (16.25 15.11	(660-650) 653.33	(102.5-97.5) 100.62	(162.5-150) 156.87	(2525-2475) 2500.0
<i>M.nicaeensis</i>	(2875-2850) 2866.66	(2.25-1.75) 2.06	(7.75-7.5) 7.56	(312.5-300) 304.16	(55-50) 51.87	(87.5-75) 79.37	(1825-1750) 1781.25
<i>M.parviflora var.cristata</i>	(1575-1475) 1506.25	(2.5-2.25) 2.41	(12.5-11.25) 11.56	(232.5-225) 228.02	(57.5-55) 56.25	(87.5-75) 81.25	(950-600) 830.75
<i>M.parviflora var.microcarpa</i>	(3250-3075) 3180	(2.75-2.25) 2.50	(12.5-12) 12.31	(384-362.5) 374.37	(80-75) 77.5	(110-100) 104.75	(1250-1200) 1226.66
<i>M.pusilla</i>	(2875-2500) 2700.11	(2.25-2) 2.166	(12.5-11.75) 12.01	(300-287.5) 295.11	(62.5-55) 57.5	(105-100) 102.25	(1010-1000) 1004.75
<i>M. sylvestris</i>	(1750-1700) 1730.12	(2.5-2.25) 2.11	(12.5-10.75) 11.43	(210-207.5) 208.75	(52.5-50) 51.84	(127.5-125) 125.62	(1000-950) 982.5
<i>M. verticillata</i>	(1500-1425) 1456.25	(3.75-2.5) 3.08	(12.75-12) 12.50	(112.5-100) 107.5	(62.5-57.5) 60.62	(100-87.5) 92.5	(700-650) 677.5

*The meaning is represented by the values between the arches, while the lowest and maximum values are represented by the values outside the arches.

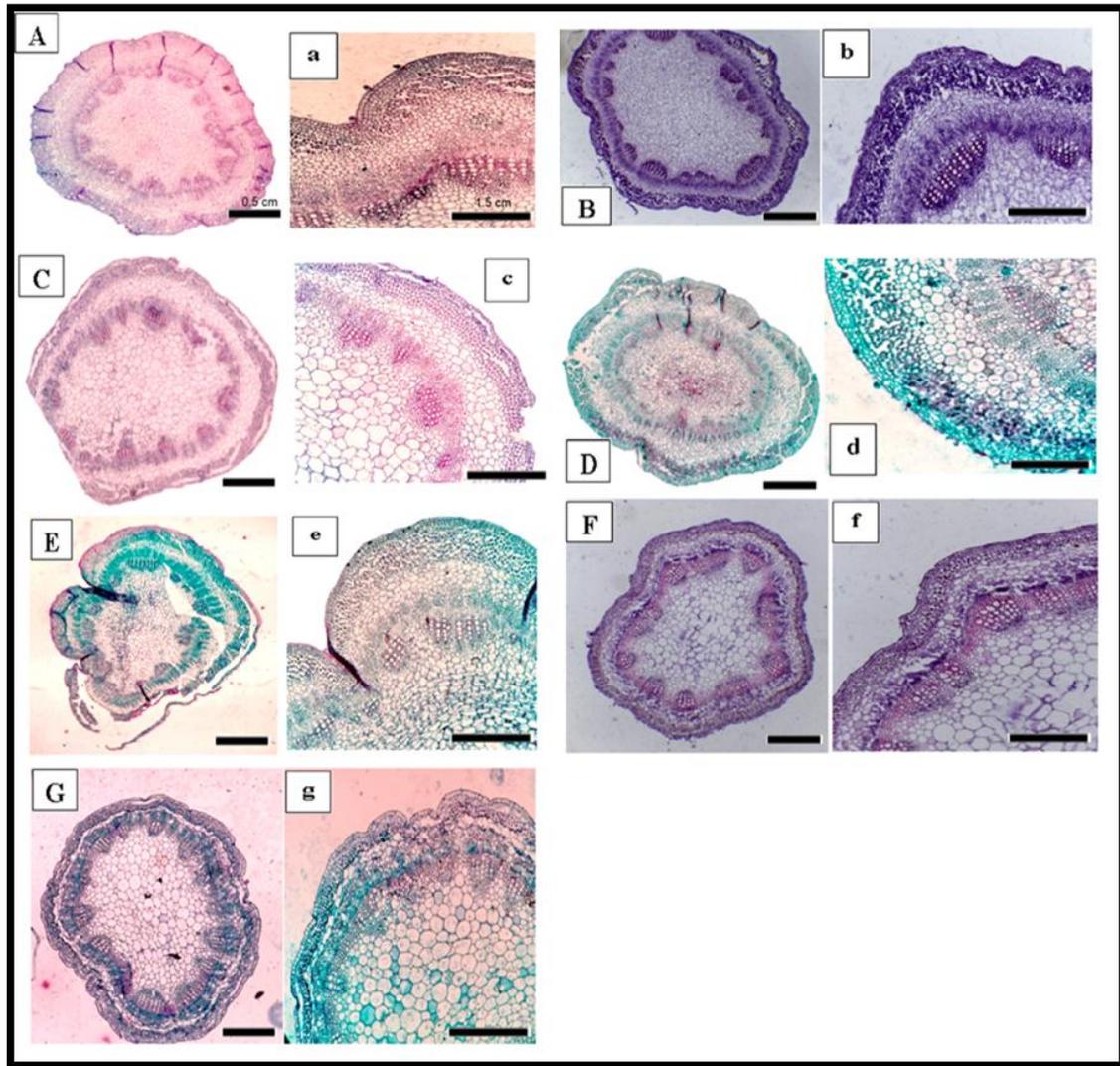


Figure (7): Transverse section of stems: (A-a) *M.neglecta* , (B-b) *M.nicansis* ,(C-c) *M.parviflora* var.*cristata* (D-d) *M.parviflora* var.*microcarpa*(E-e) *M.pusilla* (F-f) *M.sylvestris* (G-g) *M.verticillata*(scale 50 um).

Conclusion

This investigation made it possible to identify anatomical variations among *Malva* species, supporting the classification and taxonomy of these species. The anatomical characteristics differ between the species. The type of mesophyll and shape of stem were separated between two varieties of *M. parviflora*.

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