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Comparative Anatomical Study of the Species *Notobasis syriaca* Cass and *Silybum marianum* L. (Asteraceae) in Salah-Aldin Governorate (Iraq)

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ABSTRACT

The species *Notobasis syriaca* Cass. and *Silybum marianum* L. among the most important endemic plants of the Asteraceae family in the Iraqi flora. The anatomy of the stem and leaves of both species was studied using a compound microscope, due to the similarity between the two species in many morphological characteristics and grow in the same environment. Therefore, this research work was conducted to find Anatomical differences of taxonomic value that help distinguish between the two species under study. Results concluded great importance for anatomical characteristics were achieved by studying stem cross section, leaf epidermis and leaf cross section for both species and compare the anatomical characteristics between the two species under study in a scientific manner.

Keywords- *Notobasis syriaca*, *Silybum marianum*, Anatomical, Asteraceae, Iraq.

I. INTRODUCTION

Plant anatomy involves the examination of internal components of plants and has been a subject of interest for scientists from the early days of microscopy. It is crucial for comprehending plant life (Dengler, 2002). The Asteraceae family, also referred to as Compositae, is the most extensive family of vascular plants, with 2,500 genera and 25,500 species. The distribution of this plant family spans throughout all continents, with the exception of Antarctica. It comprises around 10% of all flowering plants, rivaling the Orchidaceae and Leguminosae families in terms of global plant count (Funk *et al*., 2009; Mandel et al., 2019). Iraq has a significant presence of the Asteraceae family, with approximately 430 species and 123 genera that are native to the country (Alkatib, 1988; Ghazanfer *et al*., 2019). Regarding the two species being examined, the genus *Silybum* L. includes only one species in Iraq, specifically *Silybum marianum* L. Additionally, the genus *Notobasis* Cass. encompasses a single species, precisely *Notobasis Syriaca* Cass., found in Iraq (Rechinger, 1964; Ghazanfer *et al*., 2019). *Silybum marianum*, a prominent medicinal plant in the Asteraceae family, is an annual or biennial plant that is indigenous to the Mediterranean and North African regions. However, it has now become prevalent worldwide (Sidhu & Saini, 2012). *Silybum marianum* and *Notobasis Syriaca* are extensively distributed in the Mediterranean region and significant parts of Asia (Azab, 2018; Porwal *et al*., 2019). *Notobasis syriaca* is the solitary species in the botanical genus *Notobasis* within the family Asteraceae (Snir *et al*., 2015). *Notobasis syriaca* is a highly prevalent shrub in the eastern Mediterranean region, characterized by its thorny and aesthetically pleasing blossoms. Throughout antiquity, humans and animals in numerous areas consumed it as a nourishing food source. Archaeological

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evidence suggests that humans began utilizing *N. syriaca* as a food resource approximately 23,000 years ago (Licata *et al*., 2016). the species *Notobasis syriaca* is a renowned herb in traditional medicine. In Arab society, it was traditionally utilised to cure migraines, the plague, sores of canker, dizziness, and yellowing of the skin and occasionally to improve lactation. It is a component in certain bitters recipes and acts as an antioxidant. The base of stem and leaf might be eaten when fresh. The anatomical traits are important tools in the plant systematics (Lu *et al.*, 2008). The anatomical features became widely used in systematic studies and taxonomic examinations (Noman *et al.*, 2014). There is a shortage of anatomical data on the family Asteraceae taxa, notably concerning the species *Silybum marianum* and *Notobasis syriaca* in Iraq. The current study aimed to provide a thorough description of the anatomical features of the species *Silybum marianum* and *Notobasis syriaca* found in Iraq.

II. MATERIAL AND METHOD

Plant material

The leaves and stems specimens of species *Notobasis syriaca* and *Silybum marianum* collected from different region of Salah Aldin Governorate, Iraq during April, 2023 (Table 1). many publications have been used to taxonomic the plant specimens being investigated (Post, 1933; Davis, 1975; AL-Rawi, 1964; Ghazanfer et al., 2019).

Table 1: species, locations and altitude.

Species	Location	Altitude (m)
Notobasis syriaca Cass.	Alalam, Makhul, Shirqat	100- 204
Silybum marianum L.	Siynia, Beji, Tikrit, Shirqat, Samara	90-210

Epidermis preparation

The epidermis was prepared from fresh samples of species *Notobasis syriaca* and *Silybum marianum*, after being fixed in a solution F.A.A. (Formalin Acetic Acid Alcohol). F.A.A. prepared according to (Johanson, 1940). The fixation process continued for 24-48 hours, after which the samples were washed with 70% ethyl alcohol once or twice. A part of a mature leaf was taken from the middle of the leaf, which includes the midrib and part of the blade and the leaf margin, by used the scrapping method to obtain the upper and lower epidermis use a dissection blade and fine-ended forceps. The prepared epidermis was then transferred to a Petri dish containing water to remove the remaining materials and tissue remnants stuck to the epidermis. Then it was transferred to a clean glass slide containing a drop of glycerin. The epidermis was brushed and covered with the cover slide, it was then ready for examination. The epidermis slides were examined, and measurements of the stomata and the shapes and dimensions of the epidermal cells were taken using an ocular micrometer, and the epidermis was photographed under the camera mounted on an Olympus compound microscope. The stomatal index was calculated based on (Stace, 1956).

Prepare permanent slides for cross-sections of the stem and leaf blade

The method Thammathaworn (1996) was used to prepare permanent cross sections of the stem and leaf blade as follows:

Killing and fixation

Fresh parts of the central region of the stem and leaf blade were taken directly in the field during field trips. It was placed in small glass vials of 30 mm capacity, with about 20 mm of F. A. A. solution in each vial, and the samples were left in the solution for 18-24 hours at room temperature.

Washing and Dehydration

The samples were washed twice with 70% ethyl alcohol to remove traces of the fixative solution. It was preserved in 70% alcohol. Then, small parts of the stem and leaf blade were passed through an ascending series concentrations of ethyl alcohol (80%, 90%, 96%) for a period of time three hours in each concentration, and then in absolute ethyl alcohol for two hours.

Clearing and Infiltration

The sample pieces were passed sequentially in a mixture of absolute ethyl alcohol and xylene in volume ratios (3:1, 1:1, 1:3), then in pure xylene for two hours, after that half of the xylene in the samples were poured and an amount of liquid paraffin was added instead in an oven at a temperature at 60º C for 48 hours.

Embedding and Mounting

Paper templates of suitable sizes were prepared from cardboard, and an amount of melted paraffin was poured into them, and one or two samples were placed in each of them. The templates were marked and left in a cold place for an overnight to ensure they were sufficiently hardened. Then the paraffin templates containing the samples were fixed on special wooden pieces as holders so that they were ready for sectioning using a rotary microtome, the samples were cut with a thickness ranging between 8-12 micrometers. The ribbons sections brushed on clean glass slides that had been previously coated with a thin smear of glycerin-albumin adhesive. The slides were placed on a hot plate at 40-45°C for 4-12 hours for the purpose of fixing the ribbons of sections and removing the wrinkles of them.

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Remove Wax and staining

Glass slides containing plant sections were passed through the following solutions: Xylene 2-4 hours at 50°C twice. Xylene to absolute alcohol 1:1 for 5 minutes. A descending series of ethyl alcohol (96%, 80%, 70%, 50%, 30%) for 5 minutes for each one. Safranin at a concentration of 1% is dissolved in ethyl alcohol at a concentration of 70% for 2-24 hours. An ascending series of ethyl alcohol (30%, 50%, 70%, 80%, 96%) for 5 minutes each. fast green at a concentration of 1% dissolved in absolute ethyl alcohol for 0.5-1 minute. Absolute ethyl alcohol for 5 minutes. Xylene to Ceder oil 1:1 for 5 minutes. Xylene for 3 minutes twice. Then perform Permanent mounting by placing drops of P.D.X. on the section and then put the slide cover and move the slides to a hot plate with a temperature of 40-45 ° C and for an overnight to dry. The procedure before and after staining were performed according to (Thammathaworn, 1996). The samples were examined and measurements of the sections and their dimensions were taken using the ocular micrometer and photographed under the Olympus CH3 mounted camera on an Olympus compound microscope.

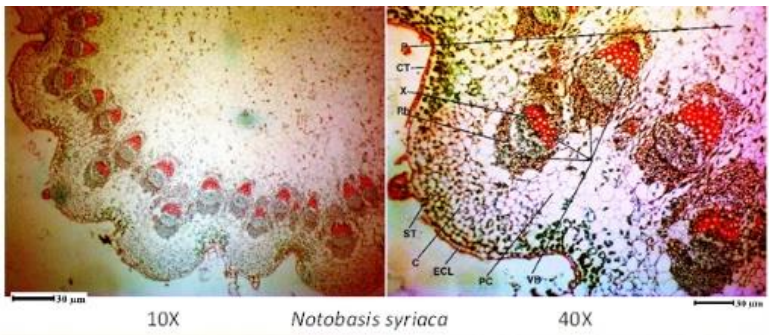
III. RESULT AND DISCUSSION

Stem anatomy

The result of stem anatomical features of species *N. syriaca* and *S. marianum* under study are demonstrated in table 2 and figure 1. The form of stem cross section of species *N. syriaca* and *S. marianum* were cylindrical ribbed or beam like, this is approvement with (Nigmatullaev *et al*., 2019). The epidermis cells in species *N. syriaca* consist of 1-2 rows, the outer wall enveloped by thin or thick a cuticle layer, the epidermis cell form of species *N. syriaca* roundish-oval with average thickness 9.76 µm, followed by 4-6 layers of collenchyma cells next, the cortex layer, composed of 3-7 rows of parenchymal cells with average thickness 152.72 µm, the vascular bundle surround by sclerenchyma cells composed bundle sheath cells, the vascular bundles arranged in two rings and the xylem tissue contain 4-7 rows. The parenchyma pith cells located in the center of stem are characterized by rounded shape with average thickness 687.48 µm. While, the epidermis in species *S. marianum* was polygonal-oblate to oval shape and consist of 2-3 rows with mean thickness 11.52 µm. The parenchyma and collenchyma cells of the cortex tissue is typically found in close of each with a varied number of rows. usually, the collenchyma layer ranging from 3-6 rows, while the parenchyma layer ranging from 3-6 rows. The average thickness of cortex 113.46 µm. The vascular bundle surround by sclerenchyma cap and arranged in 2 rings. The xylem is composed 1-3 rows. Several studies on the many taxa belong Asteraceae including Lersten and Curtis (1997), Celik *et al.* (2005) and Nigmatullaev *et al.*, (2019) have emphasized the importance of the number rows in collenchyma cells under the epidermis layer for identifying various taxa, concerning species *N. syriaca* and *S. marianum*, the identified feature is determined significance in classification.

Table 2. Stem anatomical features of species *N. syriaca* and *S. marianum*, CT: Cuticle Thickness, ECL: Epidermis Cells Length, Co.T: Cortex Thicknesses, ST: sclerenchyma Thicknesses, PhT: Phloem Thicknesses, XT: Xylem Thicknesses, PT: Pith Thickness, measurement in micrometer.

Species	CT	ECL	Co.T	ST	Ph.T	XT	PT
N. syriaca	(3.12-6.47) 4.38	(7.36-13.83) 9.76	(114.61-189.83) 152.72	(37.48-58.27) 48.37	(31.62-46.36) 39.27	(32.73-51.48) 43.72	(435.31-864.84) 687.48
S. marianum	(4.12-7.23) 5.68	(8.47-15.63) 11.52	(88.41-151.76) 113.46	(34.61-60.57) 46.72	(19.84-33.18) 26.51	(24.92-38.64) 32.81	(389.56-809.35) 627.61



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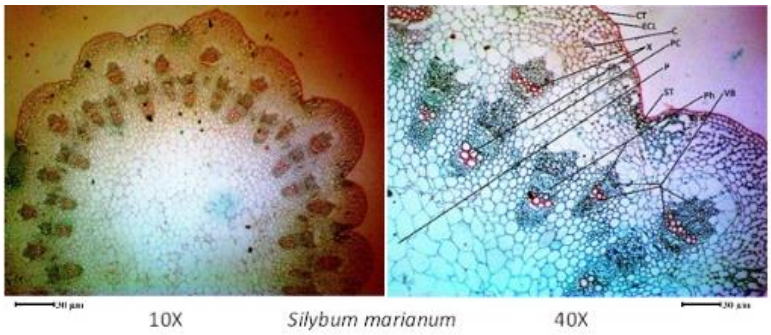


Figure 1. stem anatomy of species *N. syriaca* and *S. marianum* CT: cuticle layer, ELC: epidermis layer, C: cortex, St: sclerenchyma, PC: parenchyma cell, VB: vascular bundle, X: xylem, Ph: phloem, P: pith.

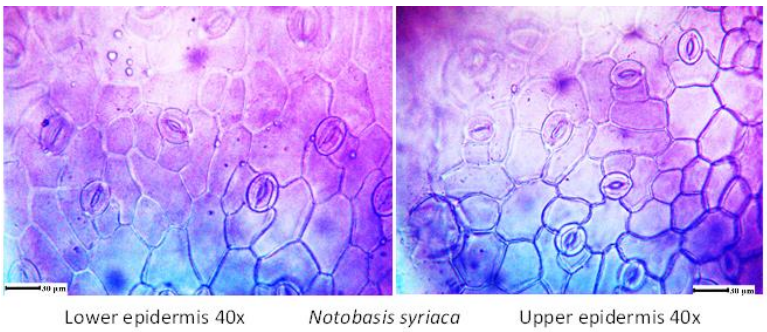
Leaf anatomy

Epidermis and stomata

Data on the longitudinal section of the leaf epidermis of the species *N. syriaca* and *S. marianum* are listed in Tables 3 and figure 2. The current examination that the leaves of the species under investigation are amphisomatic, and there is variation between the leaf epidermis of the species under study in terms of the shapes of the Anticlinal walls, Inner tangential walls and outer tangential walls of epidermal cell ordinary. In addition to the variation in the shapes of epidermal cells between the species under study, the Anticlinal walls in ordinary epidermal cells were straight or semi-straight in species *N. syriaca*, while the Anticlinal walls were slightly curved to winding in species *S. marianum*. The highest average of stomatal index was reached on the Adaxial surface and Abaxial surface, respectively 16.74, 18.58 in species *N. syriaca*. While, the lowest average of stomatal index was on the Adaxial surface and Abaxial surface, respectively 14.19, 16.26 in species *S. marianum*. The species *N. syriaca* recorded the highest average of stoma length, reaching 29.82, 30.47 µm, respectively on the Adaxial and Abaxial, while the species *S. marianum* was the lowest average of stoma length 28.67, 29.47 µm respectively on the Adaxial and Abaxial surface. The species *N. syriaca* is distinguished by anomocytic type stomatal pattern, in contrast species *S. marianum* recognized by anisocytic type. This is consistent with (Srilakshmi & Naida, 2014). The stomata are spread more on the Abaxial than the Adaxial, and this is agreed with was mentioned by (Metcalfe, 1950), that the stomata are spread on both surfaces, but they spread more on the lower surface. The current study also showed differences between the numbers of epidermal cells Surrounding the stomata, as well as in the arrangement and sizes of these cells within one species. As regard to the differences in stomatal index values for the species under study, and the reason of these differences may be a response to certain environmental conditions such as drought, humidity, or intensity of lighting, and this is confirmed by (Esau, 1953).

Table 3. epidermis cell and stomata dimension of species *N. syriaca* and *S. marianum*, SEC: Shape of epidermis cells, UELS: Upper epidermis length of stomata, UEWS: Upper epidermis Width of stomata, IS: Index of stomata, LELS: lower epidermis length of stomata LEWS: lower epidermis Width of stomata, measurement in micrometer (µm).

Species	SEC	UELS	UEWS	IS	LELS	LEWS	IS
N. syriaca	Oblong and straight wall	(26.36-32.72) 29.82	(18.41-23.69) 20.53	16.74	(25.82-33.64) 30.47	(17.27-25.38) 21.76	18.58
S. marianum	Polygonal and winding wall	(24.61-33.48) 28.67	(17.21-23.18) 19.82	14.19	(23.58-32.75) 29.47	(16.21-24.52) 20.48	16.26



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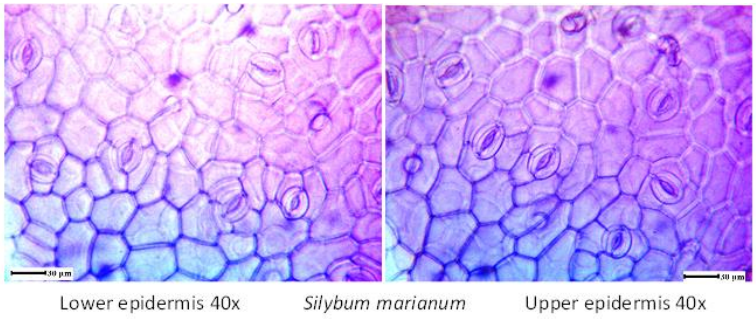

Figure 2. Epidermis and stomata of species *N. syriaca* and *S. marianum*.

Leaf blade cross section

The table 4 and figure 3 show features of the leaf blade cross section of the species *N. syriaca* and *S. marianum***.** Examination of the leaf blade anatomical characteristics of the species *N. syriaca and S. marianum* found that the epidermis was framed by the cuticle layer, the abaxial surface cuticle is thinner than the adaxial surface cuticle. the adaxial surface average cuticle thickness of the species *N. syriaca* and *S. marianum* were 3.51 µm, 3.91 µm respectively, while the abaxial surface average cuticle thickness of the species *N. syriaca* and *S. marianum* were 4.17 µm, 4.69 µm respectively. The abaxial and adaxial surface of species *N. syriaca* and *S. marianum* have a one-layer epidermis covered by a reasonably thick cuticle layer. Epidermal cells of species *N. syriaca* and *S. marianum* different in form and size, the shape of epidermal cell of species *N. syriaca* was oval and circular, and adaxial and abaxial average epidermis thickness 9.17 µm, 10.59 µm. Whereas the form of Epidermal cells in species S. marianum quadrangular and ellipsoid, and adaxial and abaxial epidermis mean thickness 12.34 µm, 13.83 µm. The palisade layer consists of parenchyma cells and present on leaf both sides, the leaf type equifacial in *N. syriaca* and *S. marianum*. The palisade layer average thickness of species *N. syriaca* 44.51 µm, while its average thicknesses in species *S. marianum* 54.78 µm. The spongy layer between the two-palisade layer consists of 1-2 rows in species *N. syriaca* with average thickness 21.85 µm, while the spongy layer of species *S. marianum* consist of 1-4 rows with average thickness 24.18 µm. The lamiana thickness of leaf blade was different of species *N. syriaca* and *S. marianum*, the average lamiana thickness of species *N. syriaca* was 138 µm, but the average lamiana thickness of species *S. marianum* reach 179.61 µm. Sajo and Menezes (1994) confirmed Asteraceae species differed in mesophyll cell layer, but Patricia et al., (2006) indicated 2-4 rows of spongy layer and palisade layer below close to adaxial surface.

Table 4. leaf anatomical features of species *N. syriaca* and *S. marianum*, UCT: Upper Cuticle Thickness, LCT: Lower Cuticle Thickness, UET: Upper Epidermis Cell Thickness, LET: Lower Epidermis Cell Thicknss, PCT: Palisade Cells Thicknesses, SCT: Spongy Cells Thickness, LT: Lamiana Thickness, measurement in micrometer (µm).

Species	UCT	LCT	UET	LET	PCT	SCT	LT
N. syriaca	(1.24-3.45) 2.16	(2.83-3.87) 3.12	(6.41-11.76) 9.17	(7.15-13.61) 10.59	(38.63-51.81) 44.51	(18.26-25.48) 21.85	(118.51-157.85) 138.
S. marianum	(1.67-3.79) 2.68	(3.11-4.28) 3.56	(7.53-16.35) 12.34	(7.43-17.11) 13.83	(43.77-67.29) 54.78	(20.91-27.41) 24.18	(136.48-204.71) 179.61

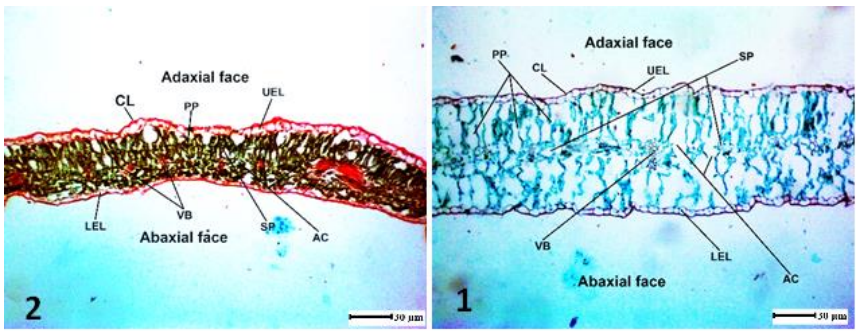

Figure 3. leaf blade anatomical features 1: *N. syriaca*, 2: *S. marianum* CL: cuticle layer, UEL: upper epidermis layer, LEL: lower epidermis layer, PP: palisade parenchyma, SP: spongy parenchyma.

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Midrib anatomical cross section

The results for the midrib cross section of the leaf blade are shown in a table 5 and figure 4. The midrib region of the species *N. syriaca* was a pyramid-shaped structure with a rounded apex, made of collenchyma tissue with average thickness 90.45 µm. The vascular cambium composed of xylem and phloem with average thickness of xylem 52.48 µm, the number of arms of xylem in it reaches 5-9 arms, and each arm contain 4-7 xylem elements. the phloem average thickness of species *N. syriaca* 25.56 µm. The midrib form in species *S. marianum* convex, dome-shaped, these results are consistent with the study of Nigmatullaev (2019) indicated that the Midrib of the species *S. marianum* contained a bundle of collenchyma supporting tissues in a dome-shaped. the average thickness of collenchyma tissue 74.13 µm. The xylem average thickness of *S. marianum* 46.85µm, the number of arms of xylem in it reaches 3-6 arms, and each arm contain 2-5 xylem elements. the phloem average thickness of species *S. marianum* 22.81 µm. These features are reliable in identifying the family and complement earlier findings by (Metcalfe and Chalk,1950; Fahn, 1979; Castro *et al.*, 1997; Noorbakhsh *et al.*, 2008; Makbul *et al.*, 2011)

Table 5. Midrib anatomical features of species *N. syriaca* and *S. marianum*, UCT: Upper Cuticle Thickness, LCT: lower Cuticle Thickness, UECL: Upper Epidermis Cells Length, LECL: Lower Epidermis Cells Length Co.T: Cortex Thicknesses, ST: sclerenchyma Thicknesses, PhT: Phloem Thicknesses, XT: Xylem Thicknesses measurement in micrometer (µm).

Species	UCT	LCT	UECT	LECL	Co.T	ST	PhT	XT
N. syriaca	(1.89-4.37) 3.51	(2.73-4.84) 4.17	(7.51-13.28) 10.84	(7.92-15.41) 12.71	(71.54-113.28) 90.45	(14.81-23.75) 19.62	(21.37-29.49) 25.56	(37.61-62.51) 52.48
S. marianum	(2.12-5.07) 3.91	(3.25-5.71) 4.69	(9.24-17.41) 13.57	(8.19-18.25) 14.77	(63.29-86.51) 74.13	(11.26-19.73) 15.25	(18.76-26.25) 22.81	(31.24-53.67) 46.85

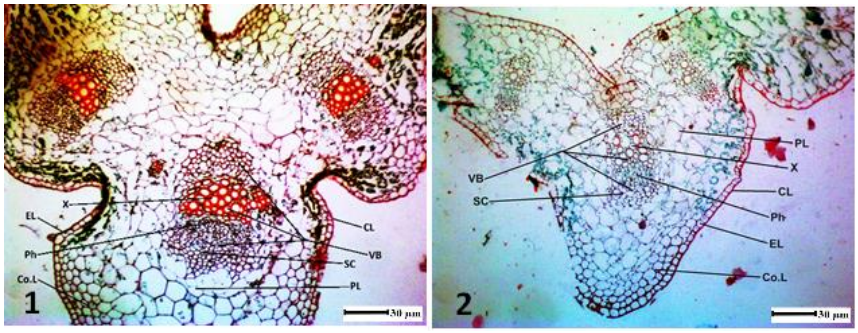

Figure 4. Midrib anatomical cross section 1: *N. syriaca*, 2: *S. marianum* CL: cuticle layer, EL: epidermis layer, Co.L: collenchyma layer, SC: sclerenchyma cell, PL: parenchyma layer, VB: vascular bundle, X: xylem, Ph: phloem.

IV. CONCLUSION

This research was beneficial and led to the examination of important anatomical difference between the species *Notobasis syriaca* and *Silybum marianum* presence variation in stem anatomical characters and leaf epidermis and stomatal patterns.

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