



Taxonomical and Anatomical Study of the Species *Nonea echioides* (L.) Roem. & Sehl (Boraginaceae) in Northern Iraq

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Received: 14/3/2021

Accepted: 22/6/2021

Abstract

The present study deals with some morphological and anatomical characteristics of the *Nonea echioides*(L.) Roem. & Sehl species belonging to *Boraginaceae*, which is recorded to have spread recently in Kurdistan region of Iraq.

This research focused on some of the important morphological characteristics of the stems, leaves, flowers, and fruits and comparing them with other studies of neighboring countries to Iraq. These morphological characteristics were found to be important in isolating the species of the field. The anatomical features of the epidermis, stomata, and trichomes were also investigated. The study shows that *Nonea echioides* belongs to C_3 plants based on the anatomical features of the leaf. In conclusion, the present study provided means for field identification and taxonomy of the plant.

Keywords: Borage family, *Nonea Medik* , Classification , Identification ,Trichomes.

دراسة تصنيفية وتشريحية للنوع *Nonea echioides* (L.) Roem. & Sehl في شمال العراق

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الخلاصة

تناولت الدراسة الحالية بعض الصفات المظهرية والتشريحية للنوع *Nonea echioides*(L.) Roem. & Sehl الذي يعود للعائلة *Boraginaceae*، والذي سجل انتشاره حديثاً في كردستان العراق. تم التركيز في هذا البحث على بعض الخصائص المظهرية المهمة للسيقان والاوراق بالإضافة الى دراسة الصفات المميزة للأزهار والثمار ومقارنتها مع دراسات اخرى في الدول المجاورة للعراق . وتبين ان لها أهمية كبيرة كصفات تصنيفية في التمييز بين الانواع في الحقل ، وكذلك تم دراسة بعض الخصائص التشريحية (صفات البشرة والثغور والشعيرات) كما اظهرت الدراسة ان النوع *Nonea echioides* ينتمي الى نباتات C_3 وذلك من خلال الصفات التشريحية للورقة واخيراً تم وصف النوع *Nonea echioides* لتسهيل تشخيصه حقلياً و تصنيفياً .

1. Introduction

Boraginaceae of the order *Boraginales* order is one of the most widespread plant families in the temperate (tropical and subtropical) regions of the Earth [1], comprising about 117-131

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genera and 2,700 species [2-5]. In Iraq, it has 26 genera and 93 species [6]. Also, the family has 29 wild species, whereas 5 species are cultured [7].

Nonea medik is one of 15 genera belonging to the *Boragineae* tribe. The *Nonea* genus spreads from Central Asia to the Atlantic region in the Iberian Peninsula and Morocco. It is also recorded in the Caucasus region, the Iranian-Turkish highlands, and Anatolia. The *Nonea* genus is recorded to be more widespread in Turkey, as the number of its species reaches 23 species, eight of which are endemic to Anatolia only [8,9]. Its spread is also noticed in the Mediterranean region and on the banks of the Aras and Qizil rivers in Iran [10]. Raedl [11] reported 13 species belonging to this genus in Iran's flora. The spread of 45 species belonging to the *Nonea* genus was also reported in the Irano – Turanian- Anatolian mountains, which are considered as the center of biological diversity [12, 13].

The spread of 9 species was recorded in the Kingdom of Saudi Arabia [14], whereas this genus was not recorded in the Lebanese and Palestinian Botanical Encyclopedia [15]. *Nonea echioides* is placed under the name *Nonea ventricosa* (Sm.) Griseb by Rechinger [16] and Al-Rawi [17]. In addition, research in Italy distinguished 12 genera and 37 species belonging to the *Boragineae* tribe [18]. There is a wide debate regarding the *Nonea* genus due to the wide and clear variation in flowers and fruits. This variation is shown in the corolla and fruit shapes, in addition to differences in the micro phenotypic characteristics of pollen grains and surface patterns. It was classified long ago by De Candolle in 1846 [19]. In addition, *Nonea* has been widely contested as being placed within the sect. *Orthocarym*, due to the difference in nutlet shapes, which are erect, curved, or reniform [20]. This includes a study conducted by Sardar [21] who indicated the presence of a new species belonging to the *Paracaryum* genus in Mount Sinjar, Iraq. While Chacon [22] studied the spread of the *Borage* family in Germany and found that there are 174 species within four groups based on DNA indicators. Al-Zubaidi [23] diagnosed the presence of a number of phenolic compounds in the *Anchusa* genus in Iraq. The two species of *E. plantagineum* and *E. vulgare* were recorded, in addition to the study of pollen grains of 35 species belonging to the *Nonea* genus, by Falatoury [24] in Iran. Notably, the study of Ahmed [25] was the first to report *Nonea ventricosa* (Sm.) Griseb spread in northern Iraq. Bothalia [26] indicated the presence of new species within the *Echium* genus in South Africa. The economic importance of this family is that it is used for decoration, while some types are used for coloring oils, medicines, and syrups, some of which are used for medicinal purposes, whereas others are weeds [23].

The aim of the current study is to provide an accurate description of *Nonea echioides* and to identify its most important phenotypic and anatomical characteristics.

2. Materials and Methods

2.1 Sample collection: Fresh samples of the wild-growing *N. echioides* were collected from three districts of Iraq, which are Erbil, Sulaimaniyah, and Kirkuk, during several field trips for the period between February - April of 2019-2020 (Figure 1). The samples were then preserved in 70% ethyl alcohol, after leaving them for 10 hours in a Formalin acetic acid alcohol (F.A.A) stabilizer solution [27].

2.2. Sample Diagnosis: The samples were diagnosed based on the information available in the Turkish, Iranian, and Italian botanical encyclopedias [28,29]. The selected samples were also compared with the species listed elsewhere [17].

2.3. Phenotypic study: Measurements of the general plant height, root, and stem, as well as leaf length and the dimensions of the calyx, corolla, and fruits of *N. echioides* were taken using a ruler. Some parts were photographed under an anatomical microscope with a power of 10X [27].

2.4 Anatomical study: The epidermis of stem and the upper and lower epidermis of the leaf were studied by the skinning method. The measurements of epidermal cells, stomata, and trichomes were recorded and photographed. In addition, cross sections of stems and

transversal sections of leaves were made after fixing them in the F.A.A solution and preserving them with ethyl alcohol at 70% concentration. These samples were passed in a series of ethyl alcohol concentrations (80%, 95% and 100%) for 2 hours for dehydration, followed by a series of alcohol and xylene with the proportions of 3: 1, 1: 1, and 1: 3 v / v for 10 minutes each. After that, it was soaked with paraffin wax, then cut into thin strips with a thickness of 5-10 μm and loaded onto clean glass slides for viewing and photographing using an optical microscope [30].

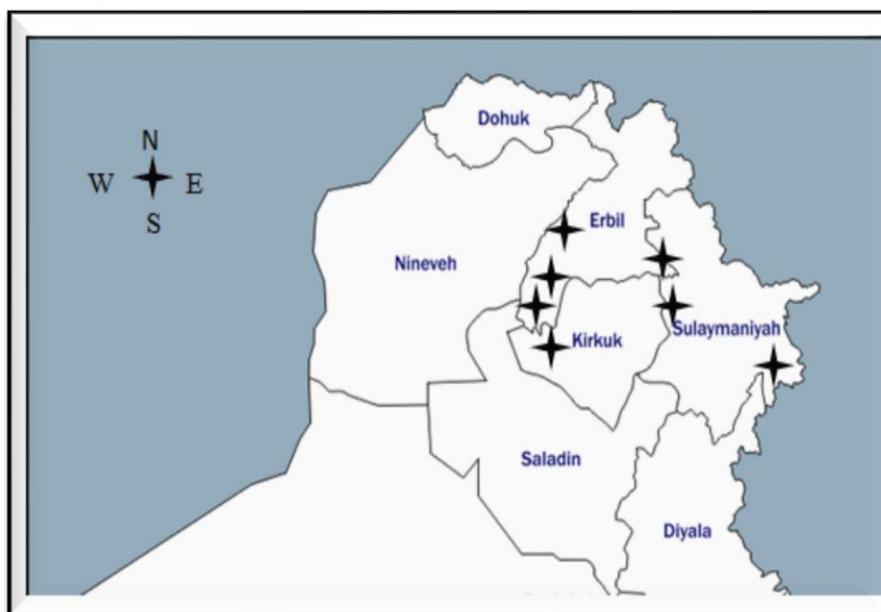


Figure 1-Distribution of *Nonea echioides* of northern Iraq.

3. Results

3.1 Morphological study

The results of the phenotypic study show that the *N. echioides* is an ephemeral herbaceous plant with many branches stemming from the base. The height of the plant is in the range of 10-20 cm, while the roots are wedged without branching. The stem is hairy and grows upward in an ascending manner of 6-8 cm long, with 5-10 branches that contain cauline leaves (lower and upper), with no basal leaves.

The leaves were oblong or lanculate, with dimensions of 5-40 \times 6-8 mm (Table 1). The leaves are sessile with an obtuse apex, having a truncate or cordate base, in addition to an entire margin.

An important observation is that the midrib vein is deeply sunken within the leaf blade at the upper surface view of the leaf. The bract is cordate with an acute apex, 3 cardiac base, a smooth filamentous tip, and dimensions of 11-15 \times 4-6 mm (Plate.1, Table 1.). The inflorescence is of spike flowers, bisexual and sessile, sometimes with short pedicles. The calyx is of a fruiting type, made up of five united sepals with teeth ends. The calyx tube has an average length of 3 mm and its teeth is 1.5 mm. The corolla is of five-petals which are united, symmetrical, and radial, having a salver form with white color and a wide limb of dimensions of 3.5 \times 7.5 mm. The stamens are 5-merious, epipetalous, free versatile, black, and blooming in length, with dimensions of 1 \times 3 mm. The filaments are linear of white color and 0.5-0.7 mm in length. The pistil has a single, ovary which is simple, globose, gynobasic style, style undivided, filiform and stigma capitate. (plate 1). The fruit is with transversely reniform nutlets, dimensions of 2-3 \times 1-2 mm, blackish to dark brown color with a white scar at maturity, and reticulate ornamentation.

Table 1- Morphological characteristics of *N. echioides* .

Morphological characteristics (Mean \pm SE)			
Plant height (cm)	Stem length(cm)	Leaf length(mm)	Leaf width(mm)
10-20 (15 \pm 5)	6-8 (7 \pm 1)	35-40 (37.5 \pm 5)	6-8 (7 \pm 1)
Bract length(mm)	Bract width (mm)	Calyx length(mm)	Corolla length (Rate L\timesW)
11-15(13 \pm 2)	4-6(5 \pm 1)	3 \pm 1	3.5 \times 7.5
Stamen length(mm)	Stamen width(mm)	Fruit length(mm)	Fruit width (mm)
1-3 (2 \pm 0.5)	1-2 (1.5 \pm 0.5)	2-3 (2.5 \pm 1)	1-2 (1.5 \pm 0.5)

**Plate 1-** *N. echioides*. 1 & 2: habit; 3: leaf; 4: bract; 5: inflorescence; 6 & 7: flower; 8: calyx fruiting; 9: stamens; 10 & 11: fruit .

3.2 Anatomical study

According to the result of the stem epidermal analysis, cells of the elongate-linear type have straight walls with oblique ends, Dimension 500-650 \times 100-140 μ m. The stomata are parallel, their dimensions 20-30 \times 10-15 μ m, Its frequency reaching 3 stomata in the microscopy field under the force 40X, while the upper and lower epidermal cells of the leaves were regular, with 4-6 angles with straight walls in the lower epidermis, their dimension 50-90 \times 30-40 μ m, it is . The epidermal cells are highly undulate in the upper epidermis, its dimension 30-50 \times 20-35 μ m. The stomata of the anomocytic type appeared in both surfaces of the stem and leaf in which the length \times width 25-30 \times 25-30 μ m in the lower epidermis and its frequency reached 4 stomata; and between 30-35 \times 20-25 μ m in the upper epidermis with frequency reaching 6 stomata. (Plate .2, Table 2).

The trichomes spread on the stem, the surfaces and margin of the leaf, and the calyx. They are similar in all parts, in which they are of the non-glandular type, single cell, curved type, papillate, flexuous, or short erect, ranging in length between 100-300 μ m in the surface leaves. They may have a multicellular cup base or the base is immersed in the epidermis

rounded by a surrounding collar, and at times it appears surrounded by only two cells. Its tip is acute or rounded in the stem. (Plate 3).

Table 2 -Stoma features and trichomes on the upper and lower epidermis .

Epidermis characteristics (Mean ± SE)			
Upper surface of leaf	lower surface of leaf	Stem surface	Trichomes
	Epidermis cells length (µm)		
30-50(40 ± 10)	50-90(75± 20)	500-600(550± 50)	
	Epidermis cells width (µm)		
20-35 (27.5 ± 7.5)	30-40(31± 5)	100-140(110 ± 20)	
	Stomata length (µm)		
30-35(32.5 ± 2.5)	25-30(27.5 ± 2.5)	20-30(25± 5)	100-250 (170± 75)
	Stomata width (µm)		
20-25(22.5 ± 2.5)	25-30(27.5 ± 2.5)	10-15(12±2.5)	

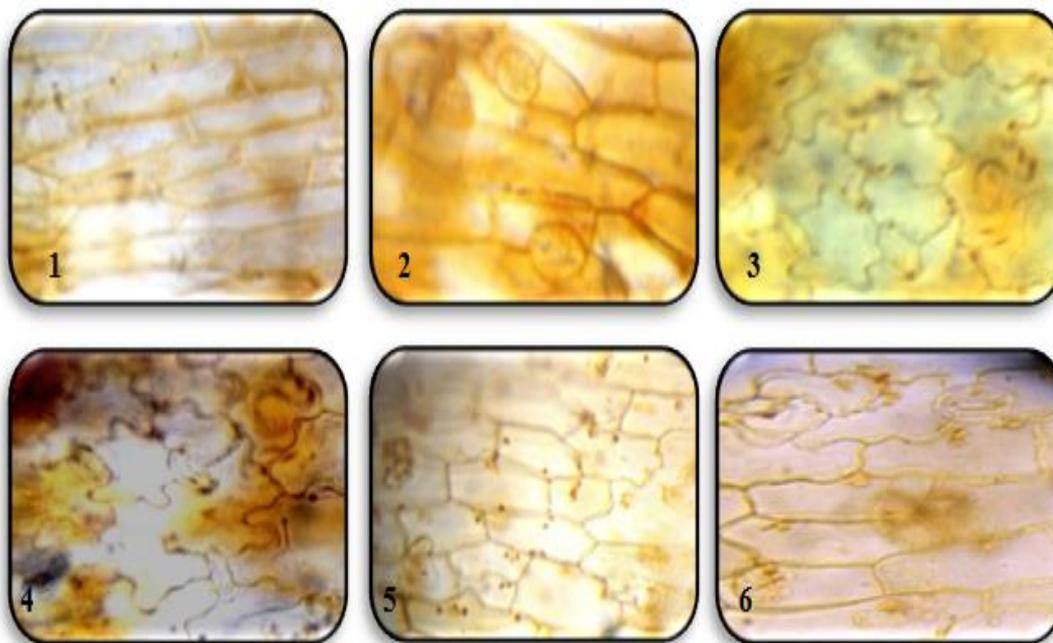


Plate 2- Surface characteristics of epidermis cells ,1- Surface view of stem epidermis cell, 2 stomata of stem , 3 .Upper surface of leaf, 4- stomata of upper surface. 5 Lower surface of leaf 6- stomata of Lower surface.

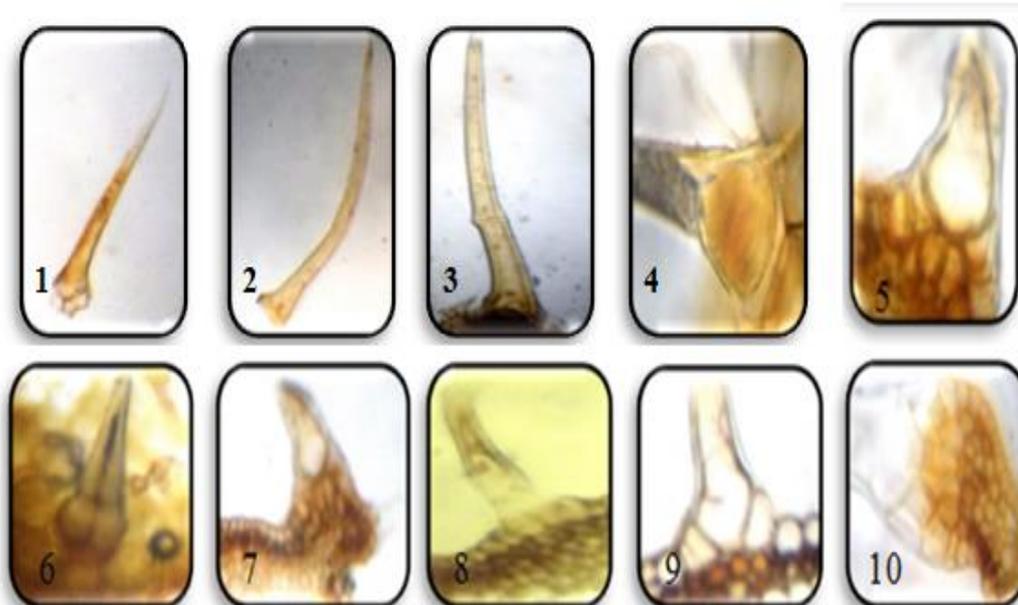


Plate 3-Morphological features of trichomes.1-3 hairs type , 4-10 hairs basic .

The results of the stem cross sections showed variation in types of cells and the method of their proliferation. The stem was circular consisting of a thin layer of cuticle (dermis) with 2.5 μm in thickness, followed inside by the epidermis which is oval, interspersed with a number of stomata that appear leveled with the epidermal cells. It is also observed that the base of the Trichomes are immersed within the epidermis with an average thickness of 10 μm . This is followed in the inside by the ground tissue representing the cortex consisting of several tissues, including lamellar collenchyma, chlorenchyma and ordinary parenchyma below epidermis and wide parenchymatous pith in the center of the stem, vascular tissues in a circular arrangement separating between cortex and pith , which forms a regular ring with the thickness of the crust reaching between 150-200 μm . As for the vascular tissue making up a number of vascular bundles arranged in closed collateral bundles without kranz anatomy (i.e., characteristic of C3 plant), and consisting of xylem and phloem and surrounded by the bundle cup with dimensions 50 \times 100 μm (Plate.4, Table 3). The transverse sections of the leaf appear to be of the winged, bifacial type, 300-350 μm in thickness, covered with a thin layer of cuticle with a thickness 2.5 μm . This is followed by the upper epidermis that is uniserate with aligned cells except for stomata regions with an average thickness of 15 μm , while the average lower epidermis thickness is 25 μm . It should be noted here that the stomata are of the anomocytic type and the proliferation of trichome is also observed. Concerning the mesophyll tissue, the thickness is between 270-300 μm consisting of two rows of palisade parenchyma with a thickness of 100 μm and several rows of spongy parenchyma reaching a thickness of 90 μm . The expansion of the voids in this tissue is observed, while the spread of small vascular bundles is observed on both sides of the leaf surrounded by a row of large parenchymal cells, reaching a thickness of 60 μm . widening of the voids in this tissue was observed, while the spread of small vascular bundles was observed on both sides of the leaf surrounded by a row of large parenchyma cells with a thickness of 60 μm . The midrib appears concave from the adaxial face, convex on abaxial face, vascular tissues arranged in oval shape surrounded by ordinary parenchyma of the storing form, The species was belonging plants C3 (Plate.4 Table 3).

Table 3- Anatomical characteristics of *N. echioides* .

Anatomical characteristics		
Thickness (Mean \pm SE) (μm)		
Stem Epidermis cells 7.5-12.5(10 \pm 2.5)	Stem cortex 150-200 (170 \pm 25)	Vascular bundle of stem 50-100 (73 \pm 25)
Cross section of leaf 300-350(320 \pm 25)	upper epidermis cells of leaf 10-20(15 \pm 5)	lower epidermis cells of leaf 20-30(25 \pm 5)
Palisade layers 100 \pm 5	Spongy layers 90 \pm 5	Vascular bundle of leaf 60 \pm 5

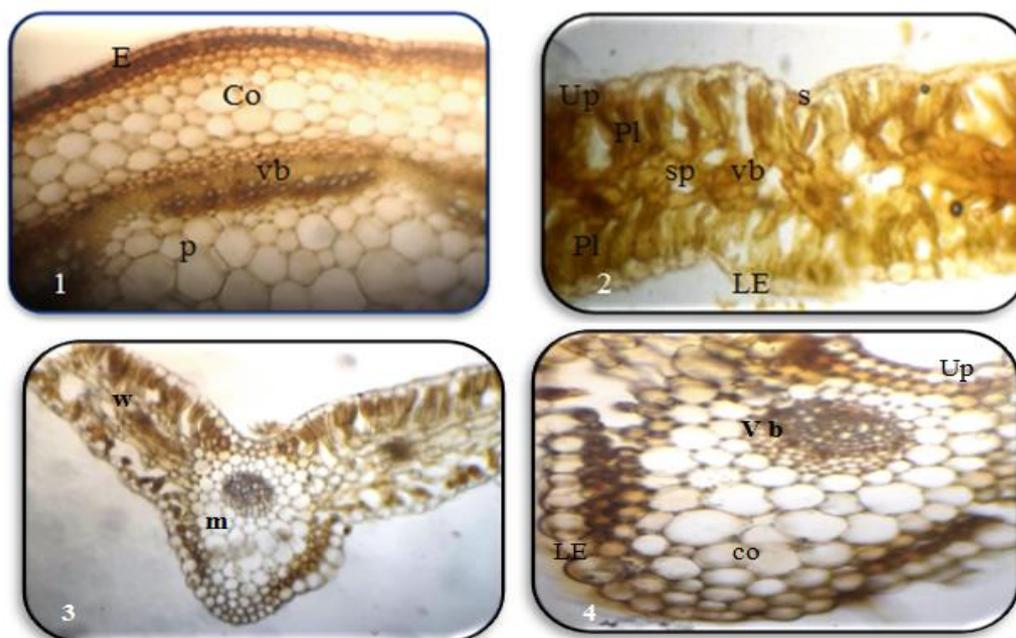


Plate 4- *Nonea echioides* , 1. cross sections of stem 2-3 cross sections of Leaf, 4- C.S. of mid rib of leaf. E: epidermis , Co: Cortex ,v b: vascular bundle, p: pith ,Up: upper epidermis cells, s: stomata sp : spongy layers, LE: lowers epidermis cells, w: wing leaf , m: mid rib.

3.3 Taxonomic key to *N. echioides*

4. Discussion

The current study deals with the *N. echioides* due to the existence of great controversy over the diagnosis and description of the species belonging to the *Nonea* genus, and has not been studied phenotypically or anatomically in Iraq. It belongs to the *Boragineae* tribe of the *Boraginaceae*, which is currently spread in northern of Iraq. *N. echioides* is a very widespread plant that extends widely from northwest Africa and Europe towards the northeast to Turkey, Iraq and Iran [28,31,32]. It should be noted here that this species grows in arid lands [28].

Luebert [33] indicates that the *Nonea* genus is characterized by usually being annual or perennial plants and rarely trees or shrubs. The calyx of the plant is hairy, the corolla is tubular, and the fruits are nutlet. The anatomical results show that the epidermis originates from a single row of cells forming a ring around the stem[34].

The *Nonea echioides* differs from *Nonea dumanii* through the absence of the glandular hairs in the stem. Thus, the results of the current study do not agree with the study by YEŞİL [35] who indicates the presence of a hair cyst of the glandular type.

The study agrees with the method of distribution and spread of other tissue systems (such as cortex, vascular bundle & pith)[31].

This indicates a common origin and anatomical convergence. The study also shows that the morphological characteristics of the *Nonea echioides* are significantly similar to the results of the study by [10] and differ in the quantitative values due to its influence with environmental factors while it differs in the morphological characteristics with other species of the same genus compared to the results of other studies, including the studies by [28,35] Anatomical properties have been shown to be of great importance as they contribute greatly to solving taxonomy problems according to the study of [35]. The difference in the shapes of the epidermal cells and their dimensions may be due to the surface tension that the plant organ is subjected to. The cells in the stem are elongated due to the nature of the longitudinal growth, while the growth is almost equal in the leaves, therefore, the cells are isodimetric or wavy.

5. Description

Ascending herbs, leaves opposite, simple, elongated or lanculate, sessile, with an indumentum eglandular. Flowers single, subsessile or shortly pedicellate, pentagonal, calyx is cup-shaped, corolla white in color with a united, symmetrical, pentamerous, lobes, actinomorphic, tube narrow, Stamens 5-merious number, epipetalous having black color anthers, pistil divided nearly to base consisting of 4 nutlets, ovary simple, globose, gynobasic style, style undivided, filiform, stigma capitate, Fruit is a black nutlets with a white eschar and a mesh reticulate wall.

6. Conclusions

1- *Stomata*: of the anomocytic type, and the spread of the superficial covering of the agglutinative type in the aerial parts of the plant body (stem, leaf, calyx).

2- *The transverse section of the stem*: it is typical and represents dicotyledonous plants. The stem is rounded by thin-walled single-row epidermal cells and surrounded by the dermis consisting of the glandular hairs. The cortex is composed of several collenchyma and parenchyma tissues that spread regularly, and the vascular bundles are of the lateral type arranged in one regular ring. The stem is of the solid type, occupied in the center by the pith and composed of large, multi-faceted, thin-walled parenchymal cells.

3- *Anatomy of the leaves*: the epidermis is of a single-row and large-celled in the upper and lower leaf surfaces, while the intermediate tissue consists of two rows of columnar cells and several rows of spongy cells, between them 2-3 rows of Chloronchyma cells are perpendicular to the columnar cells and spread by circular vascular bundles of small size and closed compared to the central bundle in the central vein.

6. Acknowledgment

This research was supported by the Biology department - Faculty of Education for Pure Sciences - University of Tikrit. Special thanks to Mr. Mustafa Mohamed Ibrahim and Mr. Neel Mustafa for their assistance in collected samples and helped advice.

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