MORPHOLOGICAL AND ANATOMICAL VARIATIONS OF FRUITS IN
SOME TAXA OF VALERIANACEAE BATSCH FAMILY
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ABSTRACT

This study was conducted at the different geographical regions at the north of Iraq; during the field trips, many samples of individuals of two genera *Valeriana* L. and *Valerianella* Miller were collected. The fruits of specimens morphologically have been inspected, and some parts of fruits were anatomically examined by paraffin method, from both studies the diagnostic characteristics of fruits were determined. This study was proved the morphological characteristics of significance within genera and species levels, while anatomical features had a large role in separation between the two studied genera, it was proved that the cross-sectional outlines of fruits played a significant role in delimiting among species of the genus *Valerianella*.

Key word: achene, diagnosis, glandular hairs, taxonomy. *Part of Ph.D. Dissertation of the 1st author.

مجلة العلوم الزراعية العراقية -2020 :51: (عدد خاص):101-111 العائلة الفاليريانية Valerianaceae Batsch تباينات مظهرية وتشريحية لثمار بعض افراد العائلة الفاليريانية Valerianaceae Batsch ميروان طه صالح الدباغ وسعيد معروان طه صالح الدين – أربيل جامعة صلاح الدين – أربيل المستخلص المستخلص

أجريت هذه الدراسة في المناطق الجغرافية المختلفة في شمال العراق. خلال الجولات الحقلية، تم جمع العديد من عينات الأفراد التابعة للجنسين Valeriana L و Valerianella Miller . تم فحص ثمار العينات مظهريا، وتم فحص بعض أجزاء الثمار تشريحيا بطريقة البارافين، ومن كلا الدراستين تم تحديد الخصائص المظهرية والتشريحية التشخيصية للثمار. أثبتت الدراسة ان الصفات المظهرية للثمار ذات الأهمية على مستوى الجنسين والأنواع، في حين كان للخصائص التشريحية ور كبير في الفصل بين كلا الجنسين المدروسين، وقد اثبتت الدراسة أيضًا أن محيط المقاطع العرضية للثمار لعبت دورًا هاما في العزل بين أنواع الجنس الجنسين المدروسين، وقد اثبتت الدراسة أيضًا أن محيط المقاطع العرضية للثمار لعبت دورًا هاما في العزل

الكلمات المفتاحية :فقيرة, تشخيص, الشعيرات الغدية، الثمار، التصنيف.

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INTRODUCTION

The Valerianaceae family is approximately 370 species belonging to 10 genera (18). This family comprises nearly 300 species within 10 genera, mostly annual or perennial herbs, the leaves are opposite, estipulate, inflorescence composed of cymes. The calyx reduced to forming pappus-like or lobes and/or teeth, the corolla is sympetalous gibbous or spurred. Stamens are 1-4, gynoecium is syncarpous, 3carpelled, ovary inferior with one fertile locule. Fruit is an achene, occasionally cypsela. Seeds non-endospermous (27). Fruits originate from a tri-carpellate inferior ovary with one single-seeded abaxial and two inactive adaxial locules. Valerianella fruits possess various modified inflated sterile locules, while mature fruits of Centranthus and Valeriana devoid of sterile locules (15), with the remnant fruiting calvces (17). The final number of genera and species, were spread worldwide and counted by Takhtajan (32) as Valeriana, 250 species; Valerianella, 50 species; Phyllactis, 25 species; Patrinia, 15 species; Centranthus, 9 species; Belonanthus, 5 species; Nardostachys, 1-2 species; Plectristis, 5 species; Stangea, 7 species. In current APG III system (3), the Caprifoliaceae expanded to encompass the families: Diervillaceae, Dipsacaceae, Linnaeaceae, Morinaceae, and Valerianaceae.In Iraq, the past researchers such as (2; 6; 12; 13; 26; and 34) emphasized the distribution of only two species of Valeriana genus (V. alliariifolia and V. sisymbriifolia) in the north of Iraq. Both species are growing naturally on the mountain in rocky wet places near or along streams, these two species grow at the elevations between (1600-2800 and 850-2900) m, respectively. Besides that, they have mentioned about 10-13 species of the genus Valerianella distributed at different geographical regions at the elevations ranges between (210-1900) m in Iraq. The anatomical characteristics can be relied upon as diagnostic characteristics for the systematic purpose and to the elucidation of phylogenetic relationships among taxa (8). The anatomical characteristics are considered as a piece of evidence that apply in the taxonomic study from a century ago (25). Stuessy (31) interpreted, the anatomical information is often quite helpful

in solving taxonomical problems, and the anatomical data can help in the interpretation of evolution. Singh (28) stated that the anatomical characteristics are observed by light using microscope, but micromorphological features are brought out by using an electron microscope, they also play an intensified role in the explanation of phylogenetic studies. Phenotypic differences have a significant relationship with molecular analyzes (1). Metcalfe and Chalk (19) worked on most of the dicotyledons families including Valerianaceae family with the identifying of characteristics which anatomical are considered to be the merit feature of each family. Cronquist (7) hinted to some of the anatomical characteristics of the Valerianaceae through the family description. There are no detailed morphological and anatomical studies in Iraq with regard to Valerianaceae taxa, therefore this study was considered as the first study about this family, in order to achieve the most diagnostic characteristics to facilitate the separation of the studied members of the family.

MATERIALS AND METHODS Morphology

The plant materials for the morphological studies were based on the fresh samples which were obtained from the 37 field trips during the fruiting stages during years 2016, 2017 and 2018, at Kurdistan region-Iraq, additionally, some preserved specimens in Iraqi herbaria were used. Fruits were experimented to discuss and describe the different aspects of morphological for taxonomic structures purposes, with available scientific methods. Photographs and macromorphological features have been taken by Canon camera (Digital IXUS 960 IS 12.1 M. P.), as well as the micromorphological features by setting up the camera on Olympus dissecting microscope, in addition to the KRÜSS (DCM35) camera with using graduated stage micrometer for measuring small parts. The terms cited here are largely descended from those used by herbalists and botanists of the past, such as (4; 5; 14; 18; 25; 30; 31 and 33). Plant fruits' structures were examined and described in detail, the resulting data is listed in a Table and used in segregation the treated species, supported with Figures and artworks.

Anatomy

Paraffin methods as adopted by Najmaddin Mahmood (23) were applied and for preparation of permanent slides for tissues as follows: The inflorescences of plants during field sampling were fixed immediately in FAA (16), and left at room temperature for 24 hrs. The samples were dehydrated by using series of concentrations of ethyl alcohol. After that, the embedding of fruit samples in paraffin was done and left in the oven at 60°C for a night. The blocks of paraffin were made by preparing the blocks of metal, and then the samples were placed in a suitable manner for cutting. Slide sections have been cut with the thickness of 8-10 micrometer using the rotary microtome (Bright, LTD), the ribbons placed and mounted on slides carefully, then transferred to a hot plate for overnight. The slides were stained by safranin and fast-green and covered by coverslips after adding a drop of DPX. The prepared slides have been examined and imaged by Light Microscopes (Olympus AC 100 with a camera, Japanese-made). The used anatomical terms were cited by (9; 10; 11 and 20).

RESULTS AND DISCUSSION

General Morphological Description of Fruits: Commonly the fruits within Valerianaceae are simple dry, indehiscent, achene or cypsela, derived from an inferior, tri-carpellate ovary of a single flower, 1 or 3 loculi ovary, 1 seeded, often crowned with persistent calyx, (Table 1). 1. Valeriana: The fruits within the genus Valeriana are cypsela, narrowly ovoid-oblong to narrowly urceolate, unilocular, slightly curved, with three narrow, shallow slit (groove) in back, and two broad, shallow slits in front; crowned with the number of separate feathery, calyx limbs acicular, acuminate apex; connate at base and forming pappus of crowning achene; yellow to brownish yellow or straw color.

V. alliariifolia and V. sisymbriifolia

The main differences between the examined species are the fruits often glabrous in *V. alliariifolia*, the number of calyx limbs is 12-14 (15), the length of fruits is between (8.0-10.0) mm. and the width is between (4.0-7.0) mm; the length of the fruit excluding calyx tube is between (4.5-7.0) mm, and the width is between (1.1-1.5) mm. While the fruit is

compressed dorsiventrally in *V. sisymbriifolia*, slightly puberulent, or densely pubescent with short, soft hairs, the number of calyx limbs is 12-14 [10], the length of fruits is between (7.5-11.0) mm and the width is between (4.0-6.0) mm; the length of the fruit excluding calyx tube is between (4.5-6.0) mm, and the width is between (1.0-2.2) mm (Table 1) and (Fig 1).

2. Valerianella: The fruits in Valerianella species are achene, tri-carpellate, tri-locules; the middle one fertile single-seeded, the two other laterals are sterile, crowned with persistent calyx. The fruits collectively showed greed morphological differences among studied species of the genus. Since the morphological characteristics of Valerianella fruits are regarded as a diagnostic feature to delimitation species, it is necessary to describe the fruit of each species solely, as listed below, (Fig 2).

V. pumila

Approximately all the fruits in V. pumila are similar in size (4.0-5.0)x(1.4-2.0) mm., globose, three angled in polar view (face view), convex at back, with deep elongated depression in the center of the front, usually the sterile locules boarder than the fertile locule, almost glabrous, or very finely pubescent, excluding calyx tube (1.8-2.8)x(1.4-2.0) mm.; calyx limbs disappear to minute or tiny teeth, often the posterior (of fertile locule limb) remains short tooth-like, and of the two other lateral (of sterile locules limbs) convert into rudimentary protrusion (0.1-0.7)x(0.4-1.0) mm.; deciduous early. (Fig 2A).

V. carinata

Almost the fruits in *V. carinata* are similar in size and shape (2.0-3.0)x(1.4-2.0) mm., oblong, tetragons, slightly curved, concave in front, keeled, with deep elongate U shaped furrow in center over entire length, convex posterior, and behind of each side with narrow, shallow furrow, the sterile locules more or less as broad as the fertile locule, scabrous, pubescent or sometimes glabrate, excluding calyx tube (1.8-2.8)x(1.4-1.5) mm.; calyx limbs disappear to minute or tiny teeth, often the posterior (fertile locule limb) remains short tooth-like, and of the two other lateral limbs (sterile locules limbs) are totally disappear;

brown or yellowish brown; deciduous early, (Fig 2B).

V. muricata

The fruits in V. muricata are similar in shape and subequal in size (1.6-3.5)x(0.7-1.7) mm., globose-ovoid or ellipsoid, scabrous, covered with straight, white hairs, sericeous around connate base of the calyx tube, almost flat in round-elliptical, with front. shallow depression, convex posterior and densely scabrous, the sterile locules smaller than the fertile locule, excluding calvx tube (0.9-2.2)x(0.7–1.3) mm.; calyx tube herbaceous, conduplicated to oblique tubular, crest-like collar, triangular (0.6-2.0)x(0.6-1.8) mm., distinctly reticulate veined, denticulate margin, acute apex, almost as broad as fruit; brown or yellowish brown; deciduous early falls. (Fig 2C).

V. coronate

The fruits in V. coronata are obcuneat or cupshaped (4.0-6.0)x(4.0-6.0) mm., circular, sometimes four angled, woolly or villous, covered with white hairs, slightly convex or almost flat at back, with deep groove in front, provides with three unequal locules, the fertile locule posterior, usually longer and broader than the two lateral sterile locules, excluding calyx tube (1.5–2.5)x(1.2–1.5) mm.; crowned with persistent fruiting calyx tube, crowncampanula shape, (3.0-4.0)x(4.0-6.0) mm. pilose beneath and glabrous inside, within 6 (3.0-4.0)x(4.0-6.0)triangular mm.. membranous, sharply reticulate veined, subequal lobes, entire, ciliate margin, acute, long, hooked mucro apex; brown seed with yellowish brown fruiting calyx, (Fig 2D).

V. kotschyi

The fruits in *V. kotschyi* are ovoid-cupule (3.0-5.0)x(1.6 -5.0) mm., woolly or villous, covered with white hairs, convex at back, and deeply oblong or V shape grooved in front, the fertile locule boarder than the sterile locules, excluding calyx tube (2.2-4.0)x(1.1-2.0) mm.; crowned with persistent oblique calyx tube of 6 broad triangular lobes (1.0-2.5)x(2.0-4.5) mm., reticulate veined, entire margin, acute hooked mucro apex, within three prominences at the ovary tip, the posterior bigger due to swelling expansion of fertile cell, and the other of sterile cells are smaller, brown-yellowish brown or shiny yellow, (Fig 2E).

V. vesicaria

The fruits in V. vesicaria are cup-like, woolly or villous (3.0-7.0)x(2.0-7.0) mm., covered with long white hairs, with round pit in front, nearly flat, obcuneate posterior, excluding calyx tube (2.2-4.0)x(1.1-2.0) mm., the fertile locule broader than the sterile locules; crowned with globular, inflated persistent calyx tube (2.5-6.0)x(2.0-4.5) mm., usually villous, membranous conspicuous reticulate nervous, the throat of calyx tubes circular furnished with 6 small, broad triangular or orbicular, acuminate, hooked mucro apices limbs, inflexed horizontally, almost meeting in the center, approximately two or little longer and broader than the seed; yellow or brownish yellow, (Fig 2F).

V. dufresnia

The fruits in V. dufresnia are short turbinate (8.0–13.0)x(7.0–13.0) mm., woolly. with densely white long hairs, one-seeded fertile locule posterior, with deltoid depression in front, of 2 distant gibbosities of laterally sterile locules and below calyx limbs, excluding calyx tube (2.2-4.0)x(1.1-2.0) mm., the fertile locule longer and narrower than the sterile locules; crowned with three broadly ovate-(6.0-9.0)x(4.0-10.0)orbicular mm.. membranous, reticulate veined, maroon colored teeth and margin, sharp-toothed apices calyx limbs, the posterior one large, the two lateral limbs small, wing-like, villose from base to ca. half of both surfaces; brownish yellow with marooned fruiting calyx, and puffs of cotton like seed; deciduous early, (Fig 2G).

V. tuberculate

The fruits in *V. tuberculata* are tubular, slightly curved, tuberculata, (4.0-5.0)x(3.0-3.5) mm., (covered with wart-like blunt outgrowths) at dorsal, usually glabrous in ventral, grooved in front with two parallel gibbosities of sterile locules, the sterile locules usually very smaller and narrower than fertile one; excluding calyx tube (3.0-3.6)x(1.0-1.3) mm., calyx reduced into 10-12 unequal limbs (1.2-1.5)x(3.0-3.5) mm., triangular, hooked teeth at tip within three distinct big lobes, irregular, making it hypocrateri or patelliform; brown, greenish brown or yellowish brown, (Fig 2H).

V. oxyrrhyncha

The fruits in V. oxyrrhyncha are narrowly cylindrical, slightly narrowed at tip, (3.2-4.5)x(4.0–6.0) mm., densely pubescent, hispidulous, or sometimes slightly hispidulous or glabrous; with a narrow slit (groove) in front, expanded into small square at base, wing-like laterally almost divergent the sterile locules usually gibbosities, narrower than fertile one; excluding calyx tube (2.5-4.0)x(1.1-1.5) mm.; calyx limbs semicircular or broadly triangular (0.8-1.6)x(3.0-8.0) mm., toothed margin, reticulate veined, with 2 long, narrowly subulate-linear, one of them longer than the other, toothed or acute apex, hooked, almost horizontally, brown, greenish brown or yellowish brown, (Fig 2I).

V. dactylophylla

The fruits in V. dactylophylla are irregularly botuliform oblong, attenuate from boarder base to collar (4.0-7.0)x(4.0-7.0) mm., densely papillate, as well as with short clavate trichomes, one-seeded fertile locule posterior, with narrow groove in front, deltoid at base with two convergent gibbosities, highly expanded at base, the fertile locule approximately as twice as broader than the sterile locules; excluding calyx tube (2.0-5.0x(1.0-2.1) mm.; crowned with oblique crown-campanula shape calyx tube (2.0-4.0)x(4.0–7.0) mm., six subulate-triangular limbs, ciliate margin, usually unequal, broad at base, membranous, sharply reticulate nerves blade, with rigid and thickened center nerve, tapering end into sharp hooked point, slightly declined, often pubescent abaxial, sometimes glabrate; dark brown or yellow, or sometimes with black seed, (Fig 2J).

V. discoidea

The fruits in *V. discoidea* are obcuneat or obturbinate, four angled (4.0-6.0)x(4.0-6.5) mm., woolly or villous, covered with long, white hairs, feather-like, slightly convex or

almost flat at back, deeply grooved in front, provides with three unequal locules, the fertile locule at middle, usually longer and broader than sterile locules; excluding calvx tube (2.0-3.0)x(1.5–2.2) mm.; crowned with persistent disc-like calyx tube (2.0-4.0)x(4.0-6.5) mm., contain of 5-7 parted and 3-4 small subordinate limbs, different in size, very wide triangular-obconical, membranous, reticulate veined, hairy at both sides, margin entire, apex with acute long, excurrent hooked; brown vellowish brown or sometimes the seeds are black (Fig 2K). The fruit and seeds of Valerianella species might take various forms of fruiting calyx adapted to wind dispersal such as in the form of a crown, spreading star and inflated calyx, or the calyx lobes apexes extended to bristles for dispersion by animals (33), certain plant species easily dispersed by winds (21). Fruits of Valeriana members are cypsela of narrowly ovoid-oblong achene with the pappus-like persistent calyx of 12-14 lobes. In Valerianella species, ovoid, obovoid, obcuneat, or tubular achene, crowned with different shape and dimensions of fruiting calyx, each species has its own shape and dimensions. Additionally, the indumentum investigation showed various aspects of individual surface coverage. Fruits of V. alliariifolia were glabrous while of V. sisymbriifolia were indumentum, whereas fruits in most species of Valerianella are indumentum. Furthermore, the hair type, shape, and intensity are also different among species. So fruit characteristics have played an important role in identification among all species of both genera. As Pandey and Misra (24) mentioned, the characters of fruits have been explored for making diagnostic keys, also noted that Cood (1967) used only fruit features in delimitation among Valerianella species as well.



Fig 1. Different morphological views and cross sections (4X) of fruit and seed parts of studied *Valeriana* species; A-C: *V. alliariifolia* A: fruit in the early stage, B: matured fruit, C: hand drawing illustration, left ventral and right dorsal view with pappus-like calyx, and D: *V. sisymbriifolia*. F: fertile locule, S: sterile locule, VB: vascular bundle, AV: accessory vascular bundle, T: non-glandular hairs

Species	Fruits with persistent calyx			Fruits excluding persistent calyx			Persistent calyx tubes		
	Length	Width	A (l/w)	Length	Width	A (l/w)	Length	Width	A (l/w)
V. alliariifolia	(8.0-10.0) 9.09	(4.0-7.0) 5.33	1.705	(4.5-7.0) 5.25	(1.1-1.5) 1.11	4.730	(3.5-5.0) 4.00	(4.0-7.0) 5.33	0.750
V. sisymbriifolia	(7.5-11.0) 9.10	(4.0-6.0) 5.12	1.777	(4.5-6.0) 5.10	(1.0-2.2) 1.38	3.696	(3.0-5.0) 4.60	(4.0-6.0) 5.12	0.898
V. pumila	(4.0–5.0) 2.41	(1.4-2.0) 1.74	1.385	(1.8-2.8) 2.14	(1.4-2.0) 1.73	1.237	(0.1-0.7) 0.35	(0.4-1.0) 0.73	0.479
V. carinata	(2.0-3.0) 2.37	(1.4-2.0) 1.17	2.026	(1.8-2.8) 2.37	(1.4-1.5) 1.17	2.026			
V. muricata	(1.6–3.5) 2.56	(0.7-1.7) 1.16	2.207	(0.9-2.2) 1.69	(0.7-1.3) 1.12	2.26	(0.6-2.0) 1.36	(0.6-1.8) 1.09	1.248
V. coronata	(4.0-6.0) 4.92	(4.0-6.0) 5.52	0.891	(1.5-2.5) 2.02	(1.2-1.5) 1.35	1.496	(3.0-4.0) 3.37	(4.0-6.0) 5.27	0.639
V. kotschyi	(3.0–5.0) 4.18	(1.6-5.0) 3.24	1.290	(2.2-4.0) 2.91	(1.1–2.0) 1.79	1.626	(1.0-2.5) 1.47	(2.0-4.5) 3.24	0.454
V. vesicaria	(3.0–7.0) 4.93	(2.0-7.0) 4.13	1.194	(2.0-3.5) 3.03	(1.5-2.5) 1.91	1.586	(2.5-6.0) 3.53	(2.0-4.5) 4.09	0.863
V. dufresnia	(8.0-13.0) 11.12	(7.0-13.0) 9.92	1.121	(4.0-6.5) 5.22	(2.5-4.5) 3.63	1.438	(6.0-9.0) 7.67	(4.0-10.0) 9.92	0.773
V. tuberculata	(4.0–5.0) 4.44	(3.0-3.5) 3.29	1.349	(3.0-3.6) 3.24	(1.0-1.3) 1.15	2.817	(1.2-1.5) 1.36	(3.0-3.5) 3.29	0.413
V. oxyrrhyncha	(3.2-4.5) 3.77	(4.0-6.0) 4.84	0.779	(2.5-4.0) 3.03	(1.1-1.5) 1.26	2.405	(0.8-1.6) 1.35	(3.0-8.0) 5.37	0.251
V. dactylophylla	(4.0–7.0) 5.95	(4.0-7.0) 5.23	1.138	(3.0-3.5) 3.35	(0.8-1.1) 0.96	3.490	(2.0-5.0) 3.38	(1.0-2.1) 1.70	1.988
V. discoidea	(4.0-6.0) 4.92	(4.0-6.5) 5.26	0.935	(2.0-3.0) 2.50	(1.5 – 2.2) 1.96	1.275	(2.0-4.0) 3.18	(4.0-6.5) 5.53	0.575

Table 1. Fruit dimensions of studied members of Valerianaceae in millimeters*

*The dimensions were taken from (20-25) specimens, the numbers inside brackets represent the minimum and maximum limit, and that outside brackets represent the average.

A (l/w), is the abbreviation of Average (length/width).



Fig 2. Variations of mature fruit and seed of studied *Valerianella* species. Different morphological views (upper, hand drawing illustration, left ventral and right dorsal view), and details of cross section shapes showing the position and size of sterile locules relatively with fertile locule (lower, 4X); A: *V. pumila*, B: *V. carinata*, C: *V. muricata* D: *V. coronata* E:

V. kotschyi, F: V. vesicaria, G: V. dufresnia, H: V. tuberculata, I: V. oxyrrhyncha, J: V. dactylophylla, K: V. discoidea. F: fertile locule, S: sterile locule, VB: vascular bundle, AV: accessory vascular bundle

General anatomical description of fruit and seeds:

The transverse sections of the Valerianaceae fruits could be categorized into two major types; the first, fruits consist of single locule, made up from fusion of three carpels, as in *Valeriana* species, and the second, fruits consist of three locules, made up from fusion of three carpels, as in *Valerianella* species. In general, in all studied species, the pericarp of fertile locules is clearly distinguished into four zones. The exocarp of the pericarp (epicarp) in all species is single continuous epidermis layer; consists of spherical, sub-spherical, elongate and rectangular cells. The epidermal cells are relatively small and isodiametric with a thick layer of cuticle. The stomata are present in epidermis of all species, but different trichomes are present in most species as shown in (Fig 3 and 4); glandular hairs consist of unicellular stalk with bi-seriate multicellular elongated head, the heads are 4, 6 or 8 celled, they occur in all fruits of the studied species, excluding *V. pumila* and *V. oxyrrhyncha* which they have glabrous fruits. *V. alliariifolia* has the pappus-like (persistent

calyx) hairs of cypsela while any type of trichomes are absent on exocarp surface, V. sisymbriifolia has the pappus hairs of cypsela, and the exocarp covered with cylindrical filiform of pilose. V. carinata covered with non-glandular hairs of short conical bristle. V. muricata covered with three types of nonglandular hairs which are cylindrical filiform of pilose, short conical bristle and long conical bristle. V. coronata covered with nonglandular hairs of cylindrical hairs of hirsute and barrel-shaped glandular hairs, besides the persistent calyx is covered with hairs of cylindrical bristle-like obtuse tip. V. kotschyi covered with non-glandular hairs of cylindrical hairs of hirsute, and the glandular hairs of capitate unicellular short tail. V. vesicaria covered with non-glandular hairs of cylindrical hairs of sericeous and cylindrical hairs of hirsute. V. dufresnia covered with nonglandular hairs of cylindrical hairs of sericeous only. V. tuberculata covered with unicellular glandular hairs of capitate short and long tails. V. dactylophylla covered with unicellular glandular hairs of striate and curved clavate, and capitate unicellular short tail. V. discoidea covered with non-glandular hairs of cylindrical hairs of sericeous and cylindrical hairs of hirsute, papillate with glandular hairs of papilla and barrel shape. It is necessary to refer macromorphology to the and micromorphology of the dermal appendages (trichomes). Clearly, the macro hairs are visible by naked eyes (20) or hand lenses (25), therefore, the fruits were morphologically described as they have been shown by naked eyes or hand lenses. Various types of glandular and non-glandular hairs in fruits of the studied taxa have been observed under microscopic investigations. The dermal of most Valerianella species contain different types of trichomes in different parts of fruits; these differences are evidence of the high taxonomic value of segregation genera and species. This is consistent with what (22 and 29) believed, that the trichomes have significant value at all the levels in plant classification, from family to even varieties. The mesocarps in all species are anatomically homomorphous; they have six vascular bundles with sclerotic cells near the phloem, and are varying in number of rows in each layer. The hypodermis consists from 6

to many rows with parenchyma thick cell wall, without intercellular spaces as V. alliariifolia and V. sisymbriifolia, whereas 2-4 rows in V. pumila, V. muricata, V. coronata, V. vesicaria and V. discoidea, and sometimes 5-6 rows in V. dufresnia. The hypodermis in the rest species consists of 2-3 rows of sclerotic cells with distinct darker layers in color. The endocarps are comprised of 2-3 and /or 2-4 layers of cross and tube cells closely combined with each other. The cross cells have thick cell walls with discernible reticulate the thickenings, which are crossly arranged with the tube cells. In all species the tube cells contain the prismatic crystals, in addition, V. alliariifolia contains the inulin also. The inner epidermis of fruits consists of a single layer of the square to rectangular or oblong sclerified cells, which is considered a seed coat or testa (Fig 5). Below the testa exist nucellus they attached integrally with each other, consists of cells with thin walls arranged in two rows of a single integument. The embryo sac at the earlier stage of the seed development is just about far from the testa then becomes firmly contacted with testa when the fruit is ripe, and the embryo entirely occupied the fertile locule. But, in Valeriana species observed through the fruit development, the ovary at the early stages appears in three locales; the large locule in dorsal and two small inactive locules in ventral, eventually, only the single-seeded locule remains, the other two locules collapse and become invisible when the fruit matures (Fig 5; 6A; 8Aa). The embryo straightly fulfilled the fertile locule and completely surrounded with the embryo sac, with conspicuously separated two cotyledons. The cotyledons homologous to leaves have upper and lower epidermis; the cells are cubic, rectangular or oblong, almost isodiametric, arranged in a single layer, and surrounds the oblong or sub-spherical storage parenchymatous cells. In some species, the oblong cells similar to palisade cells are perpendicularly arranged on the epidermis internally. The inner cotyledons storage cells contain starch grains, aleurone grains, and oil drops, with obvious nuclear, the seed is devoid from the endosperm (Fig 5; 6A). Generally, in Valerianella species there are two sterile locules of fruits, they are similar structure of

fertile locule, while they differ in some characters such as; the embryo sac and all embryo elements are devoid in locules, two of the vascular bundles are situated laterally, the sclerotic and cross cells zones are thicker, and the endodermis is absent as well (Fig 6 - 8). The upper part of the fruit in Valeriana species has a circular shape in outline and markedly six ribs, while the lower part has an elliptic shape in outline, one hump dorsally, two humps laterally and three humps ventrally, which are the locations of vascular bundles. Moreover, the accessories of vascular bundles exist near the vascular bundles of ventral or lateral ribs (Fig1). Each species of the genus Valerianella has a particular outline in transverse section as described formerly in morphological descriptions, the outline shapes of fruits were clarified by using the micrographic section as shown in (Fig 2). With respect to internal elements of fruit, the current study demonstrated that the fertile locules of all studied species are approximately similar in their content of tissue layers. The study also showed that the seed coat is comprised of the inner and outer epidermis which is derived from the mono-layered integument, this is consistent with what Karcz (17) found. Also, it showed the presence of prismatic crystals in seed coats of all species, and the inulin crystals appeared only in fruits of V. alliariifolia. In all studied species, there are six prominent carpel ribs, each rib composed of single vascular bundle, the accessory vascular bundles are present only in some ribs of both species of Valeriana, and this phenomenon has not been mentioned in the available references. According to the number of locules, the present study was able to distinguish between Valeriana and Valerianella species. The fruits in Valerianella clearly contain three almost unequal locules, whereas the fruits in Valeriana species are seen in a distinct single locule with two invisible sterile locules, as Jacobs et al. (15) cited, the sterile locules are non-existent in mature fruits. While at the species level, the outlines of cross sections have appeared in different forms, each form represents a particular species. This study revealed that the morphological results indicated that the fruits of the Valerianaceae family have a role in delimitation among examined taxa. Fruits of Valeriana species are crowned with calyx pappus-like of cypsela, but they differ from the presence or absence of other trichomes. Anatomical evidence has contributed significantly to taxonomy; also it provided valuable support to has the taxonomists in plant classification. The anatomical features are not less important than the other characters or studies such as morphological features, pollen grains and chromosome numbers in solving many problems and removing suspicion among the plants which are morphologically very close together. Many significant anatomical characteristics are obtained to be taken in account to separate the plants under the genera species levels especially and among individuals within the convergent taxa which are difficult to separate.



Fig 3. Trichome types variations in mature fruit and seed of studied Valerianaceae members.

Glandular hairs A: glandular hairs consist of unicellular stalk with bi-seriate multicellular head, the heads are 4, 6 or 8 celled, B: striate and curved clavate, C: capitate unicellular short tail, D: capitate unicellular long tail, E: barrel shape, F: papillate. Scale bars: $A-B = 10 \ \mu m \ C = 20 \ \mu m$, and $D-F = 40 \ \mu m$.



Fig 4. Trichome types variations in mature fruit and seed of studied Valerianaceae members. Non-glandular hairs A: pappus-like (persistent calyx) hairs of cypsela, B: cylindrical hairs of sericeous, C: cylindrical hairs of hirsute, D: cylindrical filiform of pilose, E: short conical bristle, F: long conical bristle, G: cylindrical bristle-like obtuse tip, Scale bars: A=1mm, B-E

=100 μm, F= 1000 μm, G= 100 μm



Fig 5. Fruit and seed anatomy of A:V. alliariifolia and B: V. sisymbriifolia. Micrograph portions of cross section (40X), fruits outer ribs with vascular bundle (VB) and accessory vascular bundle (AV), outer epidermis (Oep = exo carp), sclerified endocarp with tuber cells (tu) and reticulate walls thickening cross cells (cr), uni-layered seed coat (testa, te), nucellus (nu), and cotyledon of embryo (co).



Fig 6. Fruit and seed anatomy of A:V. pumila, B: V. carinata, C: V. dactylophylla, and D:V. discoidea. Micrograph portions of cross section (40X), fruits outer ribs with vascular bundle (VB) and accessory vascular bundle (AV), outer epidermis (Oep = exo carp), sclerified endocarp with tuber cells (tu) and reticulate walls thickening cross cells (cr), uni-layered seed coat (testa, te), nucellus (nu), and cotyledon of embryo (co).



Fig 7. Fruit and seed anatomy of A:V. muricata, B: V. coronata, C: V. kotschyi, D: V. vesicaria and E:V. dufresnia. Micrograph portions of cross section (40X), sclerified mesocrp (scm), sclerified endocarp with tuber cells (tu) and reticulate walls thickening cross cells (cr), prismatic crystals (pc), hairs of hirsute (tch), barrel shape glandular hairs (tb), glandular hairs of capitate unicellular short tail (tcs),cylindrical hairs of sericeous (tce).



Fig 8. Fruit and seed anatomy of A:V. *tuberculata* and B: V. *oxyrrhyncha*. Aa and Ba micrograph portions of cross section, (10X), Ab and Bb magnified portions, (40X), vascular bundle (VB), sterile locule (S), sclerified mesocrp (sc), sclerified endocarp with tuber cells (tu) and reticulate walls thickening cross cells (cr), prismatic crystals (pc),), uni-layered seed coat (testa, te), nucellus (nu).

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