# Taxonomic significance of anatomical characters in some species of Caryophyllaceae Family in Iraq

Sadeq Sabeeh Kareem Al-taie<sup>\*1</sup>, Sahar A. A. Malik Al-Saadi<sup>2</sup>

1. Department of Biology, College of Science, University of Misan, Iraq

2. Department of Biology, College of Science, University of Basrah, Iraq

\* Corresponding author's E-mail: sadeq\_altaie@yahoo.com

# ABSTRACT

The anatomical characters of the leaves, trichomes and stems of 12 species of Caryophyllaceae family were studied. The results showed that the average of epidermal cells length in the adaxial surface ranged between 95.00  $\mu$ m in *Agrostemma githago* and 29.5  $\mu$ m in *Agrostemma gracile*, while in the abaxial surface, it ranged between 92.5  $\mu$ m in *A. githago* and 35.62  $\mu$ m in *A. gracile*. Stomatal complex was circular and elliptic-shaped. All species have four types of stomata: diacytic, anomocytic, paracytic and hemiparacytic. Trichome and their distribution does play significant role in the taxonomic delimitation, *A. gracile* trichomes have non-glandular hairs, while *Arenaria balansae* has non-glandular and glandular hairs. Two types of mesophyll appear to be a good diagnostic characteristic isobilateral in the *Agrostemma* species and dorsiventral (bifacial) in the remaining species as well as several sclerenchyma layers including thick and thin-walled cells that surrounded vascular bundle and also the petiole contains many layers of sclerenchyma cells. Stem shape and size were different between species. The stems had the greatest size (1064.50  $\mu$ m) in *Acanthophyllum bracteatum*, while the smallest stem cross section (450.46  $\mu$ m) in *A. balansae*. Cells were rich in druses crystals. Sclerenchymatous layers in *A. gracile* and 4-7 in *A. crassifolium*. Size of pith was different. The greatest was 280.22 mm in *Agrostemma githago*, while no pith was found in *Polycarpon tetraphyllum* and *Arenaria balansae*.

Keywords: Anatomical characters, Leaf, Stem, Caryophyllaceae Family.

# INTRODUCTION

Caryophyllaceae is a large, annual, or perennial family, belongs to the tribe Caryophyllals, it is containing 96 genera with 2630 species (Walter & Keil 1988; Thorne & Reveal 2007; Mabberley 2017), distributed in temperate regions, Irano-Turanean and Mediterranean region (Bitrich 1993). In the case of Iraq, 24 genus and 135 species were distributed in its different regions. Numerous species of the family are used pharmaceutically important plants (Tutin *et al.* 1964; Davis & Cullen 1965; Zohary 1966; Mcneill 1967; Gorshkova *et al.* 1970; Rendle 1975; Kilic 2009). The first studies reported by Metcalfe & Chalk (1950), wood anatomy was reported by Carlquist (1995), while vascular bundle anatomical structures studied by Ataslar (2004). Schweingruber (2007) described and examined the vascular bundle of 88 species from Caryophyllaceae. Some anatomical properties of some species such as *Saponaria kotschyi* Boiss were also studied (Ataslar 2004). Trichome types, stomatal types of Caryophyllaceae family studied by Davis (1967) and Zarinkamar (2007) and assured that the stomatal number of some species in Iran were studied by Jafari *et al.* (2008). Epidermis features, trichomes and stomata of *Silene* species were studied by Yildiz & Minareci (2008), while Kilic (2009), Sahreen *et al.* (2010) and Fathi *et al.* (2010) reported anatomical characteristics of stem and leaf structures. Stem, root, mesophyll in leaf, arrangement of xylem elements in peduncle, and the number of sclerenchymatous layers are significant to separating species

(Shamsabad *et al.* 2013; Gucel 2013). Anatomy of stem, root, and leaves, as well as arrangement of xylem elements in peduncle, arrangement of mesophyll in leaf and the number of sclerenchymatous layers were studied by Atasler (2004). The aim of this study was to describe the variation some species of the Caryophyllaceae in Iraq using the anatomical characteristics, as well as assesses the value of anatomy in determining interrelationships between taxa.

#### MATERIALS AND METHODS

Twelve species belonging to five genera of the Caryophyllaceae family have been investigated .(TableLeaves and stems of these species were collected from herbarium specimens deposited in BAG (National Herbarium of Iraq, Baghdad, Abo-Ghraib) during 2019 -2020.The cuticles were prepared by macerating the leaves in Jeffrey's solution (equal parts of 10% chromium trioxide solution and concentrated nitric acid) and mounted in safranin stained with glycerin jelly.

Stomatal index was found using the formula mentioned in (Ditcher 1974), as follows:

(%)

Stomatal index

 $\frac{number \ of \ stomata}{number \ of \ stomata + number \ of \ epidermal \ cells} \times 100$ 

The numbers of stomata and epidermal cells were counted by microscopic view fields for both surfaces of leaves. For sectioning, fresh materials of leaves and stems were fixed in formalin acetic acid alcohol solution (FAA) for at least 18-24 h and preserved in 70% alcohol, then dehydrated in ethyl alcohol series, sectioned on a rotary microtome, stained with safranin and fast green, then mounted in Canada balsam (Johansen 1940). The sections were examined using Olympus CH4 light microscope and photographed with digital camera type DCE-2. Stomatal index was calculated as mentioned by Ditcher (1974). Anatomical terms used are cited from Esau (1965), Radford (1974) and Ditcher (1974).

Table 1. Location and information of plant species of Caryophyllaceae species.

species	Localities
Acanthophyllum bracteatum Boiss.	260 km NW. OF Ramadi alt: 500 m,7/6/1957, No. 20959
Acanthophyllum caespitosum Boiss.	Biyara, Jabel Avroman alt: 950 m 6/6/1948, No. 11742
Acanthophyllum crassifolium Boiss.	Haji Omran, alt: 1700 m, 21/6/1047, No. 9155
Agrostemma gracile Pres.	Magor range Haji Omran, alt: 2200 m, 25/8/1957, No. 24313
Agrostemma githago L.	Tawela, alt:1350 m, 8/6/1957, No. 22324
Arenaria balansae Boiss.	Erbil law, arlgirdbagh, alt: 2500-3200 m, 5/8/1947, No. 9602
Arenaria leptoclados (Reich.) Guss.	Badra, kut liwa, alt: 100 m, 18/3/1947, No.6604
Bufonia oliveriina Ser.	Penjwin 21/6/1957, No. 22531
Bufonia calycina Bioss. and Hausskn.	Eastern slopes of Arl GirdDagh, alt: 2600m, 5/8/1947, No. 9612
Polycarpon tetraphyllum L.	17 km E. of Haklaniya alt: 150 m , 17/5/1980 ,No. 52345
Polycarpon succulentum (Delile) J.Gay	Fabba, alt: 200 m, 21/4/1955, No. 14971
Vaccina pyramidata	Near Bald , 2/5/1965, No. 33710

#### **RESULTS AND DISCUSSION**

#### Epidermis

Measurements of epidermal cells of eight species of Caryophyllaceae are summarized in Tables 2-3 and Figs. 1-2). There are usually differences in cell shape and dimensions between the adaxial and abaxial surfaces of the leaf as well as between taxa examined. The epidermal cells of adaxial and abaxial epidermis of leaves are polygonal, triangular, oblong, irregular, circular and semi-circular in all species examined. The anticlinal cell walls in the upper surface were thicker in most of species, while thin in *Arenaria balansae*. The results showed that the anticlinal cell walls of the adaxial and abaxial surfaces were straight and slightly sinuate in *Acanthophyllum bracteatum* and *A. crassifolium*, curved undulate in *Agrostemma gracile* and *A. githago*, and undulate or strongly undulate in *A. caespitosum*, *Polycarpon tetraphyllum*, *Vaccina pyramidata* (Table 2, Figs. 1-2). The average of epidermal cells length in the adaxial surface ranged between 95.00 µm in *Agrostemma githago* and 29.5 µm in *A. gracile*, while in the abaxial surface, it ranged between 92.5 µm in *A. githago* and 35.62 µm in *A. caespitosum* and 83.12 cells mm<sup>-2</sup> in *A. githago*, while on the upper epidermis, it ranged between 275.83 cells mm<sup>-2</sup> in *A. caespitosum* and 78.12 in *A. githago* (Table 2). The presence of epidermal cells with straight-curved anticlinal walls on both adaxial and abaxial surfaces in some species of Caryophyllaceae (Tavakkoli 2016) agrees with the present study. Stomatal complex found on both surfaces (amphistomatic leaves), was circular and elliptic in shape.

All species have four types of stomata: diacytic, anomocytic, paracytic and hemiparacytic (Figs. 1-2). Higher lengths of stomata on the adaxial and abaxial surfaces were 50 µm and 53.43 µm in A. githago, respectively (Table 3, Figs. 1-2). The number of stomata on the adaxial surface ranged between 63 stomata/mm<sup>2</sup> in A. balansae and 18.12 in A. githago, while on the abaxial surface, it was 134.37 stomata/mm<sup>2</sup> in A. caespitosum, and 16.87 in A. githago (Table3). Diacytic type stomata usually are observed in Caryophyllaceae family (Yentür, 2003), but other type of stomata was observed in the examined species. Our results were in agreement with those obtained by Sahreen et al. (2010) who reported that diacytic type of stomata is the diagnostic character of family Caryophyllaceae (Divsar 2018; Ahwidi 2020; Arzehgar 2019; Mirzaei 2019; Pansambal 2019). Stomatal index rate (%) on the adaxial surface was high in A. balansae (22.50%) and low in A. caespitosum (13.90%), while on the abaxial surface, it was high (27.80%) in A. gracile and low in Polycarpon tetraphyllum (15.60%; Table 3). The differences in stomatal complex characteristics indicate differentiation and adaptation to ecological environments (Haraldson 1978). Trichome and their distribution plays a significant role in the taxonomic delimitation either generic or tribal level of the family Caryophyllaceae. Our results showed that the species contain unicellular non-glandular and multicellular uniseriate non-glandular, in agreement with Chandra et al. (2019). The Acanthophyllum species trichomes had multicellular and unicellular non-glandular hairs, while nonglandular hairs and unicellular in Bufonia calycina. A. gracile trichomes exhibited non-glandular hairs, while Arenaria balansae displayed non-glandular and glandular hairs (Fig. 3), some of these results were in agreement with Chandra et al. (2019).

		Epider	- Number of cells				
Species	U	pper	Lo	ower	. Indiniber of cens		
-	Length	width	Length	width	Upper epidermis	Lower epidermis	
Acanthophyllum bracteatum	(40-75)	(37.5-50)	(40-62.5)	(30-43.75)	(137.5-175)	(150-200)	
	59.30*	46.56	5187	37.81	156.25	172.5	
Acanthophyllum.caespitosum	(41.25-60)	(18.75-42.5)	(32.5-42.5)	(35-55)	(250-300)	(340-375)	
	49.68	30.62	38.32	42.50	275.83	356.87	
Acanthophyllum.crassifolium	(48.75-55)	(36.25-37.5)	(48.75-87.5)	(25-37.5)	(187.5-212.5)	(162.5-175)	
	50.75	36.87	62.25	32.75	200.00	166.25	
Agrostemma gracile	(25-32.5)	(37.5-41.25)	(30-50)	(32.5-50)	(212.5-250)	(275-312.5)	
	29.50	38.50	35.62	45.00	239.00	239.75	
Agrostemma githago	(87.5-100)	(60-87.5)	(55-122.5)	(35-41.25)	(55-87.5)	(75-90)	
	95.00	74.16	92.50	38.75	78.12	83.12	
Arenaria balansae	(20-47.5)	(18.75-75)	(36.25-55)	(25-38.75)	(200-237.5)	(162.5-212.5)	
	36.25	46.56	47.91	33.75	216.66	191.66	
Polycarpon tetraphyllum	(55-62.5)	(36.25-48.75)	(52.5-57.5)	(38.75-43.75)	(275-300)	(177-275)	
	59.37	42.50	55.00	40.83	275.00	244.25	
vaccina pyramidata	(50-100)	(37.5-50)	(50-100)	(25-65)	(150-187.5)	(212.5-250)	
	72.50	41.25	65.62	47.50	173.00	230.00	

\*The values between arches represent the mean and the values out the arches represent the minimum and maximum values.

# Transverse sections of leaves

The transverse section of the ten species of lamina revealed the following elements (Table 4, Fig. 4). Epidermis consists of a single layer of orbicular, rectangular, circular, semicircular, or square- shaped cells. Both epidermises are covered with a cuticle and trichomes. Thickness of cuticle was between 2.85 um in *B. oliveriana* and 1.11 um in *A. balansae* (Table 4). Thickness of lamina was between 320.75 um in *A. caespitosum* and 77.23 um in *A. balansae* (Table 4). The difference in leaf type appears to be a good diagnostic characteristic for the generic level. Our results have been recognized two types of mesophyll, isobilateral represented in the *Agrostemma* species and dorsiventral (Bifacial) in the remaining species (Fig. 4), in agreement with those found by Metcalfe & Chalk (1950) and Selvi *et al.* (2014). Parenchymatous layers contain druse crystals, as well as several sclerenchyma layers including thick and thin-walled cells that surrounded vascular bundle.

Leaves had collateral vascular type. The vascular bundle was solitary and surrounded by sclerenchymatous cells, in line with Metcalfe & Chalk (1950) and Paltinean *et al.* (2015).

	Number of stomata			Index of stomata				
Species			Adaxial surface Abaxia			l surface		
	Adaxial surface	Abaxial surface	Length	width	Length	width	Adaxial surface	Abaxial surface
Acanthophyllum bracteatum	(27.5- 37.5) 33.125*	(50-62.5) 56.00	(30-40) 35.62	(25-27.5) 25.93	(25-32.5) 28.32	(22.5-25) 23.75	17.40	24.00
Acanthophyllum.caespitosum	(17.5-55) 44.73	(78.5- 212.5) 134.37	(22.5-25) 24.06	(13.75- 16.25) 15.00	(22.5-25) 23.75	(10-11.25) 10.62	13.90	27.30
Acanthophyllum.crassifolium	(37.5- 42.5) 39.37	(30-35) 33.12	(32.5- 37.5) 35.93	(22.5- 23.75) 23.12	(32.5- 37.5) 35.93	(20-23.75) 21.25	16.40	16.6
Agrostemma gracile	(50-62.5) 57.50	(87.5- 100) 92.50	(30- 33.75) 32.08	(22.5-25) 23.75	(31.25- 32.5) 32.08	(25-27.5) 25.93	19.30	27.8
Agrostemma githago	(15-20) 18.12	(15-20) 16.87	(45-55) 50.00	(35-41.25) 37.91	(51.25- 55) 53.43	(36.25- 38.7) 37.81	18.80	16.8
Arenaria balansae	(60-67.5) 63.00	(45-65) 56.66	(22.5- 23.75) 22.91	(17.5- 23.75) 20.00	(20- 21.25) 20.62	(16.25- 18.75) 17.81	22.50	22.8
Polycarpon tetraphyllum	(57.5- 62.5) 60.00	(22-58) 45.22	(23.75- 25) 24.58	(17.5- 18.75) 18.33	(28.75- 30) 29.58	(21.25- 23.75) 22.50	17.90	15.6
vaccina pyramidata	(37.5-65) 49.52	(50-62.5) 58.12	(28.75- 32.5) 30.62	(18.75- 21.25) 20.00	(25-27.5) 26.25	(18.75- 21.25) 20.00	22.20	20.10

Table 3. Measurements of stomata in leaves of Caryophyllaceae species (in micrometer).

\*The values between arches represent the mean and the values out the arches represent the minimum and maximum values.

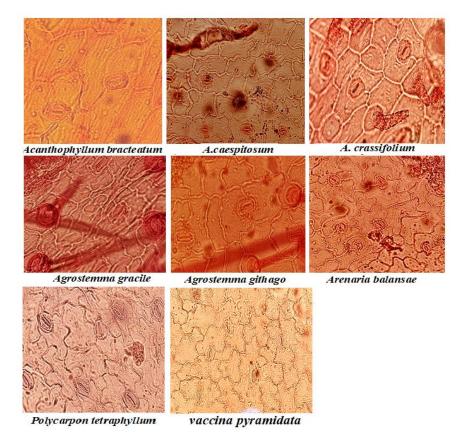
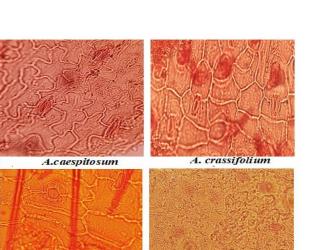


Fig. 1. Surface view of leaf epidermis in light microscope on adaxial surface.



Arenaria balansae



Polycarpon tetraphyllum

Agrostemma gracile

Acanthophyllum bracteatum

vaccina pyramidata

Agrostemma gith ago

Fig. 2. Surface view of leaf epidermis in light microscope on abaxial surface.

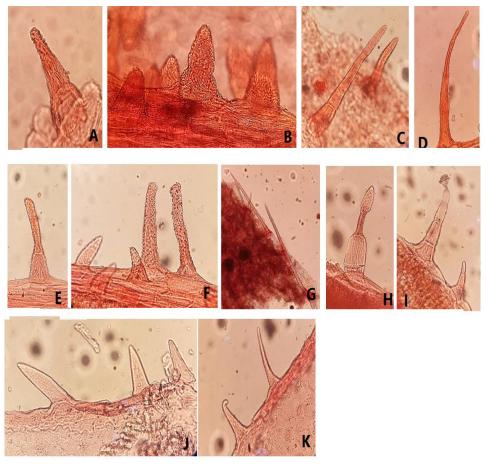


Fig. 3. Types of trichomes in some species of Caryophyllacea: (A-B) *Acanthophyllum bracteatum*, (C-D) *A. caespitosum*,(E-F) *A. crassifolium* (G) *Agrostemma gracile* (H-I) *Arenaria balansae* (J-K) *Bufonia calycina*.

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The arrangement of vascular bundle was different among the species. The size of xylem and phloem elements, were maximum in *A. bracteatum* (76.25  $\mu$ m and 38.75  $\mu$ m), while minimum in *B. calycina* (22.75  $\mu$ m and 8.12  $\mu$ m; Table 4). In the transverse section of the petiole different between species, many layers of sclerenchyma cells occupied wide area of petiole in all species expect in *A. crassifolium* which was narrow area formed from several rows of sclerenchyma layers (Fig. 4).

Species	Lamina thickness	Cuticle thickness	epidermis		Thickness	Thickness	Schlerenchyma	
			Upper epidermis	Lower epidermis	of xylem	of phloem	tissue	
Acanthophyllum bracteatum	(125-280)	(2.1-3)	(12-15)	(12-14)	(65-85)	(35-34)	(450-550)	
	250.25*	2.80	14.25	12.75	76.25	38.75	488.75	
Acanthophyllum caespitosum	(130-290)	(2-3)	(10-13)	(12-15)	(13-20)	(9-12)	(200-225)	
	230.5	2.73	11.75	13.75	15.75	10.55	208.75	
Acanthophyllum crassifolium	(270-380)	(1.2-3)	(13-20)	(9-12)	(24-35)	(11-14)	(210-430)	
	320.75	2.3	15.5	10.16	29.66	12.16	313.23	
Agrostemma gracile	(100-140)	(2-3)	(12.5-	(12.5-15)	(25-45)	(12.5-20)	(55-70)	
	120	2.75	17.5)	13.75	35	16.00	63.30	
			15.00					
Agrostemma githago	(110-120)	(1-2)	(11-12)	(11-12)	(25-35)	(15-25)	(52-78)	
	115.00	1.25	11.33	11.40	30.75	18.75	72.00	
Arenaria balansae	(75-95)	(1.1-2)	(10-13)	(8-10)	(25-28)	(15-22)	(80-179)	
	77.23	1.11	11.5	8.87	26.75	18.00	141.22	
Bufonia oliveriana	(175-200)	(2.4-3.4)	(12.5-15)	(7-8)	(20-35)	(7.5-9)	(40-49)	
	187.5	2.85	13.00	7.50	26.8	8.75	45.66	
Bufonia calycina	(160-188)	(1.9-2.75)	(12.5-14)	(7.5-8.2)	(19-30)	(6-9)	(35-45.5)	
	177.25	2.00	13.78	7.45	22.75	8.12	39.75	
Polycarpon tetraphyllum	(150-200)	(1-2.5)	(16-19)	(11-12)	(22-31)	(6-12)	(31-78)	
	181.33	1.75	17.80	11.66	33.24	9.12	62.45	
Polycarpon succulentum	(140-195)	(1.2-2.4)	(15.75-20)	(11-11.9)	(20-40)	(6-15)	(35-53)	
	173.33	1.70	18.00	11.71	34.22	10.22	44.76	

Table 3. Anatomical characters of leaves in some Caryophyllaceae species (in micrometer).

\*The values between arches represent the mean and the values out the arches represent the minimum and maximum values.

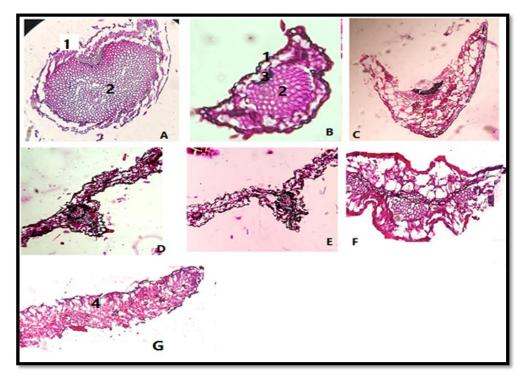


Fig. 4. Transverse section of leaf lamina and midrib. A-C: midrib (A) Acanthophyllum bracteatum, (B) A. caespitosum, (C)
A. crassifolium; D- G: (D) Agrostemma gracile, (E) Agrostemma githago, (F) Bufonia calycina, (G) Polycarpon tetraphyllum;1: Upper epidermis 2: Sclerenchyma layer 3: Vascular bundle 4: Palisade layer.

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Shape, size and numbers of sclerenchyma layers of stem gives a good character in separation of the species Polycarpon tetraphyllum and recognized by hallow stem compared with other species, many species contain large pith in A. bracteatum, A. balansae and narrow pith in Bufonia and Agrostemma species. The analysis of the stem cross-section showed that the cortex is narrow except in A. balansae and B. calycina. Polycarpon species was broad and the endodermis frequently well distinguished, while it was narrow in the rest species. Stem shape and size were different between species. In A. bracteatum, the stem in cross section was rectangular. In the case of other species, stem cross section was rectangular-undulate in Agrostemma gracile, circular in A. caespitosum and P. succulentum, semicircular-elliptic in A. crassifolium, P. tetraphyllum and A. balansae, and ovate in B. oliveriana. The stems exhibited the greatest size (1064.50 µm) in A. bracteatum and displayed the smallest stem cross section (450.46 µm) in A. balansae (Table 5, Fig. 5). The cortex, and pith rich in wide crystal (Fig. 5), calcium oxalates crystals are commonly present in the many species of Caryophyllaceae family (Metcalfe & Chalk 1950). Vascular bundle tissue is a continuous bicollateral cylinder in the most species, but it was continuous in Acanthophyllum, Agrostemma and Polycarpon genera. These results are reliable with the description given by Metcalfe & Chalk (1950). The phloem is 4-7 layered and consists of irregular cells. The xylem are irregular cells consist of large circular, semicircular, and orbicular cells (Fig. 5). Thickness of xylem was variable between 123 µm in A. bracteatum and 37.51 µm in A. caespitosum, while phloem was 44.60 µm in A. bracteatum and 10.75 µm in *B. calycina* (Table 5). The center of stems included pith which composed of isodimetric to polyhederal from parenchymatous storage cells, characteristics as thin layered cells with large intercellular spaces. Cells were rich in druses crystals. Sclerenchymatous layers were observed in all species. Their numbers were different between the species, 12-23 layers in A. gracile and 4-7 in A. crassifolium. The size of pith was different; the greatest was 280.22 mm in A. githago and no pith in P. tetraphyllum and A. balansae (Table 5). The druses crystal was presented in the leaves and stems in all species, in agreement with Metcalfe & Chalk (1950) and also similar to characters found in another species of Caryophyllaceae (Jafari et al. 2008 and Sahreen et al. 2010; Al-Saadi & Al-Taie 2014; Al-Taie et al. 2018).

Species	Stem dimeter	Cutical thickness	Epiderm Thickness	Schlerenchyma number	Cortex dimeter	Phloem thickness layers	Xylem thickness layers	Pith dimeter
Acanthophyllum bracteatum	(890- 1284) 1064.50*	(8-9) 8.62	(12-20) 17.50	7-15	(200- 300) 240	(40-48) 44.60	(100- 140) 123.00	(175- 250) 212.50
Acanthophyllum caespitosum	(498-660) 578.10	(5-12) 9.11	(12-20) 17.25	4-14	(150- 190) 176.5	(10-13) 11.80	(30-45) 37.51	(85-100) 93.39
Acanthophyllum crassifolium	(635-843) 755.42	(11-13) 12.25	(22-35) 27.5	4-7	(195- 265) 237.22	(20-28) 24.40	(50-60) 56.33	(39-42) 40.50
Agrostemma gracile	(794-988) 927.56	(7-9) 7.98	(30-35) 32.33	12-23	(160- 205) 193.25	(25-35) 28.75	(50-60) 56.25	(250- 300) 290.44
Agrostemma githago	(844.1- 1000.5) 894.75	(6.95-9) 7.00	(29.9-34) 31.99	12-16	(155- 210) 183.50	(25.2-33.25) 29.00	(50-59) 55.75	(230- 310) 280.22
Arenaria balansae	(390-478) 450.46	(2-3) 2.25	(18-20) 19.40	5-10	(50-70) 66.5	(25-30) 28.25	(60-75) 68.33	/
Bufonia oliveriana	(456-595) 536.75	(5-8.5) 7.00	(10-14) 12.25	6-8	(100- 130) 117.5	(18-20) 19.25	(45-60) 52.5	(100- 135) 120.75
Bufonia calycina	(574-734) 654.40	(1-2) 1.33	(11-13) 12.25	5-8	(100- 120) 111.25	(10-12) 10.75	(40-55) 47.25	(250- 330) 288.75
Polycarpon tetraphyllum	(466-550) 542.00	(1-2) 1.75	(13-15) 13.5	5-7	(75-90) 80.25	(14-15) 14.66	(55-60) 57.50	/
Polycarpon succulentum	(551- 692.2) 622.80	(1-1.1) 1.04	(17-20) 18.75	5-8	(70-100) 85.00	(25-35) 30.11	(75-90) 84.00	(175- 200) 185.12

\*The values between arches represent the mean and the values out the arches represent the minimum and maximum values.

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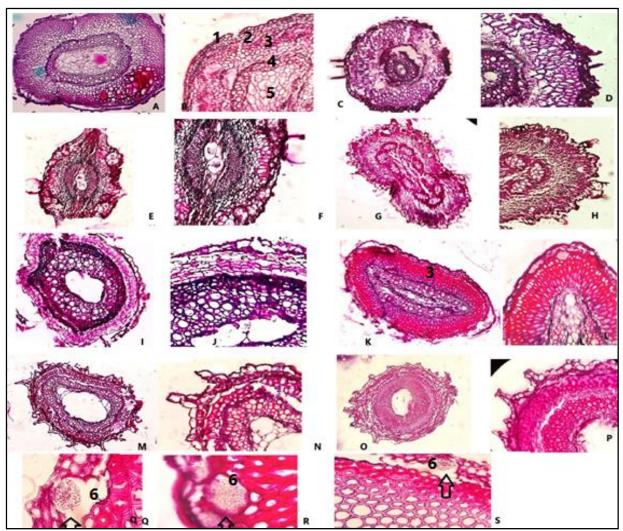


Fig. 5. Transverse section of stems: (A-B) Acanthophyllum bracteatum, (C-D) A. caespitosum, (E-F) A. crassifolium, (G-H) Agrostemma gracile, (I-J) Arenaria balansae, (K-L) Bufonia oliveriana, (M-N) Polycarpon tetraphyllum, (O-P) Polycarpon succulentum, 1: Upper epidermis 2: Collenchyma layer 3: Sclerenchyma layer, 4: Vascular bundle, 5: Pith, 6: Druses crystal.

### CONCLUSION

Anatomical leaves, stem, epidermal features can help in identification and classification of species of Caryophyllaceae family. The species differ in thickness of sclerenchyma layers, mesophyll type, trichomes type and present or absent the pith. It is concluded that anatomical characteristics having a useful taxonomic character for separating the species. Stomatal index was higher in *A. balansae* (22.50%) and low in *A. caespitosum* (13.90%), while on the abaxial surface it was high (27.80%) in *A. gracile* and low in *P. tetraphyllum* (15.60%). So, it exhibits larger stomatal size and length of epidermal cell within this species. The *Agrostemma* species can be distinguished as the type of mesophyll (isobilateral) and separated about the remaining species (Bifacial). Leaves have solitary vascular bundle surrounded by sclerenchymatous cells.

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