Comparative anatomical studies in relation to taxonomy of *Sedum s.l.* (Crassulaceae) in Iran

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**Abstract:** This study aimed to characterize the stem, peduncle, and leaf anatomy of 22 species of *Sedum senso lato* distributed in Iran. The results showed that the presence of tanniniferous storage cells in stems and leaves, distinct xylem vessels in stems, and the shape of peduncle cross sections, were taxonomically informative evidence, and isolated *Phedimus* from the other studied taxa of *Sedum s.l.*, whereas hairy leaves and peduncles containing starch storage cells were identified as *Prometheum* and *Hylotelephium* respectively. In addition, the current anatomical evidence confirmed the alliance of 2 sections of *Sedum* and *Epeteium* within *Sedum sensu stricto*. The result of numerical analysis (including 31 qualitative and quantitative anatomical characters) supported complete separation of the four allied genera and revealed significant influence of anatomical traits in taxonomy of *Sedum s.l.*

**Key words:** *Sedum s.l.*, anatomy, taxonomy, *Phedimus, Prometheum*, numerical analysis

1. Introduction

*Sedum* L., comprising ca. 420 species, is the most species-rich member of the family Crassulaceae (Thiede and Eggli, 2007; Nikulin et al., 2016). The genus is almost entirely confined to the subtropical and temperate regions of the Northern Hemisphere (t Hart, 1991; Mifsud et al., 2015). With its extreme morphological diversity and homoplasy of phenotypic features, several hybrid species, and current ploidy, *Sedum* has a confused systematic position (t Hart and Koek-Noorman, 1989; Nikulin et al., 2016), influencing the current classifications. Several previous authors (Paraeger, 1921; Froderstrom, 1936; Clausen, 1975) considered *Sedum* as a hold-all taxon, containing only a few additional genera within the subfamily Sedoideae, whereas others (Borissova, 1969; Ohba, 1977a, 1978b; Grulich, 1984) divided the genus into controversial genera (Van Ham and t Hart, 1998; Mort et al., 2001; Giuliani, 2017), among which the Asian *Hylotelephium* Ohba, *Phedimus* Rafin, *Rhodiola* L., and *Umbilicus* Dc. gained wide taxonomic recognition and were completely separated from *Sedum* based on molecular data (Mauzyumi and Ohba, 2004; Gontcharova and Gontcharov, 2009; Carrillo-Reyes et al., 2009). According to Jansson and Rechinger (1970), in Iran, the genus is represented by 16 species arranged into 2 sections: Sect. *Sedum* (Syn.: *Seda Genuina* Koch) and *Epeteium* Boiss. Akhiani (2000) reported 22 representatives of *Sedum* for the flora of Iran, mainly growing in semiarid and mountainous regions. The systematic delimitation of Iranian representatives of *Sedum* shows many contradictions, more precisely in the following species: *Sedum pallidum*, *S. pentapetalum*; *Prometheum sempervivoides*, *P. pilosum*; *Phedimus stoloniferus*, *Ph. spurius*, and *Ph. obtusifolius* (Jansson and Rechinger, 1970; Akhiani, 2000). In spite of a number of previous studies, anatomical characteristics of the genus were poorly understood. These include the general anatomical study of family Crassulaceae (Metcalf and Chalk, 1950), and the stem anatomy of selected species of *Sedum* (Zheng et al., 2016). The current survey aims to describe the anatomical structure of the stem, peduncle, and leaf of Iranian representatives of *Sedum s.l.* to determine diagnostic characters and to evaluate the extent to which these evidences can be used for separating the taxonomic ranks.

2. Materials and methods

Plant specimens were obtained from both field and herbarium materials. Measurements and observations...
were carried out for at least 5 cross-sections, selected from 2 to 3 populations per species. A list of voucher specimens is given in Table 1. Leaf samples were selected from the 2nd to 6th nodes of the stems. Stem samples were cut from about 1 cm in the middle of the 2nd to 4th nodes. Fresh materials were fixed in 50% FAA (formaldehyde/acetic acid/ethanol) for 48 h, then washed with distilled water, dehydrated in an ethanol series (30, 50, 70, 95 and 100%), and transferred to 70% ethanol for long-term preservation. Herbarium specimens were rehydrated in water before fixing in FAA. Stem peduncle and leaf cross-sections were prepared by hand cutting and then stained with methyleneblue and Congo red. Observations were carried out using an Olympus BX-51 light microscope under 100× and 400× magnifications. For cluster analysis (CA), NTSYS software (version 2.02) (Rohlf, 1997) was used to construct the dendrogram using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal 1973). Cophenetic correlation coefficient was calculated to find out to what extent cophenetic matrix fits the original similarity matrix. In this analysis, a total of 46 samples (Table 1) and 31 anatomical characteristics comprising 20 qualitative (Tables 2 and 3) and 11 quantitative characteristics (i.e. diameter of tanniniferous storage cells in stem; thickness of stem cuticle, endodermis and parenchyma layer; ratio of xylem thickness to phloem in stem; number of collenchyma layers in stem and peduncle; thickness of epidermal layer and cortex in peduncle, ratio of xylem thickness to phloem and ratio of pith thickness to cross section in peduncle) were involved.

3. Results

3.1. Cross-section of stem (Table 2, Figure 1, Figure 2)

Cross-sections were undulating circular to circular in shape with 2 lateral wings. There was a single layer of epidermal cells surrounded by a layer of cuticle on the surface. Stomata were present on the epidermis of Ph. obtusifolius (Figure 1e), S. tenellum, S. sabulatum and S. annum but absent in the other studied taxa. Trichomes were observed in the epidermis of the representatives of the genus Prometheum (Figures 1f–1h) and 2 taxa of Sedum s.s.: S. tenellum (Figure 1l) and S. elbusense (Figure 2o). Underneath the epidermis was a collenchymatous region consisting of 1-3 cell layers. This region was absent in S. caespitosum (Figure 2j) A parenchymatous cortex comprising circular compact cells was located between the collenchyma and the endodermis. Cortical bundles were observed in the parenchymatous tissue of S. tetramerum (Figure 2a) and S. pallidum (Figure 2c). One layer of endodermis separating the cortex from vascular cylinder was recognized in the stem of all examined taxa, except in Prometheum taxa. Vascular cylinder consisted of a poorly developed phloem (which were not easily recognized) and a well-developed xylem. Xylem vessels were in a continuous ring in all studied species except in Ph. stoloniferus (Figure 1a), Ph. spurius (Figure 1c) and Ph. obtusifolius (Figure 1d), which exhibited cluster vessels of unequal size. The pith region was observed in the central part of the stem of all examined taxa, except S. callichroum (Figure 2l). In some species, parenchymatous tissue of cortex and pith contained tanniniferous and starch storage cells that varied in size and density among the studied taxa (Figures 1a–1d, 2e, 2g–2i).

3.2. Cross-section of peduncle (Table 3, Figure 3, Figure 4)

Cross-sections had different shapes including circular, undulating circular, circular with 2 wings, semicircular with 3 wings and stellate. The outer surface was covered by 1 layer of cutinized epidermal cells and in some species with uni- and multicellular glandular trichomes (Figures 3d–3e, 3n–3o, 4a–4b, 4i). The cortex consisted of 1–3 layers of collenchyma and strongly developed parenchyma layers (especially in the representatives of the genus Prometheum). Some storage cells were observed in the parenchymatous region of H. caucasicum (Figure 3g). The vascular cylinder consisted of xylem, which was found in a closed ring or in clusters, a poorly developed phloem, and a pith zone.

3.3. Cross-section of leaf (Table 3, Figure 5, Figure 6)

The shapes of transverse section were convex, flat and semicircular/undulating semicircular. In some of the studied taxa of Sedum s.s., e.g. S. album (Figure 5j), S. tetramerum (Figure 6a), S. pallidum (Figure 6b), S. hispanicum (Figure 6c) and S. pentapetalum (Figure 6d), adaxial and abaxial sides were indistinguishable. In all taxa, there was a single layer of ovate-circular epidermal cells on both leaf surfaces. Trichomewas observed on the epidermis of P. pilosum and P. sempervivioides. Prometheum pilosum had multicellular glandular hairs on the lower epidermis (Figures 5e–5f); while P. sempervivioides showed a higher density of trichomes on both lower and upper epidermis (Figures 5g–5h). In some species (e.g. S. lenkoranicum and S. gracile), lower epidermal cells were larger than upper cells (Figures 5m–5o). Hypoderm was observed in the studied taxa of Phedimus, Prometheum, Hylotelephium and 3 species of Sedum s.s. (S. lenkoranicum, S. rubens and S. annum). In all examined taxa, mesophyll was isobilateral and not differentiated into palisade and spongy layers. There were some large mucilaginous cells associated with parenchymatous cells in some species (e.g. S. hispanicum, S. pentapetalum, S. album etc.), but these cells were not constant in the different populations of the same species. In the representatives of Phedimus, storage cells were found in the mesophyll (especially around the vascular bundles) (Figures 5a–5d). The midrib region was not clearly recognized in some species of Sedum s.s., while it was obvious and prominent in the examined taxa.
Table 1. Voucher specimens of the examined species. R represents the examined individuals in the numerical taxonomy.

| Species | Collection data |
|---------|-----------------|
| Ph. stoloniferus (S.G.Gmel.) \Hart, 1995 | Guilan: 25 km of Masal South, Olesbelanga, 1500 m, Mohammadi & Saedi, GUH-8299 (R1); Mazandaran: 40km of Ramsar Southwest, Garesmasar village, 1800 m, Hosaini, GUH-8300 (R2); Golestan: Azad-Shahr, Farsian village, 500 m, Mohammadi, GUH-8301 (R3) |
| Ph. Spurius (M. Bieb.) \Hart, 1995 | Guilan: Rezvan-Shahr, Paresar, Arde village, 1000 m, Mohammadi, GUH-8302 (R4); Mazandaran: Ghalus, Kuhestan, Dahir village, 2100 m, Mohammadi & Alizadeh, GUH-8303 (R5) |
| Ph. Obtusifolium (C. A. Mey.) \Ohba, 1975 | Guilan: Rezvan-Shahr, Paresar, Arde village, 1000 m, Mohammadi, GUH-8302 (R4); Mazandaran: Ghalus, Kuhestan, Dahir village, 2100 m, Mohammadi & Alizadeh, GUH-8303 (R5) |
| P. pilosum (Fischer ex M. Bieb.) \Ohba, 1978 | Guilan: 17 km of Kale bar, Pashtab village, 2000-2500 m, Ghahremani-Nejad, GUH-8334 (R8) |
| P. sempervivoides (Fischer ex M. Bieb.) \H. Ohba, 1978 | Guilan: 17 km of Kale bar, Pashtab village, 2000-2500 m, Ghahremani-Nejad, GUH-8334 (R8) |
| H. caucasicum (Grossh.) \Ohba, 1977 | Guilan: Asalem road to Khalkhal, 1800 m, Mohammadi & Nabizadeh, GUH-8315 (R28) |
| S. album L., 1753 | Guilan: Eshkevarat, Garmabdasht, 550 m, Naser, GUH-8304 (R13); Guilan: Amlash, Guraj village, 1600 m, Mohammadi, GUH-8305 (R14); Mazandaran: Ghalus, Kelardasht, 1000 m, Soleymanpour, GUH-8306 (R15) |
| S. tenellum M. von Bieberstein, 1819 | Guilan: Asalem road to Khalkhal, 1800 m, Mohammadi & Nabizadeh, GUH-8307 (R22) |
| S. sabulatum (C.A. Mey.) Boiss., 1872 | Guilan: Asalem road to Khalkhal, unknown, GUH-8307 (R22) |
| S. gracile C. M. Mey., 1831 | Guilan: Asalem road to Khalkhal, unknown, GUH-8332 (R19) |
| S. lenkoranicum Grossh., 1915 | Guilan: Asalem road to Khalkhal, 1800 m, Mohammadi & Nabizadeh, GUH-8309 (R21); Ardebil: Khalkhal, Sardal West, 2300 m, Mohammadi & Nabizadeh, GUH-8307 (R22) |
| S. tetramerum Trautv., 1881 | Fars: 15 km of Noorabad Southeast, Hrayerz, 950-1000 m, Mohammadi & Nabizadeh, GUH-8311 (R23); Fars: 23 km of Lar, Grash, 800 m, Mohammadi & Nabizadeh, GUH-8312 (R24); Kerman: Sirjan road to Bandar-Abbas, 70 m, Mohammadi & Jalali, GUH-8313 (R25) |
| S. pallidum M. B., 1808 | Guilan: Fuman road to Masuleh, 1000 m, Mohammadi & Saedi, GUH-8314 (R26); Guilan: Talesh, Arasbaran, Moradi and Gholami, GRC-847 (R27); Mazandaran: Noor, Chamestan, Vaz, 970 m, Mohammadi, GUH-8315 (R28) |
| S. hispanicum L., 1755 | Guilan: South of Chaboksar, Sarvelat village, 1200-2100 m, Mohammadi & Fahmideh, GUH-8316 (R29); Khorasan: Amiri, GUH-8317 (R30); Golestan: Ziyyarat, 1560 m, Mohammadi, GUH-8318 (R31) |
| S. pentapetalum Boiss., 1939 | Guilan: South of Chaboksar, Sarvelat village, 800 m, Mohammadi & Fahmideh, GUH-8319 (R32); Mazandaran: Noshahr, Sisangan village, 100 m, Mohammadi, GUH-8320 (R33); Guilan: Rudbar, Doghe, 1293 m, Moradi & Ladani, GRC-429 (R34) |
| S. rubens L., 1753 | Hormozgan: Bandar-Abbas, Geno mountain, 1500 m, Mohammadi, GUH-8321 (R35); Guilan: Talesh, Dokhalekuh, 1500 m, Mohammadi, GUH-8322 (R36) |
| S. caespitosum (Cav.) DC., 1828 | Guilan: Rudser, Eshkevarat, 900 m, Shahi, GUH-8323 (R37); Fars: 9 km of Shiraz road to Noorabad, Mohammadi & Nabizadeh, GUH-8324 (R38) |
| S. kotschyanum Boiss., 1845 | Lorestan: Sepid-Kuh mountain, 2300 m, Mohammadi & Roudi, GUH-8325 (R39); Fars: Safashahr, 2400 m, Khorasan & Mohammadi, GUH-8332 (R40) |
| S. callichroum Boiss., 1845 | Fars: Noorabad, Harayerz, 900-1000 m, Mohammadi & Nabizadeh, GUH-8331 (R41) |
| S. nanum Boiss., 1845 | Lorestan: Sepid-Kuh mountain, 2000 m, Mohammadi & Roudi, GUH-8327 (R42); Loristan: Makhmal-Kuh mountain, 500 m, Mohammadi & Roudi, GUH-8328 (R43) |
| S. annum L., 1753 | Guilan: Jirandeh, 1850 m, Mohammadi, GUH-8329 (R44); Fars: Rostam city, Shahi, GUH-8330 (R45) |
| S. elbursense Akhiani & Assadi | Zanjan: Tarom, Sandestan, 1900 m, Mohammadi & Saedi, GUH-8326 (R46) |
Table 2. Selected qualitative characters of stems for anatomical comparison of the examined species. + represents presence of character, - represents absence of character; numbers indicate cell layers. CS = cross-section, T = trichome, TSH = trichome shape, St = stomata, TSC = tanniniferous storage cell, CB = cortical bundle, SSC = starch storage cell, En = endodermis, XyV = xylem vessel, Pi = pith region, Cl = collenchyma.

| Species         | CS              | T              | TSh            | St | TSC | CB | SSC | En | XyV | Pi | Cl |
|-----------------|-----------------|----------------|----------------|----|-----|----|-----|----|-----|----|----|
| Ph. stoloniferus| Undulating circular | _              | _              | +  | +   | +  | +   |    |     |    | 1  |
| Ph. spurius     | Undulating circular | _              | _              | +  | +   | +  |     |    |     |    | 1  |
| Ph. obtusifolium| Undulating circular | _              | _              | +  | +   | +  |     |    |     |    | 1  |
| P. pilosum      | Circular         | + Pappilae, glandular | _  | _  | _  |     |    |    | Closed ring + | 2  |
| P. sempervivoides| Circular         | + Pappilae, glandular | _  | _  | _  |     |    |    | Closed ring + | 2  |
| H. caucasicum   | Circular         |               |               |    |     |    |     | +  |     |    | 2–3|
| S. album        | Circular         |               |               |    |     |    |     | +  |     |    | 1  |
| S. tenellum     | Circular         | + Glandular    |               | +  |     | _  | +   |    |     |    | 1  |
| S. sabulatum    | Undulating circular | _              | _              | +  |     | _  | +   |    |     |    | 1  |
| S. gracile      | Undulating circular | _              | _              |     |     | _  | +   |    |     |    | 1  |
| S. lenkoranicum | Undulating circular | _              | _              |     |     | _  | +   |    |     |    | 1  |
| S. tetrarmum    | Circular with 2 wings | _              | _              |     |     | _  | _   | +  |     |    | 1  |
| S. pallidum     | Undulating circular | _              | _              |     |     | _  | _   | +  |     |    | 1  |
| S. hispanicum   | Circular with 2 wings | _              | _              |     |     | _  | _   | +  |     |    | 1  |
| S. pentapetalum | Circular with 2 wings | _              | _              |     |     | _  | _   | +  |     |    | 1  |
| S. rubens       | Undulating circular | _              | _              |     |     | _  | _   | +  |     |    | 1  |
| S. caespitosum  | Undulating circular | _              | _              |     |     | _  | _   | +  |     |    | 0  |
| S. kotschyamum  | Undulating circular | _              | _              |     |     | _  | _   | +  |     |    | 1  |
| S. callichroum  | Circular         |               |               |    |     |    |     | +  |     |    | 1  |
| S. nanum        | Circular         |               |               |    |     |    |     | +  |     |    | 1  |
| S. annum        | Circular         |               |               |    |     |    |     | +  |     |    | 1  |
| S. elbursense   | Undulating circular | + Glandular    | _              | _  | _   | +  |     |    |     |    | 1  |

of Phedimus, Prometheum and Hylotelephium. Vascular bundles were crescent-shaped or approximately circular.

The result of the UPGMA analysis (Figure 7) showed a high cophenetic correlation (98%), indicating a great proportion between the dendrogram and original matrix, and a complete separation of the genera into two major clusters; the 1st cluster contained representatives of Phedimus, Prometheum and Sedum s.s., and the 2nd comprised Hylotelephium species. Two sections of Sedum and Epeteium could not be delimitated in this dendrogram.

4. Discussion

Environmental influences can simulate the appearance of various adaptations in plants and thus strongly affect their morphoanatomical differentiation (Anacker, 2014). These differences usually represent an adaptive response to certain habitat conditions, but in cases of highly distinct differences may have important taxonomic significances (kruckeberg, 1951; Anacker et al., 2011). Herein we used different accessions for each taxon and selected features that were constant in them.

4.1. Important anatomical characteristics used for the intergeneric delimitation and taxonomic relationships

The characteristics that contributed most to the separation of genera were the presence or absence of hairs and tanniniferous secretory cells in the stem and leaf; the number of collenchyma layers; the presence or absence of endodermis and types of xylem vessels (distinct or continuous) in the stem; the shape of the cross-section; and the presence or absence of starch storage cells in peduncles. Based on Metcalf and Chalk (1950), tanniniferous secretory cells are commonly present in non-lignified tissues, especially the cortex, pith, and phloem of the family Crassulaceae. In majority of these plants, distinct vascular bundles are rare, and the xylem forms a continuous cylinder. Our finding revealed the importance of stem and peduncle anatomical characters in the separation of Phedimus from Prometheum, Hylotelephium and Sedum.
Table 3. Selected qualitative characters of peduncle and leaf for anatomical comparison of the examined species. + represents presence of character, - represents absence of character, numbers indicate cell layers. CS = cross-section, T = trichome, SSC = starch storage cells, XyV = xylem vessel, Cl = collenchyma, Hy = hypodermis, TSC = tanniniferous storage cell, Mid = midrib region.

| Peduncle | Leaf |
|----------|------|
| species  | CS   | T  | SSC | XyV | Cl  | CS | Hy | T  | TSC | Mid | XyV |
| Ph. stoloniferus | Semicircular with 3 wings | _ | _  | Closed ring | 1-2 | Convex | + | _ | + | + | Crescent |
| Ph. spurious | Semicircular with 3 wings | _ | _  | Closed ring | 1-2 | Convex | + | _ | + | + | Crescent |
| Ph. obtusifolium | Semicircular with 3 wings | _ | _  | Closed ring | 1-2 | Convex | + | _ | + | + | Crescent |
| P. pilosum | Circular | + | _  | Closed ring | 1 | Flat | + | + | _ | + | Crescent |
| P. sempervivoides | Circular | + | _  | Closed ring | 1 | Flat | + | + | _ | + | Crescent |
| H. caucasicum | Circular with 2 wings | _ | + | Closed ring | 2-3 | Convex, flat | + | _ | + | + | Crescent |
| S. album | Circular with 2 wings | _ | _  | Closed ring | 1 | Semi-circular | _ | _ | _ | _ | Crescent |
| S. tenellum | Circular | _ | _  | Closed ring | 1 | Convex | _ | _ | _ | + | Crescent |
| S. sabulatum | Circular | _ | _  | Closed ring | 1 | Convex | _ | _ | _ | + | Crescent |
| S. gracile | Undulating circular | _ | _  | Distinct | 1 | Convex | _ | _ | _ | + | Circular |
| S. lenkoranicum | Undulating circular | _ | _  | Distinct | 1 | Convex | _ | _ | _ | + | Circular |
| S. tetrarsum | Circular | _ | _  | Distinct | 1 | Semi-circular | _ | _ | _ | _ | Crescent |
| S. pallidum | Undulating circular | + | _  | Distinct | 1 | Flat | _ | _ | _ | _ | Circular |
| S. hispanicum | Circular with 2 wings | + | _  | Distinct | 1 | Flat | _ | _ | _ | _ | Circular |
| S. pentapetalum | Circular with 2 wings | + | _  | Distinct | 1 | Flat | _ | _ | _ | _ | Circular |
| S. rubens | Undulating circular | + | _  | Distinct | 1 | Convex | + | _ | _ | + | Circular |
| S. caespitosum | Undulating circular | _ | _  | Distinct | 1 | Convex | _ | _ | _ | + | Circular |
| S. kotschyanum | Undulating circular | _ | _  | Distinct | 1 | Flat | _ | _ | _ | _ | Circular |
| S. callichroum | Undulating circular | _ | _  | Distinct | 1 | Flat | _ | _ | _ | _ | Circular |
| S. nanum | Undulating circular | _ | _  | Distinct | 1 | Convex | _ | _ | _ | + | Circular |
| S. annum | Stellate | _ | _  | Distinct | 0 | Flat | + | _ | _ | + | Circular |
| S. elbursense | Circular | + | _  | Distinct | 1 | Flat | _ | _ | _ | + | Circular |

The exclusive characters enabling the separation of the examined species of Phedimus from the others were the presence of tanniniferous storage cells in the cortex and pith zone of the stem; distinct type of xylem vessels; the 3-winged cross section shape of the peduncle (Figures 3a–3c), and the presence of secretory cells in the leaf. The current results support the classification proposed by t Hart (1995) and transfer of 3 species of Sedum (i.e. S. stoloniferum, S. spurious and S. obtusifolium) to the genus Phedimus. However, 2 Iranian floras (Jansson and Rechinger, 1970; Akhiani, 2000) and the flora of Turkey (Chamberlain, 1972) have treated these species under the genus Sedum.

The present result supports the phylogenetic distance between the 2 genera Phedimus and Sedums.s. According to phylogenetic results (t Hart, 1992; Van Ham, 1995; Mayuzumi and Ohba, 2004; Thiede and Eggli, 2007), the 1st genus is nested in the tribe Umbeliceae, clade Telephium and subclade Phedimus, while the 2nd is placed in tribe Sedeae, clades Acre, and Leucosedum. In addition, further morphological traits (especially the shape of leaf blade and margin) (Moran, 2009), palynological data (e.g. pollen size, shape and infolding patterns), and micro morphological evidences of the reproductive structures (Giauni, 2017) confirm this segregation.

Hairy leaves (Figures 5e–5h), the absence of endodermis, and 2 collenchyma layers in the stem and peduncle are the most useful traits for characterizing Prometheum. This result supports Ohba’s treatment (1978) in which Prometheum pilosum (Syn.: Sedum pilosum Bieb.) and P. sempervivoides (Syn.: Sedum sempervivoides) were excluded from Sedum. This result is also in accordance with morphological (Aminirad et al, 2017) and cytological evidence (t Hart, 1995; t Hart, 2003).

The presence of starch storage cells in the cortex of the peduncle (Figure 3g) and 2–3 collenchyma layers in the...
Figure 1. Cross-sections of stem. (a-b) Ph. stoloniferus, (c) Ph. spurius, (d-e) Ph. obtusifolium, (f) P. pilosum, (g-h) P. sempervivoides, (i) H. caucasicum, (j) S. album, (k-l) S. tenellum, (m) S. sabulatum, (n) S. gracile, (o) S. lenkoranicum. DXyV = distinct xylem vessel, TSC = tanniniferous storage cell, SSC = starch storage cell, ST = stomata, GT = glandular trichome, PT = papillae trichome. Scale bars 100 μm.
Figure 2. Cross-sections of stem. (a) *S. tetramerum*, (b-c) *S. pallidum*, (d-e) *S. hispanicum*, (f-g) *S. pentapetalum*, (h-i) *S. rubens*, (j) *S. caespitosum*, (k) *S. kotschyanum*, (l) *S. callichroum*, (m) *S. nanum*, (n) *S. annum*, (o) *S. elbursense*. CB = cortical bundle, SSC = starch storage cell, GT = glandular trichome. Scale bars 100 μm.
stem and peduncle identify *H. caucasicum* (classified in the genus *Sedum*) (Akhiani 2000), the only representative of the genus *Hylotelephium* in Iran. These results confirm the position of *Hylotelephium* in clade *Telephium*, subclade *Hylotelephium*.

The studied anatomical characters present a strong variation within *Sedum s.s.*; however, these traits do not show obvious synapomorphies in the genus.

4.2. Important anatomical characters used for infrageneric delimitation and taxonomic relationships

Within the genus *Phedimus*, *Ph. stoloniferus* was distinguished by its starch storage cells (Figure 1b), and *Ph. obtusifolius* was differentiated from the other 2 examined taxa in having stomata in its stem cross section (Figure 1e).

Following Berger’s classification (1930), some local flora, such as Flora Europea (Webb, 1964), Flora Iranica...
and flora of Turkey (Chamberlain, 1972) arranged Sedums.s into 2 sections, Sedum and Epeteium. However, the 2 sections were merged by recent studies (Clausen, 1975; Ohba, 1978; t'Hart, 1982; Akhiani, 2000). Our anatomical evidences support the later investigation concerning the alliance of 2 sections. The 2 sections have several common anatomical traits, e.g. the shape of the stem cross-section (circular, undulating circular, and circular with 2 wings); number of (0–1) collenchyma layer in the stem and peduncle; the absence of storage cells in the stem, peduncle, and leaf; the presence or absence of trichome in the stem and peduncle; the presence of endodermis in the stem; the shape of xylem vessels in the stem (a ring) and leaf (circular/crescent), and the presence of pith in the stem and peduncle. According to Berger’s classification (1930), the Sedum rubens group (including S. hispanicum, S. pentapetalum, and S. rubens) has a controversial systematic position and their delimitation is not clearly understood (t’Hart, 1985). Some previous authors (Froderstrum, 1932; Jansson and Rechinger, 1970) considered S. pallidum as a synonym of S. hispanicum, while others (Borrisova, 1939; Webb, 1964; Chamberlain, 1972; Akhiani, 2000) treated it as a distinct species. Furthermore, Zafran (1976) considered S. pallidum to be conspecific with S. rubens (t’Hart 1985). Sedum pallidum and S. hispanicum have identical morphological characters, such as sterile leaves; 10 stamens; triangular sepals with equal size; and white petals with a reddish median line. However, based on palynological (Giuliani, 2017) and phylogenetic evidence (Nikulin, 2016), S. rubens, S. hispanicum, and S. pallidum are distinct species occurring in the Leucosedum clade. According to our anatomical data, these 3 closely related taxa can be readily isolated by the cross-section shape of their stem and peduncle (circular with 2 wings in S. hispanicum (Figures 2d and 3o), but undulating circular in S. pallidum (Figure 2b, Figure 3n) and S. rubens (Figures 2h and 4c). In addition, S. pallidum is characterized by the presence of cortical bundles in the stem (Figure 2c). Metcalf and Chalk (1950) and Abdel-Raouf (2012) have reported this character in some other taxa of Crassulaceae. The absence of starch storage cells in the stem of S. pallidum and a convex cross-section of the leaf with a defined midrib region in S. rubens (Figure 6E) are other important differentiating characters in this group.
S. hispanicum and S. pentapetalum have an ambiguous taxonomic position. They were treated as 2 distinct species in both Flora Iranica (Jansson and Rechinger, 1970) and flora of Iran (Akhiani, 2000), whereas Sarvar (2004) combined the 2 later species and considered S. pentapetalum as a synonym of S. hispanicum. These species have several palynological and morphological similarities (Sarvar, 2004). However, the result of present study did not

Figure 5. Cross-sections of leaf. (a-b) Ph. stoloniferus, (c) Ph. spurius, (d) Ph. obtusifolius, (e-f) P. pilosum, (g-h) P. sempervivoides, (i) H. caucasicum, (j) S. album, (k) S. tenellum, (l) S. sabulatum, (m-n) S. gracile, (o) S. lenkoranicum. TSC = tanniniferous storage cell, MH = multicellular hair, GH = glandular hair. Scale bars 100 μm.
show significant anatomical differences between the 2 later species.

5. Conclusion
Our study shows anatomical traits can provide helpful data in solving the current problem of the taxonomy and nomenclature of Sedums.l. in Iran. The representatives of the genera *Phedimus*, *Prometheum*, and *Hylotelephium* can be easily delimitated from the species of *Sedum* s.s. using anatomical features. The results also support the alliance of 2 sections *Sedum* and *Epeteium* due to several identical anatomical characters described within the sections. An identification key was provided based on the most important diagnostic characters:

1. Tanniniferous storage cells in stem and leaf are present; xylem vessels in stem are distinct; peduncle cross-section is circular with 3 wings ........................................ 2
   - Tanniniferous storage cells in stem and leaf are absent; xylem vessels in stem are continuous; peduncle cross-section is not circular with 3 wings ...................... 4
2. Starch storage cells in stem are present .................. ................................. 3
   - Starch storage cells in stem are absent .......................... 4
3. Stem with stomata ......................... *Ph. obtusifolius*
   - Stem without stomata .......................... *Ph. spurius*
4. Stem and peduncle have 2 to 3 collenchyma layers; starch storage cells are present in peduncle .............. .......................................................... *H. caucasicum*

![Figure 6. Cross-sections of leaf. (a) S. tetramerum, (b) S. pallidum, (c) S. hispanicum, (d) S. pentapetalum, (e) S. rubens, (f-g) S. caespitosum, (h) S. kotschyanum, (i) S. annum, (j) S. elbursense. Scale bars 100 μm.](image)
- Stem and peduncle have 1 collenchyma layer; starch storage cells are absent in peduncle ........................................ 5
5. Stem without endodermis, stem have 2 collenchyma layers ................................................................................. 6
- Stem with endodermis, having 1 collenchyma layer ........................................................................................................ 7
6. Peduncle with thick cortex (380–470 μm) .................. S. album
- Peduncle with thin cortex (320.4–453 μm) .................. P. pilosum
7. Collenchyma layer in peduncle is absent, peduncle cross-section is stellate ......................................................... S. annum
- Collenchyma layer in peduncle are present, peduncle cross-section is circular/undulating circular or circular with 2 wings .............................................................. 8
8. Midrib region is clear ................................................... S. pallidum
- Midrib region is unclear ................................................. S. tenellum
9. Leaf cross-sectionis flat, stem endodermis layer 16.9–20.6 μm .............................................................. S. elbursense
- Leaf cross-section is convex; stem endodermis layer thinner (11.6–14.5 μm) .......................................................... 10
10. Stem contains stomata; peduncle cross-section is circular in shape ................................................................. S. tenellum
- Stem lacks stomata; peduncle cross-section is undulating circular in shape .......................................................... 11
11. Stem cross-section is undulating circular in shape ......................................................................................................... 13
12. Stem glabrous; leaf vascular bundles are circular in shape ................................................................. S. nanum
- Stem hairy; leaf vascular bundles are crescent in shape .............................................................................................. 14
13. Leaf with hypodermis; stem contains starch storage cells ........................................................................... S. album
- Leaf without hypodermis; stem does not contain starch storage cells .............................................................. 15
14. Peduncle glabrous and with distinct vascular bundles .................................................................................. S. tetraderanum
- Peduncle hairy and with continuous vascular bundles ................................................................................. S. rubens
15. Stem without collenchyma; adaxial epidermal cells = abaxial ................................................................. S. caespitosum
- Stem with collenchyma; abaxial epidermal cells > adaxial ..................................................................................... S. gracile
16. Leaf cross-section is semicircular, leaf vascular bundle is crescent in shape .................................................. S. album
- Leaf cross-section is flat, leaf vascular bundle is circular in shape ........................................................................ S. annum
17. Stem cross-section is circular with 2 wings, cortical bundles are present in stem .......................................................... S. tetraderanum
- Stem cross-section is circular, cortical bundles are absent in stem .................................................................... S. tenellum
18. Peduncle cross-section is circular with 2 wings; stem contains starch storage cells .......................................................... S. elbursense
- Peduncle cross-section is undulating circular; stem lacks starch storage cells .......................................................... S. elbursense
19. Stem lacks pith region, endodermis layer is thin (4.5–6.8 μm in thickness) ................................................ S. callichroum
- Stem with pith region; endodermal layer is thick (12.5–14.1 μm in thickness) ................................................... S. tetramerum
20. Stem with cortical bundles; peduncle hairy ........................................................................................................ S. pallidum
- Stem without cortical bundles; peduncle glabrous ...................................................................................... S. kotschyanum

Figure 7. Unweighted pair-group method with arithmetic averages (UPGMA) dendrogram. R represents the examined individuals R1–R3 = Ph. stoloniferus, R4–R5 = Ph. spurius, R6 = Ph. obtusifolium, R7–R8 = P. pilosum, R9–R10 = P. sempervivoides, R11–R12 = H. caucasicum, R13–R15 = S. album, R16–R17 = S. tenellum, R18 = S. sabulatum, R19–R20 = S. gracile, R21–R22 = S. lenkoranicum, R23–R25 = S. tetraderanum, R26–R28 = S. pallidum, R29–R31 = S. hispanicum, R32–R34 = S. pentapetalum, R35–R36 = S. Rubens, R37–R38 = S. caespitosum, R39–R40 = S. kotschyanum, R41 = S. callichroum, R42–R43 = S. nanum, R44–R45 = S. annum, R46 = S. elbursense; (for more details see Table 1).
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