

Comparative morphological and anatomical investigations on *Corydalis caucasica* subsp. *abantensis* Lidén and *Corydalis integra* Barbey & Fors.-Major (Papaveraceae) from Turkey

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ABSTRACT: In this study morphological and anatomical features of *Corydalis caucasica* subsp. *abantensis* and *Corydalis integra* which spreading naturally in the same area in Turkey were investigated. The morphological features that can be used to distinguish these two taxa from each other are given in a comparative table. Morphological data were obtained by using herbarium samples collected from different localities and their measurements were given comparatively. In anatomical study, cross and superficial sections from the leaves and cross sections from the stem and roots of the samples were taken and examined under the light microscope; thereby the anatomical structures were determined. Determined anatomical structures were measured and the data obtained were written comparatively. As a result, the morphological features of these two taxa were examined and information that would facilitate identification of these taxa was obtained. The anatomical properties of these two taxa, which were not encountered in any study before, have been elucidated for the first time.

KEYWORDS: Papaveraceae; *Corydalis*; anatomy; morphology; Turkey.

1. INTRODUCTION

The genus *Corydalis* DC. belongs in the family Papaveraceae. According to the latest information, 529 *Corydalis* species, naturally occurring in temperate regions in the northern hemisphere, have been recorded [1]. All taxa of the genus *Corydalis* are geophytes, they can be annuals or perennials, with tap roots, tubers or rhizomes. According to recent data, in Turkey 22 *Corydalis* taxa are naturally growing and 8 of them are endemic [2].

Like many members of the Papaveraceae family, *Corydalis* species are used for various medicinal purposes. A number of alkaloids (of which protopine and bulbocapnine are among the most important) are responsible for the medicinal powers of several of the tuberous species. In pure form they have antihypertensive, sedative and myorelaxant effects. *C. cava* is still used locally as a cure for Parkinson's disease, neurological aberrations, vertigo, and muscle tremor [3]. The Chinese Yanhusuo, the boiled and dried tubers of *C. yanhusuo* and several other tuberous species, 'one of the most famous chinese herbal drugs' is an analgesic and antiarrhythmic drug used for a variety of indications, including coronary heart disease [3,4]. *C. solida* (L.) Clairv tubers in Anatolia are used as emmenagogue, anthelmintic, and as strengthener in tuberculosis [5]. In recent studies, it has been reported that some *Corydalis* species can be used against Alzheimer's disease. It has been proven that isoquinoline alkaloids isolated from various *Corydalis* species have acetylcholinesterase inhibitory effects [6-9].

C. caucasica subsp. *abantensis* Lidén is endemic to Turkey [10]. Floristic studies in recent years have shown that this new subspecies, defined in Bolu region, is actually spreading to wider areas from Çanakkale to Kastamonu [11-14]. *C. integra* Barbey & Fors.-Major is a widespread species and its native range is S. Bulgaria to NW. Iran [14-17]. The distribution areas of these two taxa overlap in some regions of Turkey (Figure 1). In Flora of Turkey and East Aegean Islands were stated that these two taxa were confused with each other [18-19].

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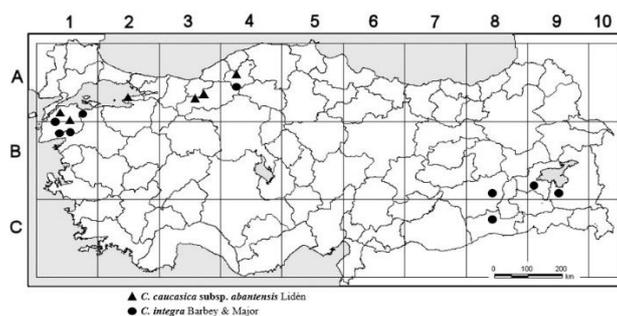


Figure 1. Distribution map of *C. caucasica* subsp. *abantensis* and *C. integra* in Turkey.

The comparative study of plant structure, morphology and anatomy, has always been the basis of plant systematics, which endeavours to elucidate plant diversity, phylogeny and evolution. Plant anatomy studies of fresh materials have played an important historical role in identifying and classifying medicinal plants [20]. Some comparative anatomical studies have shown that anatomical structures can play a role in accurate identification among *Corydalis* species [21-24].

It is important to reveal the morphological and anatomical differences and distinctive features of medicinal plants in terms of correct identification. The aim of this study is to reveal the differences by examining the morphological and anatomical aspects of two medicinal important *Corydalis* species, which are similar to each other and have been confused before.

2. RESULTS AND DISCUSSION

2.1. Morphological characteristics

2.1.1. Descriptions

C. caucasica subsp. *abantensis* Lidén: Tuber simple, \pm spherical, 8-15 \times 9-13 mm. Stems suberect 10-20 cm, with one yellowish scale leaf at the base. Leaves 1 (-2) \times ternate, the segments broadly ovate, palmatifid into oblong to narrowly obovate laciniae, petioles not sheathing. Inflorescence lax, 5-15-flowered, Corolla 22-32 mm, spur almost straight, lower petal broadly truncate, bracts entire, narrowly elliptic to narrowly obovate, semiamplexicaul, exceeding the fruiting pedicels. Lower petals broadly truncate inner petals only internally tipped with purple. Flowers purple- pink. Sepals indistinct. Capsule very narrowly elliptic to very narrowly obovate to almost linear, 6-11 \times 1-2 mm. Seeds up to 2 mm in diameter. Endemic.

C. integra Barbey & Fors.-Major: Tuber \pm spherical, 10-27 \times 15-26 mm, globose, undivided. Stems ascending to erect, occasionally branched, 9-20 cm, with one yellowish scale leaf at the base. Cauline leaves 2, alternate, biternate, the segments \pm ovate, palmatifid into narrowly obovate laciniae. Inflorescence dense, 8-22-flowered, Corolla 12-24 mm; spur strongly curved bracts entire, narrowly elliptic, semiamplexicaul. Lower petals with a narrow claw gradually dilated into a broad, usually emarginate limb, inner petals externally tipped with dark purple. Flowers pale purple. Sepals indistinct. Capsules linear, 16-19 \times 2 mm. Seeds up to 2 mm in diameter.

According to Lidén [10] *C. caucasica* subsp. *abantensis* was confused with *C. integra* from which it differs in the broadly lanceolate (not linear- oblong) fruit, absence of a dark apex to the inner petals, and the form of the lower petal (Table 1). According to Davis and Cullen [18] *C. caucasica* (from Bolu) is similar to *C. integra* except for large petals (Figure 2, Figure 3).

2.2. Anatomical characteristics

2.2.1 Anatomical properties of root

C. caucasica subsp. *abantensis*: Located as the outer protective tissue periderm consist of 2 to 3 layers of cells, round to oval with an average width of 13.05 μ m and a length of 18.31 μ m. It consists of shaped, cork cells. Epiderma cannot be distinguished because it is broken down and replaced by periderm. The cortex consists of 6-7 layers of large thin-walled parenchyma cells with an average width of 23.35 μ m and a length of 28.21 μ m. Endoderma is consists of single layer of parenchyma cells. Pericycle is consists of only one layer of parenchyma cells. Central cylinder is covered with phloem and xylem. Phloem cells are observed very intensively. Some protoxylems are connected by a row of distinctive small tracheids. Protoxylem cells consists of rounded or elliptic elements and they extend radially to the tip of the arms of the xylem. The lumen of tracheary elements of the metaxylem is larger than the protoxylem elements. The tracheary elements have an average width of 5.17 to 16.85 μ m and a length of 5.82 to 20.38 μ m (Figure 4A).

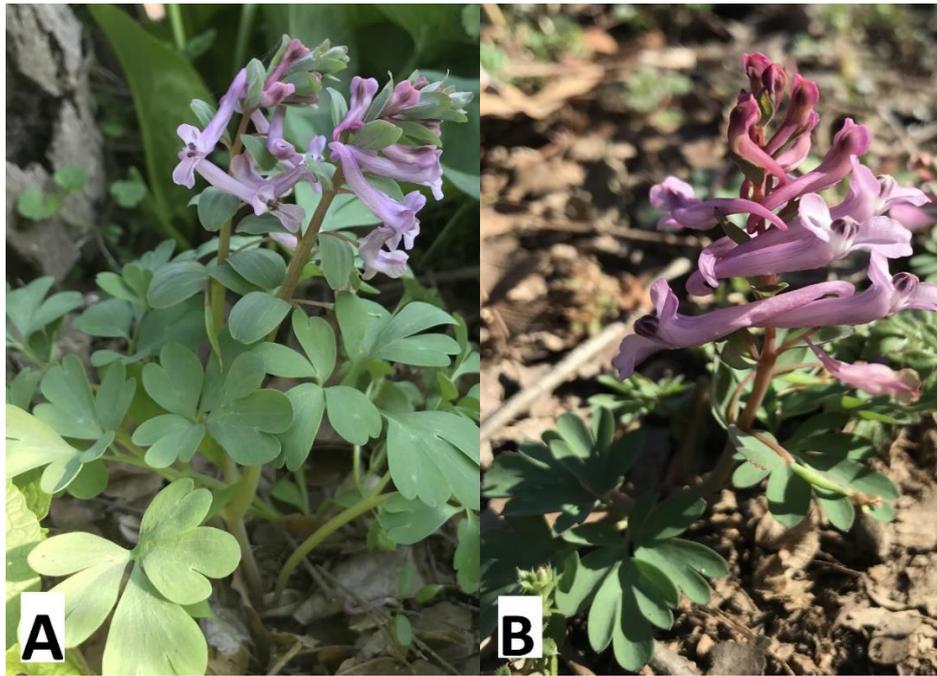


Figure 2. General view of the plants; A *Corydalis caucasica* subsp. *abantensis*, B *Corydalis integra*.



Figure 3. Herbarium specimen from Royal Botanic Garden Edinburgh, identified as *C. caucasica*, and then corrected by P.H. Davis and M. Lidén as *C. integra* [25].

C. integra: The outermost layer contains a thick periderma tissue consisting of 3-4 rows of cells with an average width of 15.42 μm and a length of 13.13 μm . Epidermal cells cannot be distinguished in this section, too. The cortex consists of 4-5 layers of large parenchyma cells. Endoderma and pericycle are consisting of single layer of parenchyma cells with an average width of 17.85 μm and a length of 29.21 μm . Central cylinder is covered with phloem and xylem. Protoxylem consists of annular and helical elements. Metaxylem tracheids have multiseriate, circular-bordered pits. Metaxylem elements are at the center and at the edges of the xylem arms, the protoxylem elements are located radially. The lumen of tracheary elements of the metaxylem is larger than the protoxylem elements. The tracheary elements have an average width of 5.83 to 15.53 μm and a length of 7.38 to 19.39 μm . The number of the layers of parenchyma cells in cortex, the number, size and shape of tracheary elements are prominent differences between the anatomical features in the cross-sections of these two roots. (Figure 4 B).

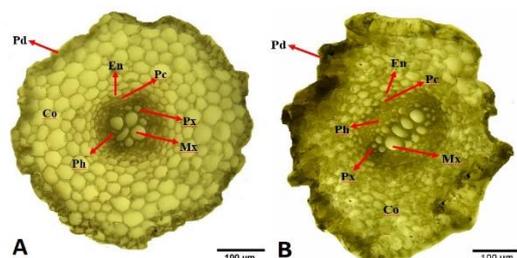


Figure 4. Cross sections from the roots of A *C. caucasica* subsp. *abantensis*, B *C. integra*, Co Cortex, Endoderma, Mx Metaxylem, Pc Pericycle Pd Periderma, Ph Phloem, Px Protoxylem

Table 1. Comparative morphological properties of the two *Corydalis* taxa according to examined specimens.

| | <i>Corydalis caucasica</i> subsp. <i>abantensis</i> | <i>Corydalis integra</i> |
|---------------|--|---|
| Tuber | Tuber \pm spherical 8-15 \times 10-18 mm | Tuber \pm spherical, 10-28 \times 13-24 mm |
| Scale leaf | Present | Present |
| Stem | Suberect, 10-25 cm | Suberect, rather robust 10-20 cm |
| Leaves | Leaves 1(-2) \times ternate, the segments broadly obovate, palmatifid into oblong to narrowly obovate laciniae | Leaves biternate, the segments \pm ovate, palmatifid into narrowly obovate laciniae |
| Inflorescence | Racemes lax 5-15- flowered | Racemes dense, 8-22- flowered |
| Corolla | 22-32 mm, spur almost straight, rarely curved | 12-24 mm; spur strongly curved |
| Inner petals | Only internally tipped with purple | Externally tipped with dark purple |
| Lower petals | Broadly truncate | With a narrow claw gradually dilated into a broad, usually emarginate limb |
| Fruit | Capsule very narrowly elliptic to very narrowly obovate to almost linear, 6-11 \times 1-2 mm | Capsule linear to narrowly lanceolate, 16-19 \times 2 mm |
| Bracts | Entire, ovate | Entire, broadly elliptic |

2.2.2. Anatomical properties of stem

Corydalis caucasica subsp. *abantensis*: At the outermost, there is an epiderma consisting of 1 layer of isodiametric cells, tangentially elongated with an average width of 6.88 μm and a length of 8.08 μm . The epidermis is covered with a very thick cuticle layer. The epidermis is followed by cortex: 12-18 layers of parenchymatous cells irregular in shape. The parenchymal cells with an average diameter of 21.6 μm that form the cortex are oval or round in shape and towards the center, the cells grow and their intercellular spaces

increase. The 5 vascular bundles are of collateral type but in different sizes. Cambium not clearly observed. Pith small, hollow in the center with an average diameter of 60.75 μm (Figure 5 A, C).

Corydalis integra: Epidermis consists of 1 layer of isodiametric cells, tangentially elongated with an average width of 6.24 μm and a length of 8.26 μm . The epidermis is covered with a very thick cuticle layer. Cortex parenchymatous: 10–15 layers of parenchymatous cells irregular in shape. The parenchymal cells with an average diameter of 15.96 μm that form the cortex are oval, round or elliptic in shape and towards the center, the cells grow and their intercellular spaces increase. The 5 big and 3–4 small vascular bundles are of collateral type. Cambium not clearly observed. Pith relatively large, hollow in the center with an average diameter of 182.3 μm . The number of the layers of parenchyma cells, the number of vascular bundles, and the size of the central hollow pith are prominent differences between the anatomical features in the cross-sections of these two stems (Figure 5 B, D).

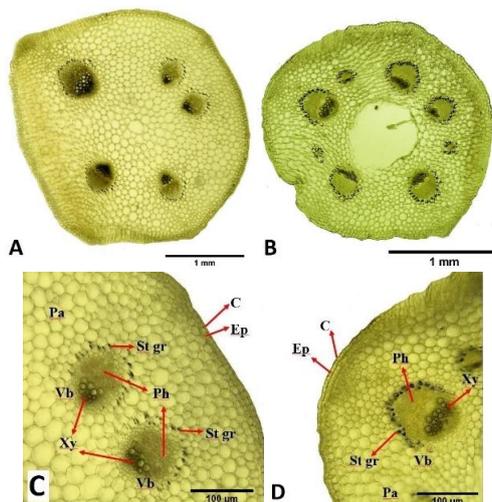


Figure 5. Anatomical structures of stem cross-sections of A, C *C. caucasica* subsp. *abantensis*; B, D *C. integra*. C Cuticle, Ep Epidermis, Pa Parenchyma, Ph Phloem, St gr Starch grain, Xy Xylem, Vb Vascular bundle.

2.2.3. Anatomical properties of leaf

Corydalis caucasica subsp. *abantensis*: When the cross section of the leaf is examined, it is seen that the upper and lower epidermis are covered with a thin cuticle. The upper epidermal cells with an average width of 9.45 μm and a length of 16.76 μm and the lower epidermal cells with an average width of 9.69 μm and a length of 17.13 μm . The mesophyll is composed of palisade parenchyma with an average width of 15.53 μm and a length of 30.11 μm an upper layer of elongated chlorenchyma cells and spongy parenchyma a lower layer of spherical or ovoid cells with an average width of 14.67 μm and a length of 18.04 μm . When the superficial section of the leaf is examined, it is seen that the leaf is amphistomatic and bifacial. It has been observed that there are stomata on both leaf surfaces and denser on the lower surface (stomatal index is 13.9534) than on the upper surface (stomatal index is 13.3333). Stomata are oval shaped and anomocytic and surrounded by 4–5 epidermal cells (Figure 6 A, C, E).

Corydalis integra: The upper and lower epidermis are covered with a thin cuticle. The upper epidermal cells with an average width of 7.48 μm and a length of 12.04 μm and the lower epidermal cells with an average width of 6.14 μm and a length of 11.08 μm . The mesophyll is composed of palisade parenchyma with an average width of 18.61 μm and a length of 31.24 μm an upper layer of elongated chlorenchyma cells and spongy parenchyma a lower layer of spherical or ovoid cells with an average width of 16.38 μm and a length of 25.79 μm . When the superficial section of the leaf is examined, it is seen that the leaf is amphistomatic and bifacial. It has been observed that there are stomata on both leaf surfaces and denser on the lower surface (stomatal index is 13.1868) than on the upper surface (stomatal index is 12.8205). Stomata are oval shaped and anomocytic and surrounded by 4 epidermal cells. The fact that the number of stomata in the upper epidermis exposed to direct light is less than the number of stomata in the lower epidermis is a normal finding for regulating sweating [26]. The size of palisade parenchyma cells, number and size of stomata on upper and lower surfaces of the leaves, shape of epidermal cells are prominent differences seen in the cross and superficial sections these two leaves (Figure 6 B, D, F).

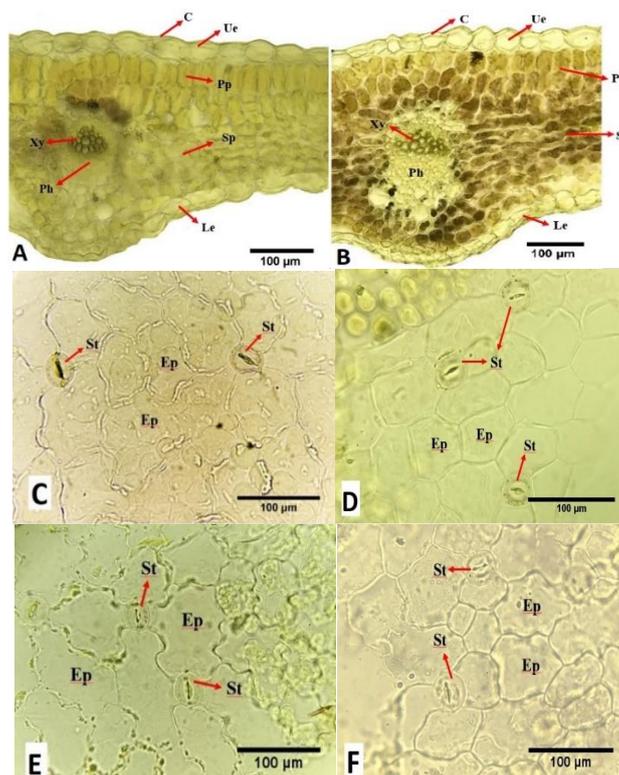


Figure 6. Cross section from the leaf of A *C. caucasica* subsp. *abantensis*, B *C. integra*. Superficial sections from the upper surface of the leaf of C *C. caucasica* subsp. *abantensis*, D *C. integra*. Superficial sections from the lower surface of the leaf of E *C. caucasica* subsp. *abantensis*, F *C. integra*. C Cuticle, Ep Epiderma, Le Lower epiderma, Ph Phloem, Pp Palisade parenchyma, Sp Spongy parenchyma, St Stoma, Ue Upper epiderma, Xy Xylem.

3. CONCLUSION

The anatomical structures of *C. caucasica* subsp. *abantensis* and *C. integra* have been elucidated for the first time with this study. The morphological and anatomical features of these two taxa that were confused with each other were examined and compared. Morphologically features like: the length of spurs, leaf shape, shape of bracts and how the inner petals are tipped, are important to distinguish these to taxa. Apart from the description in the flora of Turkey, it has been observed that in some members of *C. caucasica* subsp. *abantensis* stem length can reach 25 cm in size, rarely spurs can be strongly curved, and in some members upper petals can be deeply emarginate. In anatomical studies, the number of vascular bundles, the size of the hollow pith seen in the cross section of the stem, and the shapes of the epidermal cells differ (Table 2,3). Illuminated anatomical structures indicate that the anatomical features of the *Corydalis* taxa may also play an important role in taxonomy.

4. MATERIALS AND METHODS

Plant samples of *C. caucasica* subsp. *abantensis* and *C. integra* were collected from Çanakkale and Yalova districts in Turkey in field works conducted in 2019 and 2020. The collected plant samples were identified according to the Flora of Turkey and the East Aegean Islands Vol I and vol XI. Prepared voucher specimens were kept in Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE). For morphological investigations, specimens from ISTE herbarium, collected from different localities were examined (Table 4). For anatomical investigations plant materials collected from 2 different localities for each taxa stored in 70% ethanol, and then all cross sections and superficial sections were cutted by hand with razor blade. Roots and stems were examined by taking cross sections. Upper and lower surfaces of the leaves were examined by taking superficial sections. All sections were examined in Sartur Reagent [27,28] by light microscope, Olympus BH-2. Photographs were taken with a light microscope and measurements were made with ImageJ© program. Stomatal indices were calculated by formula: $SI (\%) = (S/S+E) \times 100$ where, S is number of stomata per unit area E is number of epidermal cells per unit area. For anatomical descriptions, the terminology proposed by Metcalfe & Chalk (1957) is followed [29].

Table 2. Anatomical measurements of *C. caucasica* subsp. *Abantensis*.

| | | Width(μm) | | | Length(μm) | | |
|-------------|----------------------------|------------------------|-------|------------------|-------------------------|-------|------------------|
| | | Min | Max | Mean \pm S.D. | Min | Max | Mean \pm S.D. |
| Root | Periderm cells | 8.52 | 18.18 | 13.05 \pm 3.44 | 9.37 | 23.48 | 18.31 \pm 9.23 |
| | Parenchyma cells | 9.36 | 32.51 | 23.35 \pm 9.02 | 13.22 | 37.47 | 28.21 \pm 8.91 |
| | Protoxylem cells | 3.30 | 7.62 | 5.17 \pm 1.58 | 3.85 | 7.71 | 5.82 \pm 1.30 |
| | Metaxylem cells | 9.34 | 23.69 | 16.85 \pm 5.97 | 10.47 | 25.22 | 20.38 \pm 5.81 |
| Stem | Epidermis cells | 6.04 | 7.71 | 6.88 \pm 0.62 | 6.20 | 10.52 | 8.08 \pm 1.56 |
| | Parenchyma cells(diameter) | 8.85 | 34.32 | 21.60 \pm 8.87 | | | |
| | Pith(diameter) | 58.12 | 61.45 | 60.75 \pm 1.81 | | | |
| Leaf | Upper epidermis cells | 7.87 | 11.25 | 9.45 \pm 1.17 | 13.42 | 21.26 | 16.76 \pm 2.64 |
| | Lower epidermis cells | 6.36 | 13.25 | 9.69 \pm 2.24 | 10.56 | 24.64 | 17.13 \pm 4.83 |
| | Palisade parenchyma cells | 13.28 | 18.82 | 15,53 \pm 1.90 | 26.57 | 35.43 | 30.11 \pm 3.18 |
| | Spongy parenchyma cells | 13.83 | 15.52 | 14,67 \pm 0.62 | 14.94 | 21.58 | 18.04 \pm 2.17 |

S.D.: Standart Deviation

Table 3. Anatomical measurements of *C. integra*.

| | | Width(μm) | | | Length(μm) | | |
|-------------|----------------------------|------------------------|-------|------------------|-------------------------|-------|------------------|
| | | Min | Max | Mean \pm S.D. | Min | Max | Mean \pm S.D. |
| Root | Periderm cells | 8.81 | 21.49 | 15.42 \pm 4.66 | 10.47 | 23.21 | 13.13 \pm 2.49 |
| | Parenchyma cells | 10.47 | 23.14 | 17.85 \pm 5.12 | 12.12 | 29.21 | 29.21 \pm 8.91 |
| | Protoxylem cells | 3.85 | 8.81 | 5.83 \pm 1.76 | 5.51 | 9.92 | 7.38 \pm 1.58 |
| | Metaxylem cells | 9.92 | 20.94 | 15.53 \pm 3.72 | 12.13 | 25.35 | 19.39 \pm 4.71 |
| Stem | Epidermis cells | 5.51 | 7.16 | 6.24 \pm 0.68 | 6.06 | 11.57 | 8.26 \pm 1.87 |
| | Parenchyma cells(diameter) | 8.32 | 23.83 | 15.96 \pm 5.26 | | | |
| | Pith(diameter) | 174.3 | 195.4 | 182.3 \pm 5.26 | | | |
| Leaf | Upper epidermis cells | 5.71 | 9.12 | 7.48 \pm 1.12 | 10.14 | 13.35 | 12.04 \pm 1.12 |
| | Lower epidermis cells | 4.16 | 8.89 | 6.14 \pm 1.65 | 8.13 | 14.24 | 11.08 \pm 2.19 |
| | Palisade parenchyma cells | 14.39 | 24.42 | 18,61 \pm 3.31 | 26.01 | 36.41 | 31.24 \pm 3.57 |
| | Spongy parenchyma cells | 13.28 | 18.82 | 16,38 \pm 2.33 | 22.14 | 33.21 | 25.79 \pm 3.89 |

S.D.: Standart Deviation

Table 4. Examined specimens from ISTE herbarium.

| Species | ISTE number | Grid | City | Species | ISTE number | Grid | City |
|---|-------------|-------|-----------|-------------------|-------------|-------|------------|
| <i>C. caucasica</i> subsp. <i>abantensis</i> | 65749 | A4 | Bolu | <i>C. integra</i> | 62631 | A1(A) | Çanakkale |
| | 21359 | A3 | Bolu | | 41769 | C8 | Mardin |
| | 57849 | A3 | Bolu | | 34449 | B8 | Diyarbakır |
| | 101131 | A2(A) | Yalova | | 67352 | B1 | Balıkesir |
| | 116562 | A2(A) | Yalova | | 117063 | A1(A) | Çanakkale |
| | 117058 | A1(A) | Çanakkale | | 117076 | A1(A) | Çanakkale |

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