

ANATOMICAL NOTES ON *Vinca soneri* KOYUNCU (APOCYNACEAE) IN TÜRKİYE

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Abstract

The genus *Vinca* L. (Apocynaceae), which is native to Europe, north-west Africa and south-west Asia is represented by five species in Türkiye. It includes deciduous perennial subshrubs, low creeping evergreens, and herbs that are not assessed based on their anatomical characteristics. The goal of the current study was to examine *Vinca soneri* Koyuncu's anatomical characteristics for the first time. A rotary microtome or hand was used to cut the transverse sections, and paraffin sections were stained using the safranin-fast green staining method. Handmade sections were dyed using HCL and floroglucin. Chosen best sections were photographed by a light microscope and the measurements were made using Kameram programme. *V. soneri* had rounded shaped stems in cross-section. The epidermal layer of species was consisting of a single row of oval-rectangular shaped cells. The collenchymatic layer was encloses stem in three or four in row and the vascular bundles were designed in a single rowed. There is a pith cavity in the center of stem in cross sections. The shape of leaves was determined as linear in type. The stomata type of *V. soneri* was anomocytic. In conclusion, the anatomical characters of the stem, leaf and petiole were examined in detail and their differences from other *Vinca* species were revealed.

1. INTRODUCTION

The family Apocynaceae is represented by 378 genera and 5350 species in the world [1] in major biomes, except arctic tundra, and especially in dry and humid tropics [2-7]. This vast pantropical plant family is distinguished by a wide range of growth forms, from 80-meter-tall tropical trees to smaller trees and shrubs, lianas, succulents, and herbs. Members of the genus *Vinca* L., is a small plant group in Apocynaceae, are evergreen or deciduous perennial subshrubs or herbs. With seven distinct species, they can be found in Europe, north-west Africa, and south-west Asia. These species; *V. ispartensis* Koyuncu & Eksi, *V. difformis* Pourr., *V. herbacea* Walld. & Kit., *V. erecta* Regel & Schmalh., *V. major* L., *V. minor* L. and *V. soneri* Koyuncu. Five of the species are distributed in Türkiye. One of these species, in 2012, the woody rootstock of *Vinca soneri*, an endemic

perennial subshrub, was described [8]. Many *Vinca* species have long been used to treat diabetes and for their sedative, hemostatic, and antihypertensive effects [9]. Although phenolic acids, iridoids, flavonoids, and lignans have also been isolated from the genus *Vinca*, it is known to contain primarily alkaloids [10,11]. *Vinca soneri* was evaluated by its phenolic and volatile compounds [12], palynologically [13], and morphologically comparing with close relative *V. herbaceae* [8] but no anatomical study was conducted. The aim of this study is to present an anatomical investigation of *Vinca soneri* and to contribute to the literature.

2. MATERIALS AND METHODS

Some of the plant specimens were placed in plastic bottles filled with 70 ethanol after being collected from the species' type locality (Sivas: between Ulaş and Zara, around Değirmenboğazı village, dry stony slopes, 1490 m B. Çıtak-168a). For light microscope (LM) observations aid of Leica DM1000 light microscope equipped with Canon EOS 450D camera, 100× or 400× magnifications, cross sections of the stem and leaf were obtained using the paraffin method obtained [14,15]. Using a Thermo Scientific Shandon Finesse 325 rotary microtome, these plant parts were dehydrated in increasing ethanol series (70%–100%), embedded in paraffin blocks, and cut into 10–12 µm thicknesses. Safranin-fast green was used to stain the cross-sections, and some were manually cut with a razor blade. The process described in [15]'s was used for the handmade sections. The cell groups were measured at least 30 times, and each type of section of the 20 pieces of plant material received an average of 20 preparations (Table 1).

The leaves' superficial sections were used to obtain stomata sections, and the stomatal index (SI) was calculated according to the formula of Salisbury (1927) where *S* is the number of stomata per mm² in leaf area and *E* is the number of epidermal cells per mm² in leaf area [16].

$$\text{Stomatal index (SI)} = (\text{Stomata (S)} / (\text{Stomata (S)} + \text{Epidermis (E)}) \times 100$$

$$\text{Stomatal index percentage (\%)} = \text{Upper stomatal index} / \text{Lower stomatal index}$$

3. RESULTS AND DISCUSSION

3.1 Stem anatomy

Four anatomical parts stood out in the anatomical sections of the stems that count as epidermis, cortex, vascular bundles, and pith region from inner to outer.

The epidermal layer enclosed the stem with a single row of oval-rectangular shaped cells. Underneath the epidermis, the collenchyma layer was enclose stem in three or four in row. The cortex layer, consisting of parenchymatous cells, consisted of seven or eight rows of oval-shaped cells. The vascular bundles were arranged in a single row and without interruption on the stem. Phloem and xylem occupied same area in the stem. The pith was composed of parenchyma cells with starch grains (Figure 1).

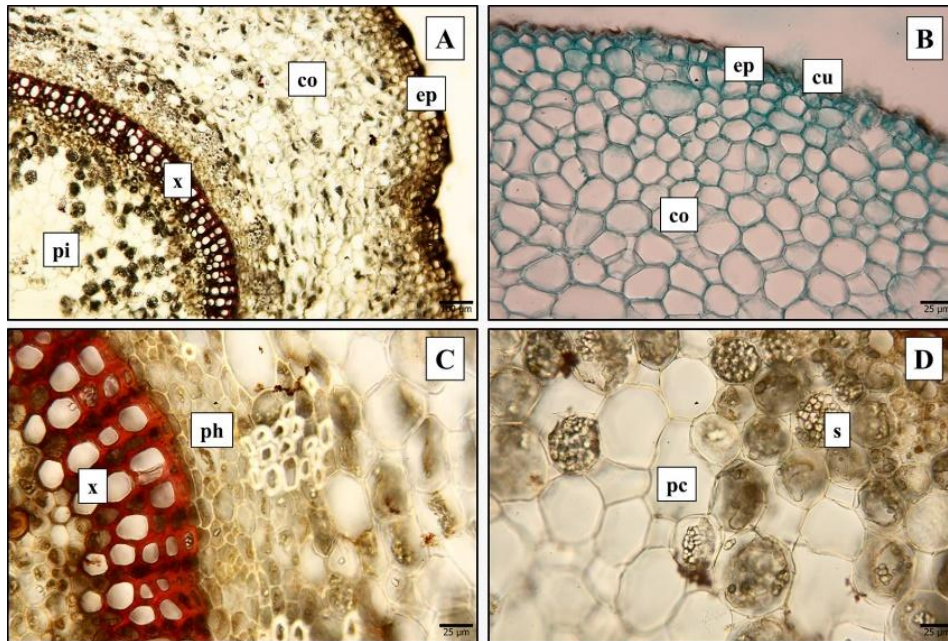


FIGURE 1. The microphotographs of stem of *V. soneri* **ep**: epidermis, **cu**: cuticle, **co**: cortex parenchyma, **ph**: phloem, **x**: xylem, **pi**: pith region, **pc**: pith cell, **s**: starch.

3.2 Leaf anatomy

The cross-sections taken from leaves showed three main anatomical parts: epidermis, mesophyll, and vascular bundles. The epidermis enclosed the leaves both upper and lower surface. The shape of leaves was determined as linear in type. The palisade and spongy parenchyma were placed in mesophyll tissue as dorsiventral type. The thickness of mesophyll was measured 223 µm (Table 1). The collateral type vascular bundles were sunken between spongy parenchyma cells (Figure 2).

TABLE 1. The measurements of anatomical cells of *Vinca soneri*.

Vegetative/Cells		Length (µm)			Width (µm)		
		Min	Max	Mean	Min	Max	Mean
Stem	Epidermis	9.5	14.98	12.22 ± 1.41	6.02	15.51	12.12 ± 3.12
	Collenchyma	-	-	-	18.43	29.4	22.41 ± 3.34
	Cortex	-	-	-	18.26	31.91	24.02 ± 3.41
	Trachea	-	-	-	14.52	22.88	19.58 ± 2.67
	Pith region	-	-	-	20.72	35.38	27.73 ± 4.51
Leaf	Upper epidermis	34.88	56.69	41.94 ± 8.53	20.61	28.94	24.35 ± 3.04
	Lower epidermis	12.32	26.94	19.35 ± 7.32	7.29	9.47	8.02 ± 0.98
	Palisade parenchyma	52.82	86.09	68.25 ± 9.38	5.11	10.91	8.29 ± 1.63
	Spongy parenchyma	76.56	94.2	85.75 ± 6.34	-	-	-
	Mesophyll tissue	217.01	228.5	223.18±4.03	-	-	-
Petiole	Epidermis	8.84	14.63	11.73 ± 1.77	6.85	15.14	10.61 ± 2.68
	Collenchyma	-	-	-	13.55	28.26	19.34 ± 3.79
	Cortex	-	-	-	19.68	28.99	24.65 ± 3.12
	Vascular bundles	-	-	-	11.94	26.85	18.56 ± 3.73
	Pith region	-	-	-	17.71	32.51	25.25 ± 5.05

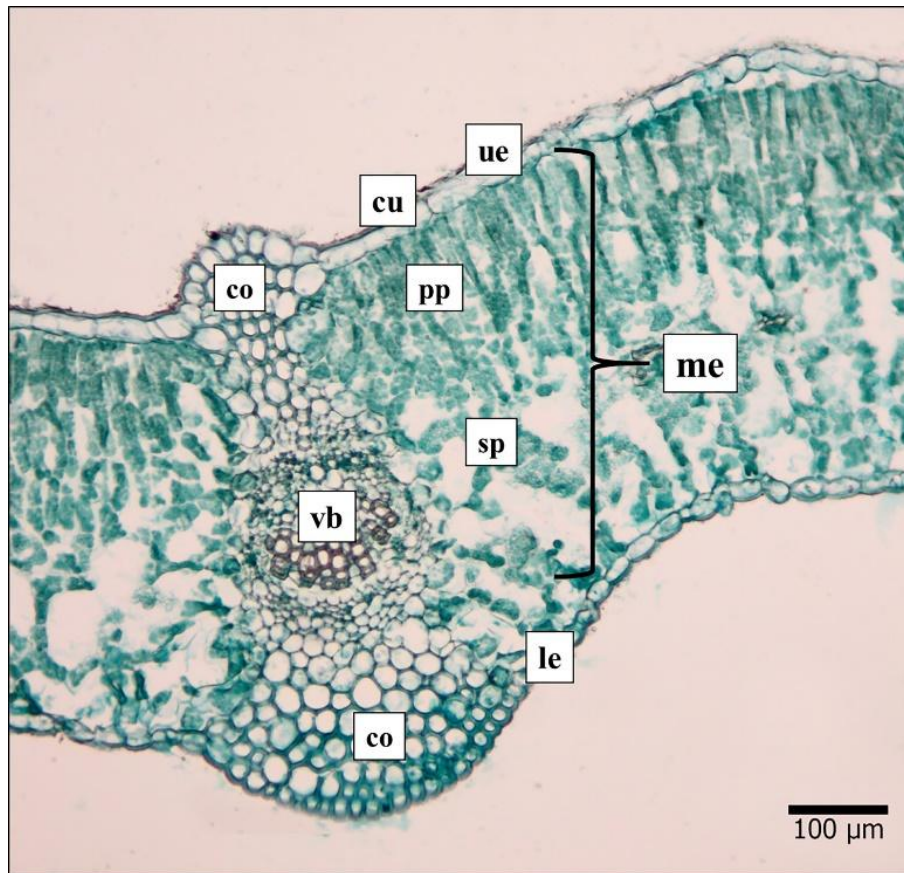


FIGURE 2. The microphotograph of cross section of leaves in *V. soneri*.

co:collenchyma, **cu:** cuticle, **le:** lower epidermis, **me:** mesophlly **pp:** palisade parenchyma, **sp:** spongy parenchyma, **ue:** upper epidermis, **vb:** vascular bundle,

3.3 Petiole anatomy

Four anatomical tissue were determined in petioles as epidermis, cortex, vascular bundles, and pith region. The epidermal layer of petiole was a single row of oval-rectangular shaped cells. The collenchyma layer was limited in four or five in row. The cortex was composed of 6 layers of parenchymatic cells. The vascular bundles were designed in a single rowed. Phloem occupies in a small area in the stem while xylem occupies a large area. The pith was composed of parenchymatic cells with starch grains (Figure 3).

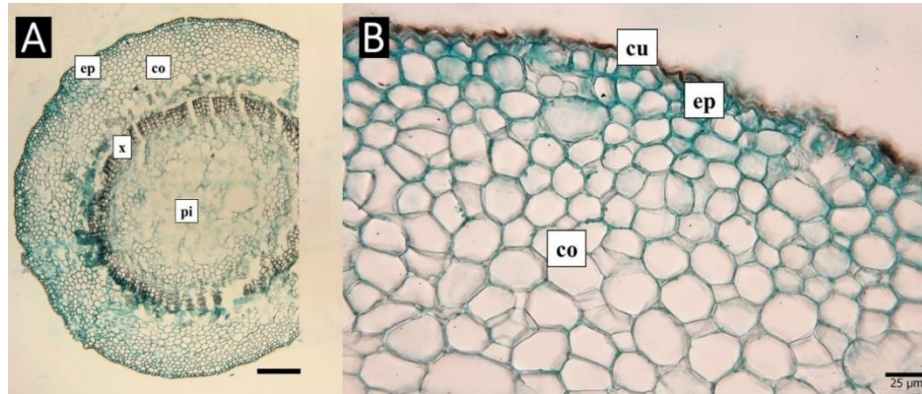


FIGURE 3. The photographs taken from the petiole. (A) the general view of petiole (scale bar= 200 µm) (B) the close view of petiole, **ep**: epidermis, **co**: cortex parenchyma, **cu**: cuticle, **x**: xylem, **pi**: pith region.

3.4 Stomata characteristics

In superficial sections, stomata were observed on both surfaces of the leaf. Therefore, the leaves were determined as amphistomatic. The stomata type is anomocytic (Figure 4). Stomatal index was calculated as 22% and the species was found to be xerophytic.

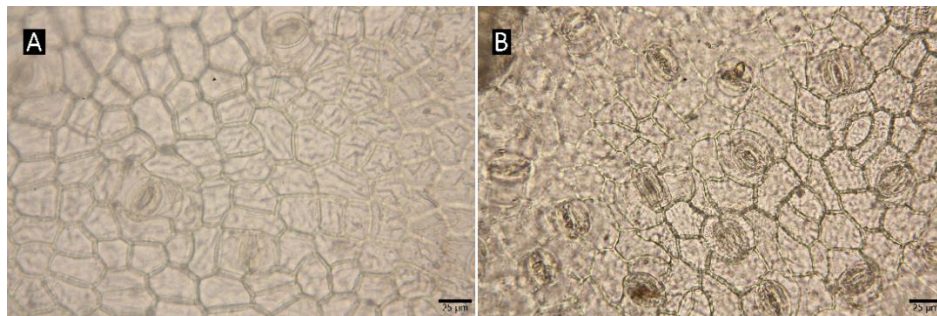


FIGURE 4. The superficial section of (A) upper surface and (B) lower surface of *V. soneri*.

4. CONCLUSIONS

In current study, the anatomical properties of the *V. soneri* was presented for the first time and the results obtained were supported the discrimination of close species of genus. The cross sections of stems of *V. soneri* had a round shape while *V. minor* was round, and *V. herbacea* was characterized by tapered, curved portions at the ends of the oval-shaped opposite ribs [17]. Moreover the pith cells of stem of *V. soneri* was composed of parenchymatic cells with starch grains. *V.*

soneri had dorsiventral mesophyll type that average thickness of the mesophyll tissue was measured as 223.18 μm (Table 1). In a previous study, *V. minor* was 210 μm [18] that differ from *V. soneri*. Parenchyma dimensions could be used to separate species that the length of palisade parenchyma of *V. soneri* was measured 68.25 μm (Table 1) while *V. minor* was 32.19 μm [18]. The area of spongy parenchyma covered was changeable 85.75 μm in *V. soneri* (Table 1) and as 135.57 μm in *V. major* [19]. The cross sections of shapes of petiole of *V. soneri* were found round shape. *V. minor* has relatively small arches on the ribs and lobes, whereas *V. herbacea* is distinguished by elongated lobes and cone-shaped ribs. Round cells with a thin sheath and dense structure make up the main tissue of the petiole of *V. herbacea* and *V. minor* species. Idioblast cells, complex grains of starch and chlorophyll, and raphides are found in the parenchyma of major tissues [17]. However, these structures had not been observed in the *V. soneri* like above mentioned species. It was determined that the stomata type of *V. minor* was diacytic [17], while the stomata type of *V. soneri* was anomocytic.

In conclusion, based on this study, it was concluded that stem, leaf and petiole anatomical characters can be used to distinguish species of the *Vinca* genus.

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Author Contribution Statements Both authors contributed equally conducted and written to the study.

Declaration of Competing Interests The authors declare no conflict of interest.

Ethical Statement This research did not involve human participants or animals. Therefore, no ethical approval was required.

Use of Artificial Intelligence No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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