

Anatomy of *Paliurus spina-christi* Mill. (Blackthorn) (Rhamnaceae)

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ABSTRACT

Objective: This investigation aimed to explore the anatomical structures of the stem, leaf, petiole, petal, and fruit of *Paliurus spina-christi*.

Methods: Plant specimens were collected from Uzundere/Erzurum (Turkey) in June 2016. Subsequently, standard herbarium techniques were employed to dry and preserve the samples stored at the Herbarium of Atatürk University, Biodiversity Application and Research Center. For anatomical analysis, the materials were preserved in 70% alcohol. Characteristic elements of these plant parts were identified through sectioning, and their structures were visually documented with photographs.

Results: The leaf is bifacial, with stomata located in the lower epidermis. Abundant cluster crystals of calcium oxalate, very dense, and unicellular trichome (only stem, leaf, petiole) were shown in stem, leaf, petiole, and petal. Lignified structures were observed in the samara fruits pericarp.

Conclusion: This research thoroughly delineates the anatomical characteristics of *P. spina-christi*. The findings derived from this investigation imply that the observed anatomical variations could have valuable implications for taxonomic classification.

Keywords: Anatomy, *Paliurus spina-christi*, Rhamnaceae

INTRODUCTION

Paliurus spina-christi Mill. belongs to the Rhamnaceae family and is represented by a single species in Turkey. *Paliurus spina-christi* Mill. is known as “karaçalı” or “çaltı dikenli” in our country. It is grown naturally in the south of Europe, Turkey, Crimea, Caucasus, Syria, Iran, and Iraq.^{1,2} The plant is a spiky shrub that grows to a height of 2-4 meters. The fruits of the plant are used in Turkish folk medicine as antidiabetic, diuretic, constipating, and stone-lowering agents. Moreover, it is known that it has been used in the past against eye diseases.^{3,4} Flavonoids, tannins, amino acids, alkaloids, and sterol content, in addition to the antibacterial and antioxidant activities of this species, have been reported in previous studies.⁴⁻⁷

Paliurus spina-christi is used among people as an anti-diarrheal, diuretic, and remedy against rheumatism. In addition, the samara-type fruits of the plant are used as an anti-inflammatory against kidney stones, chest infections, and eye infections, and the leaves are used externally for boil inflammations. In the Kastamonu region, it is known that the decoction prepared from ripe fruits is used in cases of respiratory failure. The fruits have a constipating and diuretic effect. They are thorny shrubs with yellow, small flowers in May–July, 2-4 m tall, deciduous in winter, and rarely up to 5 m tall. Young branches, axillary buds, petioles, leaf main veins, and pedicels \pm dense brown short soft hairs, mature branches \pm bare, often \pm strongly curved. The branches are thin and weak. The stipules took the form of spines, and the spines were 2 in each nodule; the long one is straight and about 2 cm long, and the short one is curved like a hook. Petioles are 3-13 mm long. The leaves are symmetrical to asymmetrical, ovate to elliptic, 2-4 cm long, 1.5-3.5 cm wide, leaf base obtus to cordate variable, apex obtuse, thin, and paper-like structure. Flowers glabrous, 3-6 mm in diameter, pedicels 4-8 mm long, receptaculum disc-shaped. Fruits are circular, bulging in the middle, winged, 3-seeded, and a dry samara. It is an invasive, cheery plant that often forms impenetrable woodlands on alluvial soils. This species can be found in a wide range of habitats, including sparse oak forests, shrublands, heathlands, scrubby forests, valley slopes, gorges, river valleys, cleared forest areas, degraded forested areas, and even vacant lands. It can be found at altitudes ranging from 1500 to 2300 m above sea level. ⁶ Since the plant develops well in arid soils, it is very effective in combating erosion. There are 5 known species, and only *P. spina-christi* is found in the flora of Turkey. *Paliurus spina-christi* is a plant that spreads in Turkey, southern Europe, the Balkans, and the Caucasus.⁸

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In this study, anatomical structures of the stem, leaf, petiole, petal, and fruit of *P. spina-christi* were investigated. Characteristic elements of stem, leaf, petiole, petal, and fruit were identified by taking the sections. Their structures are illustrated with photographs. The leaf is bifacial with its stomata located in the lower epidermis. Abundant cluster crystals of calcium oxalate and very dense and unicellular trichome (only stem, leaf, and petiole) were shown in stem, leaf, petiole, and petal. Lignified structures were observed in the samara fruits pericarp.

METHODS

Plant Material

The widely distributed *P. spina-christi*, registered in the Flora of Turkey, was collected from Erzurum/Uzundere. According to the Grid map, it was collected from Erzurum-Uzundere located in square A8. A8: Erzurum-Uzundere-Altincanak Mah. wooded areas within its borders (40°33'49" N, 41°35'47" E). It is at an altitude of 1060 m. The flowering time of the plant was observed on June 8, 2016. It was diagnosed by forest engineer Mehmet Önal from the Eastern Anatolia Forestry Research Institute. The herbarium specimens have been preserved at the Biodiversity Application and Research Center of Atatürk University with the AUEF 1348. Plant specimens for anatomy studies were taken into 70% ethanol during the collection of the plant (Figure 1).

Abbreviations for Figures

d → Druse, *e* → Epidermis, *es* → Ergastic substances, *g* → Glandular trichome, *mc* → Mucilage cell, *ph* → Phloem, *s* → Stomata, *sc* → Sclerenchyma, *st* → Starch grains, *t* → Trichome, *xy* → Xylem, *v* → Vessel

Anatomical Studies

To determine the anatomical characteristics of this species, the below-mentioned aerial part and fruit of *P. spina-christi* were collected from Erzurum, East Anatolia, Turkey, and the samples were put on 70% ethanol. Transverse and superficial sections were taken from the plant specimens manually. These sections were examined with various reagents (e.g., Sartur Reagent, Chloralhydrate). The anatomical features of the stem, leaf, petiole, petal, and fruit were determined, and photographs of the specimens and the characteristic elements were determined with the aid of ZEISS Primostar 415500, Germany. Preparations were

prepared from the stem, leaf, petiole, petal, and fruit, respectively, from the samples contained in alcohol. After the specimens were placed in styrofoam, manual sections were taken with a razor blade and put on a slide with reagent dripped on it. Then, after being covered with a coverslip and heated, tests were made.

RESULTS

In this study, anatomical structures of the stem, leaf, petiole, petal, and fruit of *P. spina-christi* were investigated. Characteristic elements of stem, leaf, petiole, petal, and fruit were identified by taking the sections. Their structures were illustrated with photographs.

The leaf is bifacial, with stomata located in the lower epidermis. Abundant cluster crystals of calcium oxalate and very dense and unicellular trichome (only stem, leaf, and petiole) were shown in stem, leaf, petiole, and petal. Lignified structures were observed in the samara fruits pericarp. The findings gained in this study propose that this anatomical diversity may be beneficial in taxonomical classification.

The stem has a cylindrical shape, and vascular bundles are regular. Mucilage cells are large and found on both vascular bundles and the central cylinder. The cuticle is thin and the central cylinder is diminished. Tracheitis, druse, endodermis, and unicellular trichome are on the stem (Figure 2).

The leaf is bifacial. Mucilage cells, druse, tracheitis, and parenchyma with starch grains are on mid vein. The cuticle is thin and the mid-vein is glabrous (Figure 3).

The corners of the petiole are round and rectangular. Its cuticle is thin and mucilage cells are on the petiole. One of the edges has a concave and there are trichomes on concave. Collenchyma is more noticeable on the petiole than on the stem. There is no central cylinder or tracheitis, while there is druse. The vascular bundles are intensified in the middle (Figure 4).

The leaf is bifacial. There is no stoma on the upper surface. The stoma type is anomositic on the under surface (Figure 5).

Druses are on all surfaces, and vessels are noticeable on petal anatomy (Figure 6).

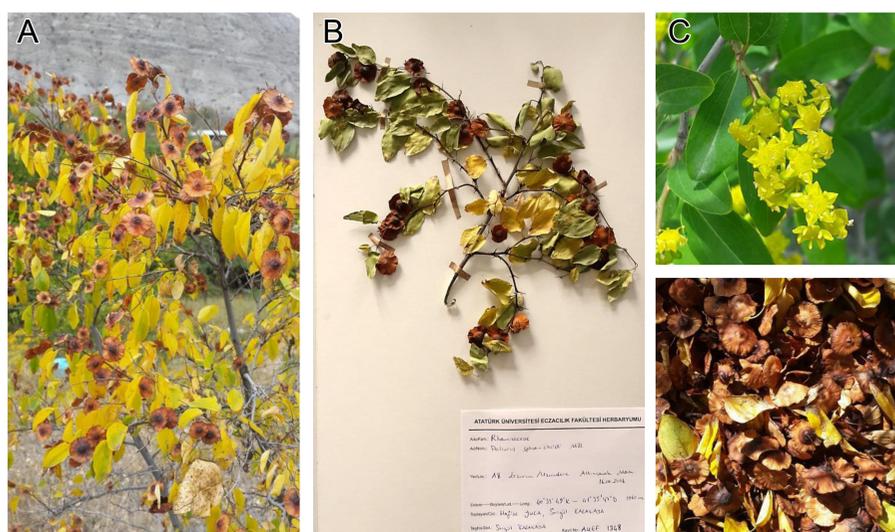


Figure 1. The general appearance of (A) *Paliurus spina-christi*. (B) Herbarium specimen. (C) Flowers and fruits.

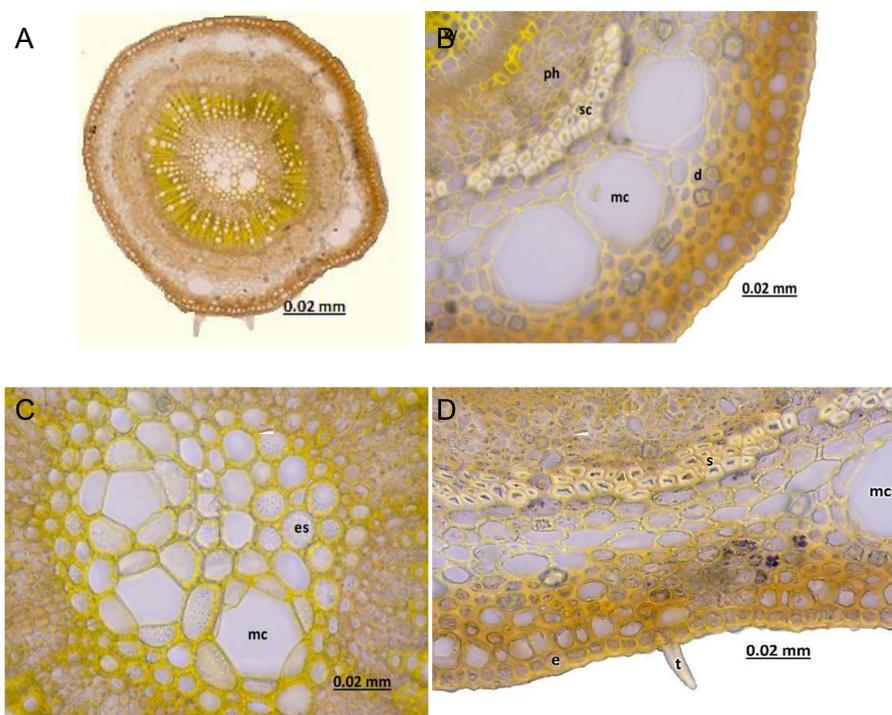


Figure 2. (A) Anatomy of stem. The stem is cylindrical; vascular bundles are regular. (B-D) Mucilage cells are large and found on both vascular bundles and the central cylinder. The cuticle is thin and the central cylinder is diminished. Tracheitis, druse, endodermis, and unicellular trichome are on stem.

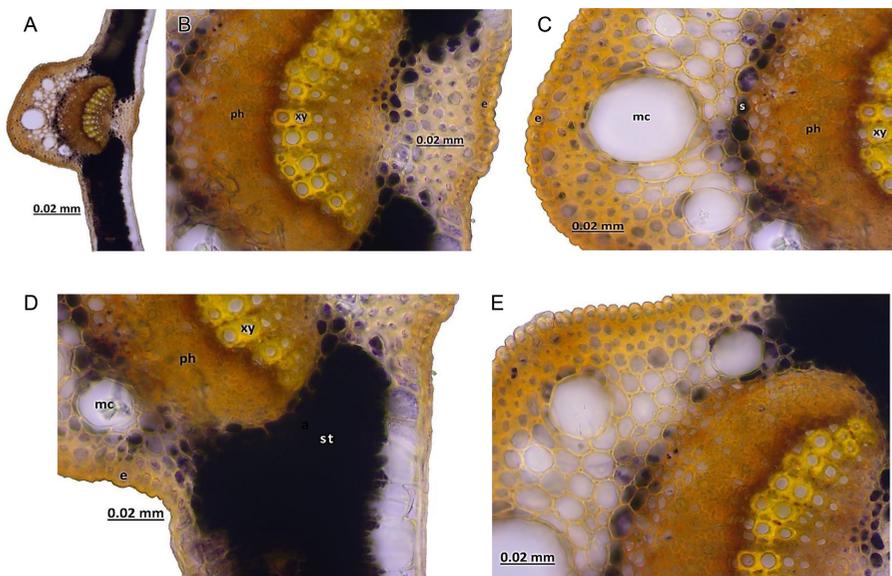


Figure 3. (A) Mid-vein anatomy. The leaf is bifacial. (B-E) Mucilage cells, druse, tracheitis, and parenchyma with starch grains are on mid-vein. The cuticle is thin and the mid-vein is glabrous.

The pedicel is cylindrical and the cuticle is thick. There are mucilage cells, phloem parenchyma, glandular trichome, trachea, and tracheitis. The sclerenchyma is arranged as cylindrical. The vascular bundles are regular, and the central cylinder is too narrow (Figure 7).

The fruit is samara. The exocarp is thick and the mesocarp is small on the wings of the fruit (Figure 8).

DISCUSSION

According to our literature research, this study represents the first examination of the anatomy of *P. spina-christi*.

Ziziphus paliurus Willd., *Z. spina-christi* Georgi, and *Z. spina-christi* var. *microphylla* Hochst. ex A.Rich. are the synonyms of *P. spina-christi*.⁹ It has been reported that the epidermis of *Z. spina-christi* var. *spina-christi* is glabrous and consists of 1 layer of isodiametric cells with a thick wall. Stomata are submerged and the mesophyll is isobilateral. Vascular bundles are collateral and surrounded by bundle sheaths with thin cell walls. Also, it has been found that the abaxial surface of *Z. spina-christi* var. *aucherii* is pubescent and the cuticle is thick on both surfaces. The epidermis contains 1 layer of cells with a thick wall adaxially. Papilla with high frequency is available abaxially. The mesophyll is isobilateral. Vascular bundles are

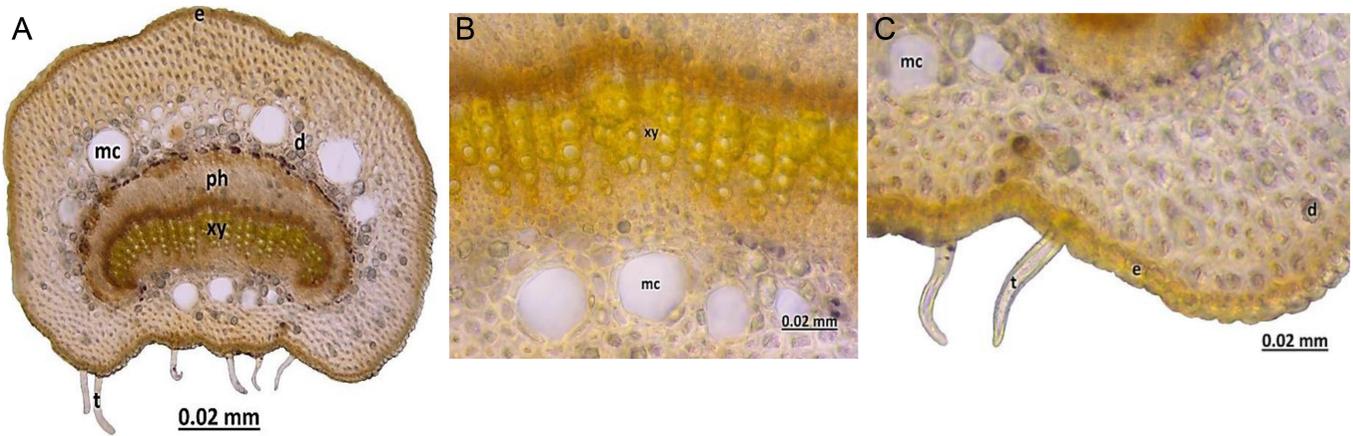


Figure 4. (A) Petiole anatomy. The corners are round and rectangular. (B-C) The cuticle is thin and mucilage cells are on the petiole. One of the edges has a concave and there are trichomes on concave. Collenchyma is more noticeable on the petiole than on the stem. There is no central cylinder and tracheitis while there is druse. The vascular bundles are intensified in the middle.

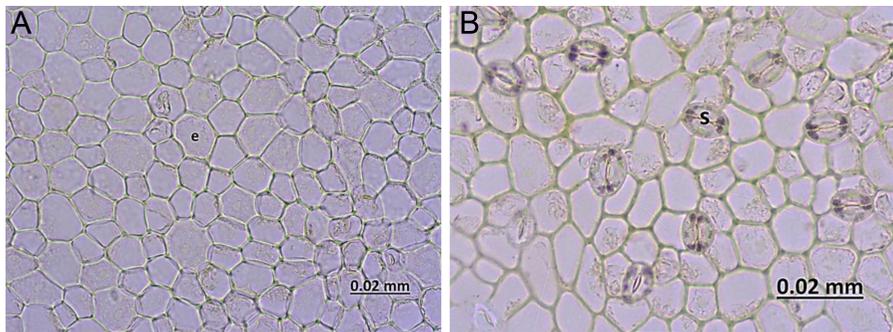


Figure 5. (A) Upper surface and (B) under surface anatomies. The leaf is bifacial. There is no stoma on the upper surface. The stoma type is anomocytic on the under surface.

collateral and surrounded by a bundle sheath. The stem is glabrous and the single epidermis includes isodiametric cells with thick outer walls. The cortex includes 3-4 layers of sub-epidermal collenchyma and a large canal. In the process of vascular cylindrical secondary growth, it's worth noting that the stem develops a cylindrical shape with additional layers of vascular tissue. The innermost part of the stem, known as the pith, contains parenchymatous cells.¹⁰ An anatomical examination of *Z. spina-christi* wood revealed certain characteristics. The wood of this species is diffuse-porous, showing distinct growth rings, simple preformation plates, polygonal openings, and parenchyma arranged either in bands or dispersed aggregates.¹¹

In another study, it was mentioned that *P. spina-christi* Mill., with 2 distinct varieties, namely *P. spina-christi* L. var. *spina-christi* and *P. spina-christi* var. *macrocarpa* Beck, is naturally found in the mountainous regions of Kurdistan, occasionally extending to the upper plains of northern Iraq. Sampling from diverse locations within the Kurdistan region yielded a collection of 15 plants, serving as the basis for comparison. Precise measurements of various attributes, including leaf, inflorescence, flowers, fruit, seeds, and leaf anatomical characteristics, were obtained—30 measurements per trait—enabling a comprehensive evaluation of the 2 varieties. Remarkably, the fruit diameter of var. *macrocarpa* outshines that of var. *spina-christi*, while larger dimensions are also

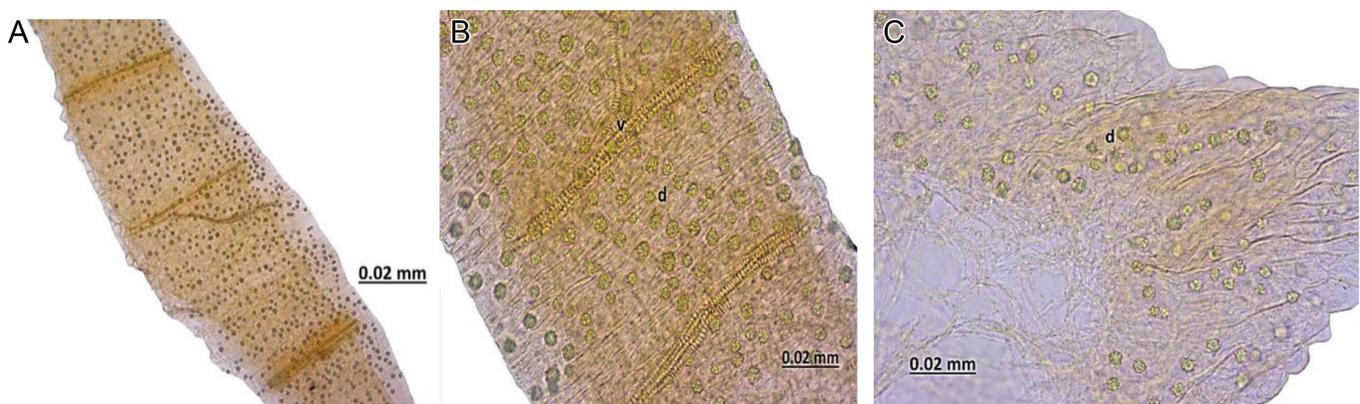


Figure 6. (A-C) Petal anatomy. Druses are on the all surfaces. Vessels are noticeable.

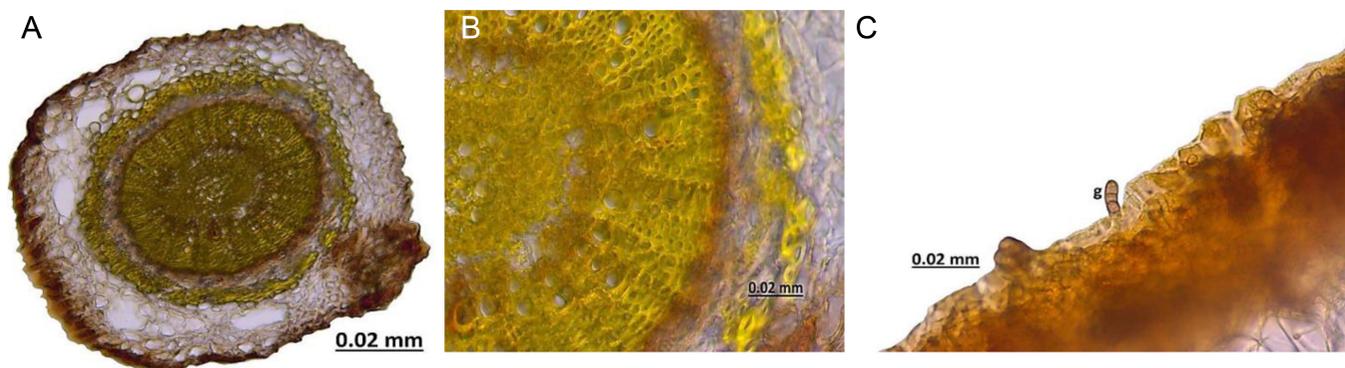


Figure 7. (A) Pedicel anatomy. The pedicel is cylindrical and the cuticle is thick. (B-C) There are mucilage cells, phloem parenchyma, glandular trichome, trachea, and tracheitis. The sclerenchyma is arranged as cylindrical. The vascular bundles are regular, and the central cylinder is too narrow.

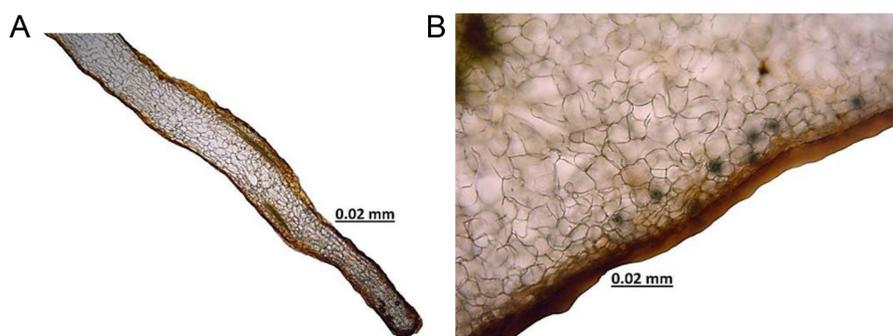


Figure 8. (A) Fruit anatomy. The fruit is samara. (B) The exocarp is thick and the mesocarp is small on the wings of the fruit. Also, exocarp is thick interior of fruit.

evident in the ovary and seed size of the former. The upper epidermal layer consistently exhibits greater thickness compared to the lower epidermal layer within the same blade. A balanced mesophyll structure emerges, featuring 2 layers of elongated palisade cells on the upper surface and 2-3 layers of shorter cells on the lower surface. Furthermore, the mesophyll houses well-distributed vascular bundles. With the exception of simple hairs exclusively present on the adaxial side of the petiole in var. *spina-christi*, absent in var. *macrocarpa*, other anatomical features appear non-taxonomically significant. Notably, the stomatal density on the adaxial leaf side reaches up to 19 stomata per mm^2 in var. *spina-christi*, contrasting the denser 38 stomata per mm^2 found in var. *macrocarpa*. Conclusively, both the fruit diameter and the stomatal density on the adaxial leaf side serve as key diagnostic indicators, facilitating the differentiation between the 2 varieties of *P. spina-christi* Mill.

The characteristics of the epidermis, stomata, and vascular bundles were similar to these studies. However, fruit, lower-upper leaf epidermis, petiole, and pedicel anatomy were not examined in these studies. While mucilage, starch cells, and glandular trichomes were seen in our study, they were not mentioned in these studies.

“Plant anatomy,” which examines the internal structure of organs, is also called “internal morphology.” “Plant systematics” studies plant diversity, “plant biochemistry” studies plant chemistry, “pharmacognosy” studies the drug properties of plants, and “plant ecology” studies the relationships of plants with their environment and each other. The correct diagnosis of medicinal plants is very important for their use in treatment. One of the

characteristics used in the diagnosis of plants is their anatomical features.¹²

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Declaration of Interests: The authors declare that they have no competing interest.

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