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RESEARCH ARTICLE

THE PHARMACEUTICAL BOTANICAL STUDIES ON THE ENDEMIC *Asperula pestalozzae* Boiss. (RUBIACEAE)

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Abstract

In this study, endemic *Asperula pestalozzae* Boiss. (Rubiaceae) was investigated in aspects of pharmaceutical botany. The morphological, anatomical, micromorphological, palynological, and chemical (antioxidant activity and total amount of phenolic substance) characteristics were reported in detail for the first time. Expanded descriptions and images of *A. pestalozzae* were given. The anatomical description was presented in detail and supported by photographs. The stem is usually angular or orbicular shaped and the leaf is monofacial. Its trichome and pollen micromorphology were examined with a scanning electron microscope (SEM). The pollen grains of *A. pestalozzae* are monad, radial symmetry, isopolar, and hexacolpate (sometimes heptacolpate). DPPH and ABTS radicals were used to determine antioxidant activity. The DPPH 50% inhibition concentration (IC_{50}) value was found to be 0.0011±0.0002 mg/mL for standard gallic acid and 0.134±0.017 mg/mL for *A. pestalozzae* extract. 0.1 mg/mL extract calculated 0.022±0.36 mM TEAC activity; 1.437±0.51 mM TEAC activity was determined in 10 mg/mL extract. The total amount of phenolic substances was calculated as 53 mg gallic acid equivalent (GAE).

Keywords

Anatomy,
Antioxidant activity,
Asperula,
Morphology,
Palynology

Time Scale of Article

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1. INTRODUCTION

The Rubiaceae family includes approximately 620 genera and 13,000 species in the world [1]. It is a cosmopolitan family and fourth family in terms of the number of species [2, 3]. There are 13 genera and 234 taxa in Türkiye [4]. The species of this family have been used in dyeing since ancient times; for example, *Rubia tinctorum* L. [5]. Some species, such as coffee, have economic value. Pharmaceutical raw materials are obtained from some genera, such as *Uncaria* Schreber, *Cinchona* L., and *Coffea* L., and are used for treatment purposes. [6].

Asperula, one of the important genera in this family, has many therapeutic properties. In Turkish folk medicine, some *Asperula* species have been used as tonic, antidiarrheal and diuretic [7]. The species representing the genus *Asperula* is *Asperula arvensis*. Thanks to polyphenol acids, iridoids, tannins, and flavones, it is used as a blood and lymph system cleaner and for skin diseases and cancer treatments [8]. The main ingredients in the dry extract of *A. odorata* L. utilized in Ukrainian traditional medicine are chlorogenic acid and cynaroside. The plant's dry extract has twice the antihypoxic impact of the medication that was used as a reference. This plant also has a sedative effect [9].

The genus *Asperula* represents 183 species and 230 taxa in the world [8]. In Türkiye, there are 58 taxa belonging to the genus, and almost half of them are endemic [10]. *Asperula* is an annual or perennial

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herb, a low shrub, with 4-6 or multiple circumscribed leaves. Stipules are rarely reduced, and flowers are in loose paniculates or spikes. The calyx is missing. The corolla is composed of (3-)4-5 parts. The corolla lobes are shorter than the tube [11]. The inflorescence is mostly used to define this genus. *Asperula cynanchica* L. has an enzyme on the tips of flowering branches that are used to ferment milk, and this enzyme is used in yogurt production. Thus, its traditional name is "yoğurt otu" in Türkiye [8]. However, one of the common names is "yapışkan otu". In current sources, it is referred to as "belumotu" [10]. *Asperula pestalozzae* Boiss. is an endemic species that is in the *Cynanchicae* section of the genus *Asperula.* It grows on rocky slopes, steppes, limestone, sandstone, calcareous soil, and clayey areas at altitudes of 300 to 2300 m, an euxine element, and it spreads in areas around the Black Sea and Central Anatolia. The Turkish name of the plant is "has belumotu" [10, 11].

There are some floristic [12, 13], anatomical [5, 14], karyological [14, 15], chemical [16- 21], and biological activity studies [7, 21- 23] on *Asperula* species in the literature. Morphological, anatomical, and pollen structures, as well as the antioxidant activity of *A. pestalozzae* have not previously been studied. Therefore, the present study aims to give a detailed account of the internal and external morphological properties and pollen characteristics of *A. pestalozzae*, which is endemic to Türkiye. It also determined whether it had antioxidant effects in terms of chemicals.

2. MATERIALS AND METHODS

2.1. Plant Material

During the flowering and fruiting period (May–July, 2020), *Asperula pestalozzae* was collected from the Bozüyük district of Bilecik province (30˚0'49.485" E-39˚54' 48.482"N). For plant identification, P.H. Davis's [11] "Flora of Turkey and the Aegean Islands" was used. Some of the dried plants in the press were numbered and turned into herbarium samples. They were placed in the Anadolu University Faculty of Pharmacy Herbarium (ESSE No. 15610).

2.2. Morphological, Anatomical Studies

Herbarium specimens and fresh specimens were used for defining morphological findings. Morphological features were described in detail. General appearance, stem structure and hairs, leaf structure and hairs, flower state, corolla structure, longitudinally opened corolla, anther, ovary, fruit, bract, and bracteole shapes were drawn in detail. A WILD TYP 181300 stereo microscope and drawing tube were used for the drawings in Figure 1. In morphological terminology, Baytop (1998)'s English-Turkish Botanical Manual [24] was used.

The micromorphology of the stem, leaf, corolla, and ovary surfaces was viewed with a scanning electron microscope in the Anadolu University Plant, Medicine, and Scientific Research Center (BIBAM). Stems and leaves were preserved in 70% alcohol, and they were used for anatomical studies. Transverse sections were taken from the stem, and superficial sections were taken from both the transverse and lower upper parts of the leaves. All cells in preparations were exposed to chloralhydrate to render them transparent. Then they were stained with Sartur reagent. The preparations were photographed with an Olympus BX51T brand trinocular microscope and described. For anatomical descriptions, the terminology proposed by Metcalfe & Chalk (1950) [25] was followed.

2.3. Palynological Study

For palynological findings, pollen from dry corolla samples was placed on double-sided tape. The samples were coated with gold (SBC-900 Single Target Plasma (Gold) Sputtering Thin Film Coating) and examined with a table-top scanning electron microscope (TM3030 Plus Tabletop Microscope, HITACHI). The polar axis and equatorial axis lengths of the pollen were measured, and the shape of the

pollen was determined by dividing the polar axis value (P) by the equatorial axis value (E). The terminology for data proposed by Walker and Doyle (1975) [26] was followed.

2.4. Extraction

The aerial parts of the plants that dried at room temperature were turned into coarse powder. Then 100 mL of 70% ethanol was added to 20 g of powdered plant. It was shaken for 24 hours (ORBITAL, shaker) At the end of the period, the mixture was filtered, and 100 mL of ethanol was added to the filtrate. The process was repeated three times and terminated [18, 27]. The filtrates were collected in an Erlenmeyer flask, and the ethanol in their contents evaporated for 30 minutes at 41 °C under pressure. The water in the structure of the samples was crystallized in a deep freeze. A lyophilizer was used to remove the ice water.

2.5. Total Content of Phenolic Substances and Antioxidant Activity Tests

The total amount of phenolic substances was determined with the Folin-Ciocalteu method. This test is based on the color reaction, and a blue-colored complex formation is observed in the reaction [28]. A solution with a concentration of 10 mg/mL was prepared by dissolving 20 mg of plant extract in 2 mL of methanol. Solutions at different concentrations (0.1 mg/mL, 0.35 mg/mL, 0.5 mg/mL, 0.7 mg/mL, and 1 mg/mL) were prepared from the standard gallic acid. 20 µL of the solutions were placed in the microplate wells, and 1560 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added. After waiting for a while, the reaction was started by adding 300 µL of 20% sodium carbonate (Na_2CO_3). After 2 hours in the dark at room temperature, the absorbance values were measured at 760 nm [29, 30] (ELISA Microplate Reader, Biotek Synergy HTX).

The extract's antioxidant activity was assessed by the $DPPH^{\bullet}$ scavenging effect and $ABTS^{\bullet}$ scavenging effect assays. A serial dilution was made from the plant sample dissolved in MeOH (methanol) for the determination of the DPPH scavenging effect. Radical scavenging activity was calculated using the 50% inhibition concentration equation [28]. A plant sample (10 mg/mL concentration) dissolved in 200 µL MeOH was transferred to the well of a 96-well microtitration plate. 100 µL of MeOH was added to the other wells. 100 µL of the sample was taken from the first well, and 10 serial dilutions were made, respectively. 100 µL of $DPPH^{\bullet+}$ solution was added, and the mixture was left in the dark for 30 minutes. It was measured at 517 nm with a spectrophotometer [31].

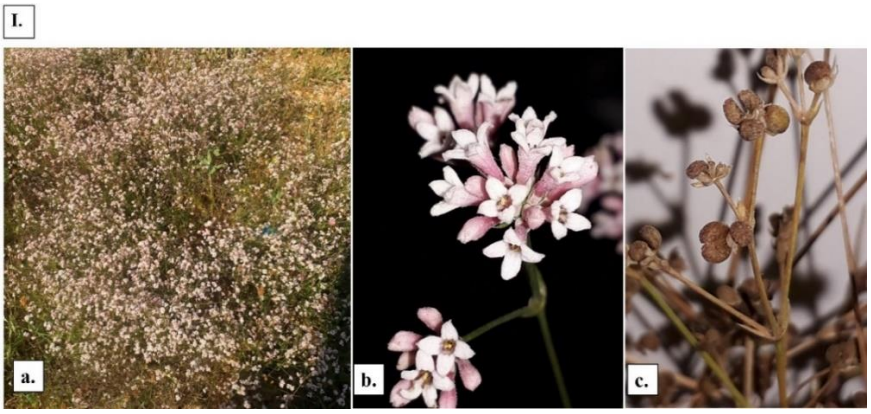
In the ABTS scavenging effect test, it was mixed with 7 milliMolar $ABTS^{\bullet+}$ and 2.5 mM sodium persulfate ($Na_2S_2O_8$). The mixture was kept in the dark for 12-16 hours, and blue-green radical formation was achieved. Solutions at concentrations of 0.1 mg/mL and 10 mg/mL were obtained from the plant extract. Then, different concentrations (2.5 mM, 2 mM, 1.5 mM, 1 mM, 0.5 mM, and 0.1 mM) were prepared from 3 mM Trolox to be used as a standard. Gallic acid with concentrations of 0.1 mg/mL and 1 mg/mL was used as the positive control. 10 µL of the prepared samples were placed in the microplate wells, respectively, and 990 µL of $ABTS^{\bullet+}$ was added. After 30 minutes, its absorbance was measured at 734 nm [32].

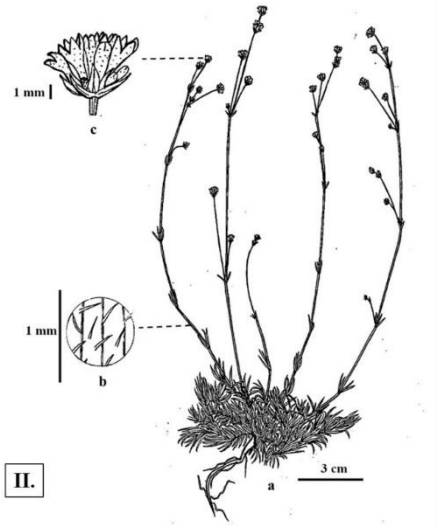
3. RESULTS

3.1. Morphological Features

A. pestalozzae is perennial, semi-shrub (Figure 1 I-a), stems ascending to erect, basally densely leafy (Figure 1 II-a), flowering and vegetative branches. Flowering stems are 18-63 cm, quadrangular or slightly rounded on the undersides, puberulent-hispid haired on the underside (Figure 1 II-b), and glabrous on the top.
Leaves are 4, verticillasters (Figure 1 III-a), linear or filiform (Figure 1 III-b), strongly revolute (Figure 1 III-c), base leaves are 5-20 x 0.2-1 mm, upper leaf is 5.8-16 x 0.3-0.8 mm, apex aristate, arista 0.2-0.7

mm and hyaline, straight-sided, base truncate; generally dense hispid hairy, erect or slightly tilted back, glabrous or few hairy (Figure 1 III-d).
The inflorescence is cymose at the tip of the branch consisting of paniculate (Figure 1 II-c), simple or branched, clusters 3–15; inflorescence stalk 5–35 mm.
Bract is lanceolate-ovate, 2-4.3 x 0.5-1.3 mm, bottom with flaps, margin straight, glabrous, aristate, arista 0.2-0.7 mm, hyaline, straight or slightly curved (Figure 1 IV-d,e,f).
Bracteole is oblong-lanceolate, 1.5-3.3 x 0.4-1 mm, margin straight, aristate, hyaline and 0.2-0.5 mm (Figure 1 IV-g,h).
Calyx none.
Corolla is gamopetalous, funnel (Figure 1 III-e,f), 2.2-3.3 mm; tube 1-2.2 mm, equal to or 2 times longer than lobes, lobes 1-1.2 x 2-3 mm, ovate-oblong, apex acute, lobes pinkish-white tube pink (Figure 1 I-b), dense hispid-tooth hairy, few or glabrous on lobes.
Stamen is 4, epipetalous, alternate with lobes (Figure 1 III-g), anther is dorsifixed, linear, 1-1.2 mm (Figure 1 IV-a) brown, filament adhered to the corolla.
The ovary is 1 x 0.5 mm, dark brown, densely papillate (Figure 1 IV-b); style 0.5-1.6 mm, bipartite equally branched, pinkish-purplish, stigma 2 and round.
Mericarp is ovate-oblong, 1.2-2 x 1 mm, green at first, dark brown in maturity (Figure 1 I-c), and densely warty (Figure 1 IV-c).
Flowering Time: July- August
Habitat: Rocky slopes and steppes, limestone, sandstone, marl, and clay areas
Altitude: 300-2300 m
Endemic





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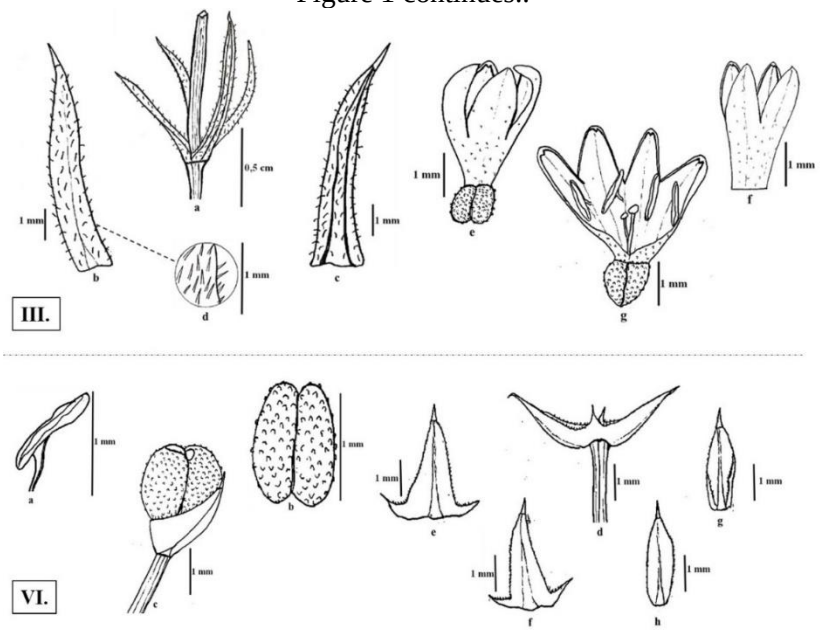

Figure 1. Morphological photographs and drawings **I-a)** General view of the plant **b)** flowers **–c)** fruits **II-a)** plant general drawing **-b)** stem hairs **-c)** flower status **III-a)** the arrangement of the leaves **-b)** the upper surface of the leaf **-c)** the lower surface of the leaf **-d)** hairs **-e)** corolla with ovaries **-f)** corolla without ovaries **-g)** inner surface of corolla **IV-a)** anther **-b)** ovary **-c)** mericarps (fruit) **-d)** bracts surrounding the flower **-e)** inner surface of the bract **-f)** outer surface of the bract **-g)** inner surface of the bracteole **-h)** outer surface of the bracteole

3.2. Trichome Morphology

The hairs on the organs of the plant and their characteristics are summarized in Table 1. The hairs are denser on the lower part of the stem.

Table 1. Hair characteristics and density in vegetative and generative organs

The Organs	Characteristics		Density
Stem	88.3 µm-196 µm Hispid, Unicellular	Upper	±
		Lower	++
Leaf	55.2- 298 µm Hispid,Unicellular	Upper Surface	+
		Lower Surface	+
Corolla	32.9 µm-91.3 µm Dense hispid-Tooth hairy	Tube	++
		Lob	± or -
Ovary surface	Round and convex papillary	All surface	++

(-) none, (±) rare , (+) intense, (++) instenser

Trichomes are simple, unicellular, usually short-conical, erect or oblique, hispid, 88.3 µm- 196 µm, and with dense cuticle blisters (Figure 2a). Trichomes are dense on both surfaces of the leaf. They are unicellular, long, with dense cuticle ridges, oblique or slightly tilted back, hispid, 55.2- 298 µm (Figure 2b).

The outer surface of the corolla appears as wavy lines formed by epidermal folds. The part from the bottom of the corolla lobes to the ovary is covered with dense hispid and tooth hairs (Figure 2c). Trichomes are 32.9 µm- 91.3 µm long and above with cuticle blisters. The ovary is 1 x 0.5 mm in size and contains dense papillae, the surface of which is generally rounded. Large and small convex papillae have straight, or curved lines formed by epidermal folds (Figure 2d).

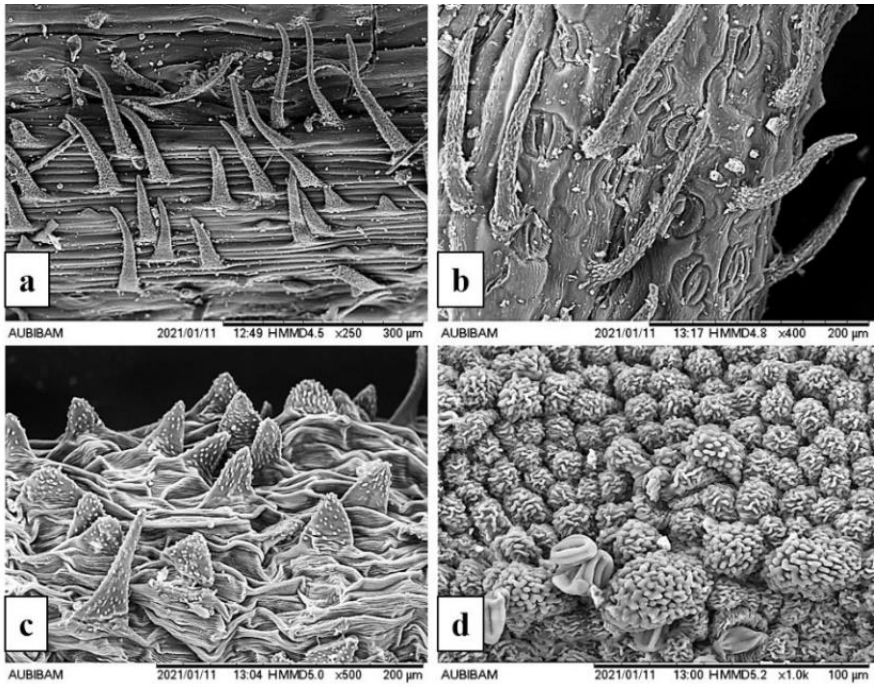

Figure 2. SEM images of hairs **a)**hispid hairs on stem **b)**hispid hairs on leaf **c)** tooth hairs on corolla **d)** papillae on ovary

3.3. Anatomical Features

3.3.1. Stem anatomy

The stem is usually angular or orbicular in transverse sections from the middle part of the stem (Figure 3a). The epidermis is composed of a single layer of almost square, compactly arranged cells. The upper surface is covered with a relatively thick cuticle and is covered with trichomes. Trichomes are simple, one-celled, short, or long (Figure 3b). Collenchyma tissue is located under the epidermis at the corners and is 5-10 layered (Figure 3c). The parenchymatic tissue is 3-5 layers and ring-shaped. It contains intense ergastic substances. The endoderma is single-layered and prominent. These cells are large transverse, and rectangular shaped. Vascular bundles surround the stem. Phloem is 3–5 layered and consists of irregular cells. Cambium is 1-2 layered or inconspicuous. Xylem comprises the regular trachea and tracheid cells. Rays are 1-2 layered. Those cells underlying the xylem are narrower. Pith is composed of large, orbicular, or polygonal parenchymatic cells, often with abundant intercellular spaces or cavities formed by the rupture of cell walls (Figure 3d).

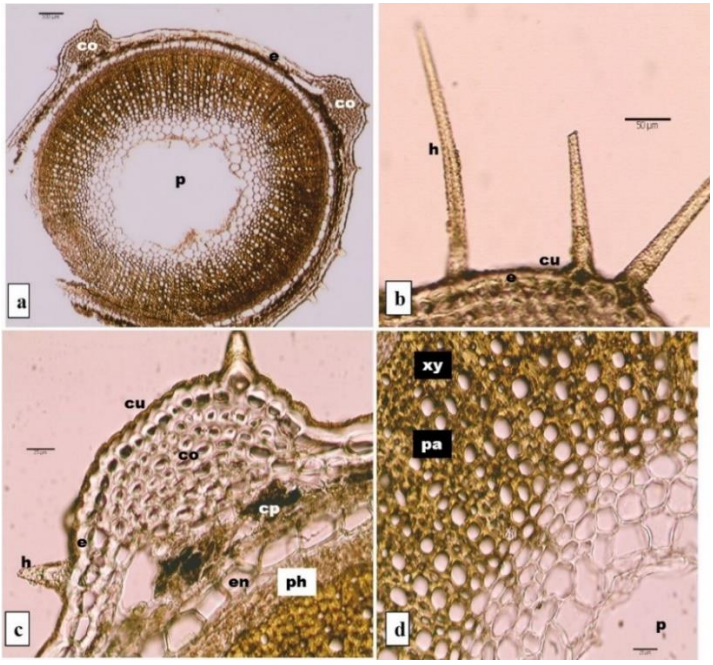

Figure 3. Anatomical structures of stem cross-sections **a)** cross-section general view of the body(100µm) **b)** hairs (cross-section) (50µm) **c)** stem corner detail(25µm) **d)** tracheal elements and center(25µm) (cu: cuticle, co: collenchyma, cp: cortex parenchyma, e: epidermis, en: endodermis, h: hair, p: pith, pa: pith arm, ph: phloem, xy: xylem)

3.3.2. Leaf anatomy

The general view in the cross-section is transverse oval-rectangular (Figure 4a). The backward-curved leaves are slightly indented on the lower surface. The upper and lower epidermis consist of uniseriate oval and rectangular cells in transverse sections. The upper walls of the epidermis are thicker than the lower and lateral walls. Both epidermis surfaces are covered with an almost thick cuticle. The cells of the upper epidermis are larger than those of the lower. In superficial sections, the upper and lower epidermis cells are long and rectangular in shape and with many simple passages; the lower epidermis is longer and more wavy-walled than the upper epidermis. Trichomes are simple, unicellular, and conical-long (Figure 4b). The stoma (amphistomatic) located on both sides of the epidermis is of the parasitic type (Figure 4 c, d). The stomata are more common on the upper surface of the leaf than on the lower face. The leaf is monofacial. Under the upper epidermis in the mesophyll layer, there are 2 seriate of long, cylindrical, palisade parenchyma, and it continues up to the lower epidermis as uniseriate on the sides. In the lower epidermis, 3-4 seriates of longitudinally cylindrical or oval, loosely arranged sponge parenchyma are located above the palisade parenchyma. Under the midrib, a large layer of collenchyma is distinguished. The vascular bundles are of the collateral type and occur in a narrow area, surrounded by a bundle sheath and 3-5 small vascular bundles to the left and right of the central bundle.

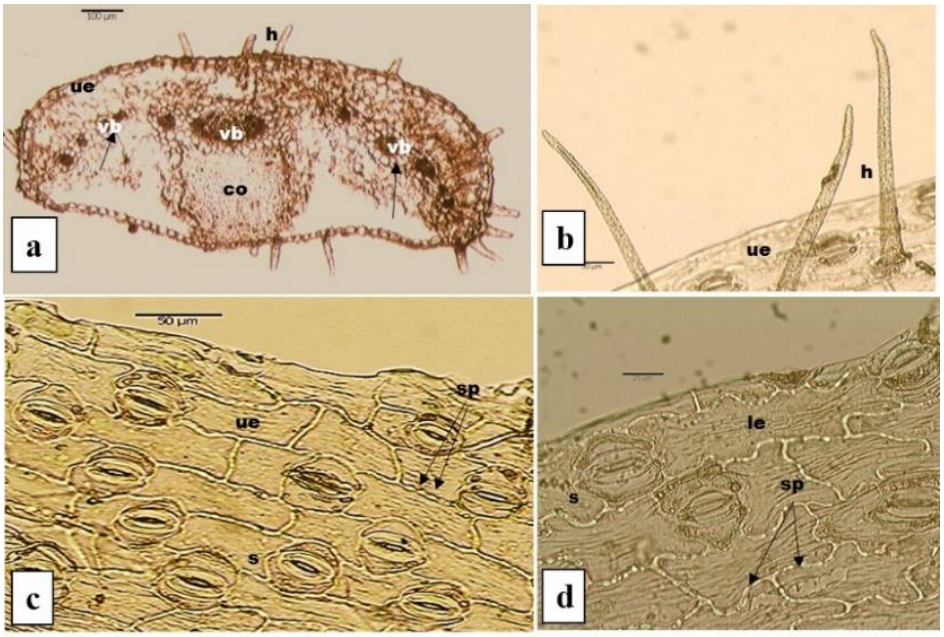

Figure 4. Leaf anatomical images **a)** leaf cross-section overview (100µm) **b)** hairs in leaf upper superficial section (50µm) **c)** upper superficial section(50µm) **d)** underside superficial section (25µm) (co: collenchyma, h: hair, s: stoma, sp: simple pass, le: lower epidermis, ue: upper epidermis, vb: vascular bundle)

3.4. Pollen Characteristics

The pollen grains of *A. pestalozzae* are monad, radial symmetry, isopolar, and hexacolpate (sometimes heptacolpate). The polar axis (P) is 16.9-20.6 µm, and the equatorial axis (E) is 10.9-14.3 µm. The P/E ratio is 1.44-1.55 µm. The shape of the pollen grains is euprolate. Equatorial images of pollen grains are elliptical (Figure 5a), whereas polar images are almost circular (Figure 5b). Colpus length is 12.5 µm-15.8 µm, and colpus width is less than 1µm. Skulptur (ornamentation) is scabrate-perforate (Figure 5c).

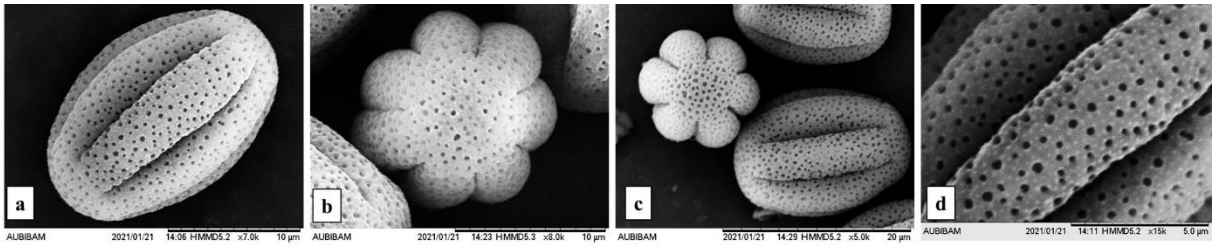

Figure 5. SEM microphotographs **a)** equatorial view **b)** polar view **c)** hexacolpate pollen **d)** close view of pollen surface

3.5. Total Content of Phenolic Substances and Antioxidant Activity Tests

The total amount of phenolic substances was found to be equivalent to 53 mg of gallic acid (GAE) in 1 g of *A. pestalozzae* extract. It was observed that the color complex of the extract was darker.
The antioxidant properties of the methanol extract tested with the scavenging effect of the 1,1-diphenyl-2-picrylhydrazil radical ($DPPH^{\bullet+}$) and the scavenging effect of the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^{\bullet+}$). The results of antioxidant tests are shown in Table 2 and Table 3.

Table 2. Results of DPPH antioxidant test

Antioxidant Capacity Test	Extract	IC_{50} value (mg/mL)
DPPH test	*A. pestalozzae* (10 mg/mL)	0,134±0.017
	Gallic acid (1 mg/mL)	0,0011±0.0002

Table 3. Results of ABTS antioxidant test

Antioxidant Capacity Test	Extract	mM TEAC (mg/mL)
ABTS test	*A.pestalozzae* (0,1 mg/mL)	0,022±0.36
	A.pestalozzae (10 mg/mL)	1,437±0.51
	Gallic acid (0,1 mg/mL)	1,436±0.45
	Gallic acid (1 mg/mL)	2,630±0.07

4. DISCUSSION

In this study, the morphology, anatomy, palynology and antioxidant activity of the endemic *A. pestalozzae* in Türkiye was investigated for the first time and the results are discussed below.

Morphological results have been compared to the Flora of Turkey in Table 4, and some differences were observed. While the plant height was 13-60 cm in the Flora of Turkey, it was 18-63 cm in our study. While the four-cornered stem is compatible with our results, a slightly rounded shape was observed in the lower parts. The leaf width was found to be larger (0.2-1 mm) than the flora (0.3-0.75 mm), and the hyaline tip was found to be smaller. In the flora, the bract was 2-4 mm; in our results, it was 2-4.3 x 0.5-1.3 mm. The corolla size (2.2-3.3 mm) was almost the same as the flora. The color of the corolla was pinkish-white in the lobes and pink in the tube. In our study, the mericarps were smaller, and they were dense with warts. These differences are thought to be caused by geographical and climatic-edaphic factors. Also, leaf number, leaf margin and base, flower number, inflorescence stem, bract tip, bracteole, corolla lobe length, stamen, anther, filament, ovary length and color, stylus, stigma, and fruit shape characters were determined for the first time by our study. All morphological images were drawn, and the deficiencies were resolved in the flora.

Table 4. Comparison of our morphological findings with the Flora of Turkey

Characters		Our Results	Flora of Turkey
Plant Height		18-63 cm	(13-)20-60 cm
Stem	Shape	Ascending to erect, angular or slightly rounded on the undersides	Ascending to erect, quadrangular
	Hairs	Puberulent-hispid on the underside; glabrous on the top	Puberulent or scabrid-hispid in lower parts; glabrous or few hairy
Leaf	Size	Base leaf 5-20 x 0.2-1 mm, upper leaf 5.8-16 x 0.3-0.8 mm	4-15(-25) x 0.3-0.75 mm
	Number	4 and verticillastrum	-
	Arista	0.2-0.7 mm	0.7 mm
	Margin	Straight	-
	Base	Truncate	-
	Hairs	Dense hispid, erect or slightly tilted back, glabrous or few hairy	Scabrid to hispit or glabrous; slightly tilted
Inflorescence	Number of flowers	Cluster 3–15	-
	Stalk length	5-35 mm	-
Bract	Size	2-4.3 x 0.5-1.3 mm	2-4 mm
	Margin	Straight	Straight or denticulate
	Tip	0.2-0.7 mm, aristate, hyaline	-
Bracteole	Size	1.5-3.3 x 0.4-1 mm	-
	Shape	Oblong-lanceolate	-
	Margin	Straight	-
	Apex	Aristate	-
	Tip	0.2-0.5 mm, hyaline	-

Table 4. Comparison of our morphological findings with the Flora of Turkey (Continues)

Corolla	Size	2.2-3.3 mm	2-3 mm
	Color	Lobes pinkish-white, tube pink	Pale pink
	Tube size	1-2.2 mm, equal to or 2 times longer than lobes	2 times longer or shorter than ovate-oblong
	Lob size	1-1.2 x 2-3 mm	-
	Hairs	Dense hispid-tooth hairy, few or glabrous on lobes	Hispid, sometimes glabrous
Stamen	Number	4	-
	Shape	Dorsifixed	-
	Position	Alternating with lobes	-
Anther	Length	1-1.2 mm	-
	Shape	Linear	-
	Color	Brown	-
Filament	Length	< 1mm	-
Ovary	Size	1 x 0.5 mm	-
	Color	Dark brown	-
Style	Length	0.5-1.6 mm	-
	Shape	Bipartite equally branched	-
	Color	Pinkish-purplish	-
Stigma	Shape	Round	-
	Number	2	-
Mericarp	Size	1.2-2 x 1 mm	2 mm
	Shape	Ovate-oblong	-
	Color	Dark brown	Dark brown
	Surface	Densely warty	Warty

"The features shown with lines in the table were determined for the first time."

The anatomical views of the stem and leaf were presented and supported by the photographs. There were collenchymatic corner projections, prominent endoderma, absence of pericycles, presence of raphide crystals, and long unicellular trichomes in the stems. The leaf was monofacial, and parasitic stomata on both epidermal surfaces of the leaves were observed. According to Metcalfe and Chalk (1950), the stems of the genus have a polygonal shape with collenchymatic corner projections, and no fungal structure is observed. The endoderma is well developed; however, the sclerenchymatic pericycle is not observed. The xylem is narrow and cylindrical, and the tracheal elements are small in diameter. A centric (*Asperula*) or homogeneous (Borreria G.F.W. Mey.) structure can be seen in some members of the family, which generally have a dorsiventral leaf structure. The hairs are long, unicellular, and sometimes curled at the ends in species of *Asperula* and some genera. Stomas are also located on the lower and upper sides of *Asperula* and some species of genera. They are of the rubiaceus (parasitic) type. Epidermal cells have smooth or wavy walls. Family members contain crystals and are extremely important in species identification. In *Asperula* stems the crystals consist only of raphides [25]. The stem and leaf anatomical features of the Rubiaceae family and the *Asperula* genus described by Metcalfe and Chalk, are usually compatible with our study results. Our findings coincide with those of Gucel (2015) [14]. The cell groups in the stem and leaf anatomy were similar.

The characteristics of the pollen, which have been mentioned by many authors, have taxonomic significance for Rubiaceae species. The pollen shapes of *A. serotina* (Boiss. Et Heldr.) Ehrend. and *A. purpurea* subsp. *apiculata* (SM.) Ehrend. have been recorded as spheroidal, the structure of the sexine was perforate, and the ornamentation was microechinate [33]. The pollen of *A. cankiriense* B.Şahin & Sağıroğlu was subprolate (P/E= 1.22), the number of colpus was 6-7, the colpus width was more than 1 µm, and its ornamentation was microechinate-perforate [34]. The pollen shape of *A. comosa* Schönb.-Tem was oblate-spheroidal; the pollen grain of *A. anatolica* was defined as spheroidal [1]. The pollen type of the endemic *A. daphneola* O. Schwarz was stefanocolpate, and the pollen shape (P/E=1.06) was spheroidal [14]. The pollen forms of some taxa were prolate-spheroidal and oblate-spheroidal; it has

been reported that the ornamentation was scabrate and perforate. The pollen shapes were different from our findings. Additionally, the pollen ornamentation in our study was scabrate-perforate. Our results showed similar ornamentation to previous research [8]. In previous research, the number of colpus in *Asperula* species has been given as usually 6-8, rarely 9 [1, 8, 14, 33, 34]. In our results, it was 7-8, compatible with other studies.

Some *Asperula* species have antioxidant activity. Minareci et al. (2011) investigated the antioxidant activities of five endemic *Asperula* species. The highest antioxidant activity value belongs to *A. pseudochlorantha* var. *pseudochlorantha* and is 1.88 mg/mL. The lowest antioxidant activity value belongs to *A. serotina* and is 1.22 mg/mL. In this study, it was determined that *Asperula* taxa showed different levels of antioxidant activity [16]. This result supports the antioxidant activity feature in our study. In another study, Halimi and Nasrabadi (2015) used the species *Asperula oppositifolia*. The antioxidant activity of methanol extracts of the aerial parts of the plant was tested. A high inhibitory effect was observed at high concentrations [17]. As a result of this study, it was stated that the *Asperula* taxon showed antioxidant activity. This situation is similar to ours. Loizzo et al. (2008) examined the *Asperula glomerata* species from the Rubiaceae family among plants with medicinal uses. The amount of total phenolic compounds was determined by the Folin–Ciocalteu method. The total phenolic component amount of *A. glomerata* extract was found to be 81.5 ± 0.13 mg/g [23]. It seems that the total phenolic substance amount in *A. glomerata* extract is higher than the result of our extracts.

At the same time, flavonoids and iridoids were determined in taxa whose components were examined [18- 21]. Kırmızıbekmez et al., (2014) worked with the aboveground parts of the *Asperula lilaciflora* species. They identified a new iridoid with a new flavonol glycoside. The name of the flavonol glycoside is lilacifluoracid; they reported iridoid as asperulogenin [20].

In all studies, different *Asperula* species have antioxidant properties. The fact that *Asperula pestalozzae* species also exhibit antioxidant activity suggests that they may contain flavonoid and iridoid structures in their phenolic components. For this reason, the subject of our future study will be the detection of flavonoid, iridoid, and other phenolic components.

5. CONCLUSION

The anatomical, morphological, micromorphological, palynological, and chemical properties of the endemic *A. pestalozzae* were compared to Flora of Turkey and other studies in detail for the first time in this study. Working on it for the first time increases its original value. Also, it shows that it is a pioneering work that contributes to further research.

When we looked at the results of all our studies, it was determined that our data matched the literature information, but some differences were determined. Our plant has shown antioxidant properties as a result of chemical studies. So, it could be used as a new source of antioxidant agents. The determination of its components is considered the subject of our future study.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Kader Kayiş: Investigation, Resources, Writing – original draft, Visualization. **Ayla Kaya:** Resources, Writing – Review & Editing, Supervision, Project administration.

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