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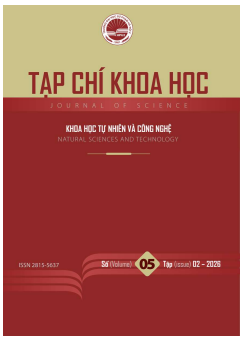

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Morphology and anatomy characterization of *Ehretia acuminata* R.Br in Viet Nam

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Abstract

Ehretia acuminata R.Br. (Boraginaceae) is a medicinal plant species widely distributed across tropical and subtropical regions, including Vietnam. However, detailed anatomical data for taxonomic identification and pharmacognostical applications remain limited. This study aims to investigate the morphological and anatomical characteristics of the vegetative organs of *E. acuminata* collected in Northern Vietnam. Our findings reveal that the leaves exhibit a bifacial structure with well-developed palisade mesophyll and loosely arranged spongy mesophyll. The epidermis consists of a single cell layer covered by a distinct cuticle, with stomata predominantly distributed on the abaxial surface. Stem anatomy demonstrates typical secondary growth with clearly differentiated vascular tissues arranged in a continuous ring, while the root structure shows characteristic development of secondary xylem. Key anatomical features, including the organization of the mesophyll, the structure of vascular bundles, and epidermal characteristics, are identified as valuable diagnostic markers for species identification. This study contributes to the anatomical database of the species in Vietnam and provides a foundation for subsequent taxonomic research and medicinal material standardization.

Keywords: *Ehretia acuminata*, morphological characteristic, micro-anatomical structure, morphology

1. Introduction

The genus *Ehretia* P. Browne (Boraginaceae) comprises approximately 50–60 species, primarily distributed across tropical and subtropical regions of Asia, Africa, and the Americas. Numerous species within this genus are recognized for their ecological significance and medicinal value, having been employed in traditional medicine to treat hepatic disorders, inflammation, and bacterial infections [1]–[4]. In Vietnam, *Ehretia acuminata* R.Br. (*Cườm rụng nhọn*) is widely distributed in secondary forests

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and utilized in folk medicine [5]–[7]. Previous research on this species has focused predominantly on its phytochemical constituents and pharmacological activities, highlighting its noteworthy therapeutic potential.

Despite its significant medicinal importance and relatively broad distribution, detailed morphological and anatomical studies on *E. acuminata* remain limited. This lack of information poses challenges for accurate species identification and hinders the establishment of anatomical standards for the quality control and authentication of medicinal materials.

Therefore, this study was conducted to characterize the morphological and anatomical features of the vegetative organs of *E. acuminata* collected in Vietnam. The research aims to identify diagnostic anatomical traits of taxonomic and pharmacognostic value, while providing baseline botanical data for the genus *Ehretia* in Southeast Asia.

2. Materials and Methods

2.1. Materials

Specimens of *E. acuminata* were collected in Ngu Hiep commune, Hanoi city, Vietnam.

The collected materials included leafy branches with flowers for herbarium specimen preparation, as well as fresh roots, stems, leaves, flowers, and fruits for morphological and anatomical investigations.

Number of samples: 40–50 sections were performed per organ.

Specimen ID: E. AC 01, currently deposited at the Laboratory of Botany, Faculty of Biology, Hanoi Pedagogical University 2.

2.2. Experiment design and methods

Habitat characteristics and morphological features of vegetative and reproductive organs (roots, stems, leaves, flowers, and fruits) were observed and recorded, followed by photographic documentation and sample collection. Fresh anatomical sections were prepared following the methods of Klein and Klein (1979) and Nguyen Nghia Thin (2007) [8], [9], then examined under an optical microscope. Microscopic measurements of cell dimensions were performed using stage and ocular micrometers according to the procedure described by Pausheva (1974). Microphotographs were captured for detailed analysis.

Morphological observation of vegetative organs: roots, stems, leaves, flowers, and fruits were observed, photographed, and collected. Fresh anatomical specimens were prepared via hand-sectioning and subjected to double-staining according to the methods described by Klein R.M. and Klein D.T. (1979) and Nguyen Nghia Thin (2007) [8], [9]. The samples were then examined under an optical microscope. Cell dimensions were measured using a stage micrometer and an eyepiece micrometer following the method of Pausheva (1974). Micrographs were captured.

3. Results and Discussion

3.1. Roots

The root system of *Ehretia acuminata* exhibits characteristic features of Dicotyledonous plants, featuring a long and robust taproot system. The vigorously developed primary root is designated as the first-order root. Numerous lateral roots are present, with their hierarchy determined by the sequence of development; those emerging earlier are assigned lower order numbers.

During the primary growth stage, the root system exhibits a predominant development of the piliferous zone (root hair zone) with a high density of root hairs, optimizing the surface area for the absorption of water and mineral salts. Upon transitioning to the secondary growth stage, the epidermis is completely replaced by the periderm. Within this structure, the phellem (cork) is strongly developed, characterized by layers of rectangular cells arranged in concentric rows; their cell walls are heavily impregnated with suberin, forming a robust protective barrier against microbial invasion and desiccation.

The cortex is extensively differentiated, functioning as a storage site for secondary metabolites and starch granules [10]–[12].

The stele dominates the anatomical structure, accounting for approximately 50%–60% of the total transverse diameter.

This characteristic reflects the prioritized development of vascular tissues typical of woody plants. The vascular system is organized into collateral bundles. These bundles do not function in isolation but are interconnected via a system of medullary rays (xylem rays), which facilitates efficient nutrient transport. The number of vascular bundles ranged from 30 to 35 per section, with irregular sizes; the metaxylem was larger than the protoxylem. The xylem vessels were large, with an average size of 183.46 ± 0.56 µm; their walls were thin but pre-lignified, which optimizes the flow rate of crude sap during the rapid growth phase of the plant.

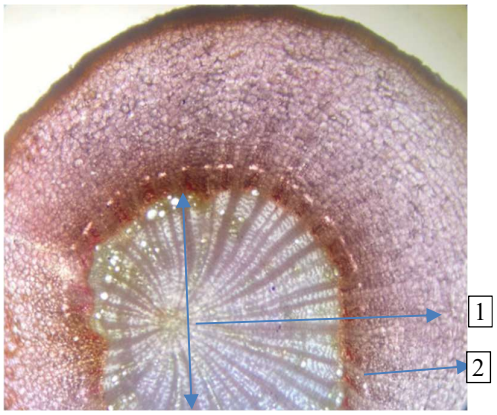

Figure 1. Transverse section of the root of *E. acuminata*; 1: Stele (Vascular cylinder); gtc: Secondary xylem; 2: Xylem ray (Medullary ray). (Source: D.T.L. Huong, N.K. Oanh).

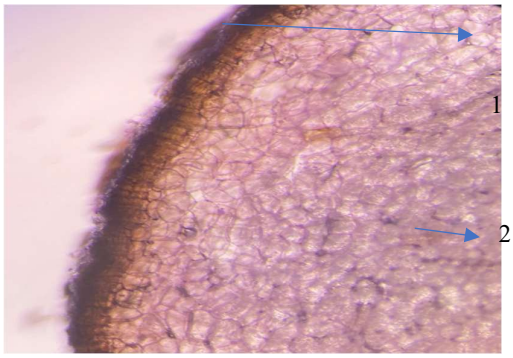

Figure 2. Root bark of *E. acuminata*; 1: Periderm, 2: Parenchyma
(Source: D.T.L. Huong, N.K. Oanh).

3.2. Stem

E. acuminata is an arborescent species, reaching an average height of 6–12 m. Young branches are characterized by a glabrous surface and prominent lenticels. The bark is typically dark gray, often exhibiting longitudinal fissures and exfoliating in irregular flakes. Upon maturity, the stem turns light brown with a smooth, glabrous surface [13]–[15].

During the primary growth stage, the stem surface is enveloped by the epidermis, consisting of tabular cells closely packed without intercellular spaces. The outer epidermal walls are covered by a resilient cuticle layer, which plays a pivotal role in regulating transpiration and preventing the infiltration of pathogens.

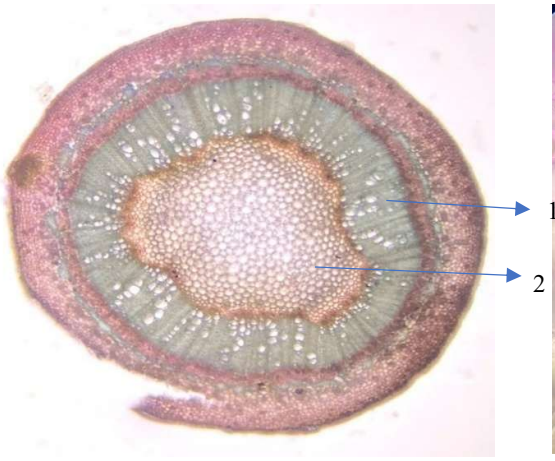

Figure 3. Transverse section of *E. acuminata* stem. 1: xylem vessel; 2: vascular bundle; 2: pith parenchyma (Source: D.T.L. Huong).

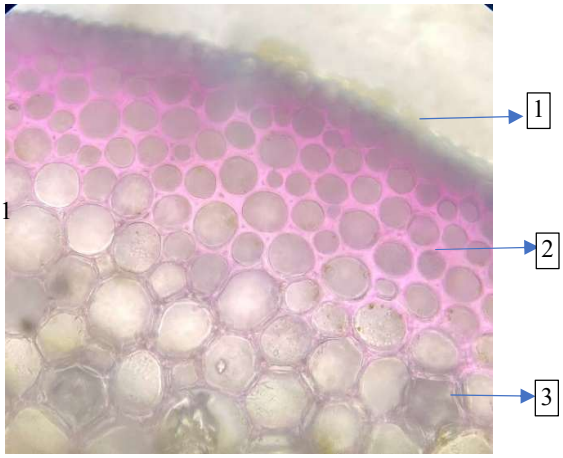

Figure 4. Bark section of *E. acuminata*. 1. Epidermis; 2. Collenchyma; 3. Calcium oxalate crystals (Source: D.T.L. Huong).

A distinguishing feature of this species is the high density of elongated multicellular trichomes (protective hairs), which form a protective buffer for internal tissues and minimize thermal radiation. Situated beneath the epidermis is a prominently developed angular collenchyma system, consisting of 4–7 cell layers. This structure ensures mechanical stability for the young stem before the completion of the lignification process (Figure 4).

In the primary stem of *E. acuminata*, the vascular system is organized into collateral bundles, with the phloem positioned exteriorly and the xylem interiorly. This represents a significant structural divergence from the root anatomy, reflecting the functional differentiation of vascular bundles in providing mechanical support for the canopy. As the plant transitions to maturity, the robust activity of the phellogen (cork cambium) leads to the formation of the periderm system. Adjacent to this are large, thin-walled parenchyma cells containing numerous starch granules and calcium oxalate crystals (a common diagnostic feature of the Boraginaceae family), which serve as vital metabolic reservoirs during periods of drought. The sclerenchyma forms a continuous ring encircling the stem, characterized by thickened, lignified walls (Photo 2). The stele is extensively developed, with both the vascular system and xylem rays occupying a significant portion of the stem's cross-sectional area. At the center lies the pith parenchyma, which functions as a storage tissue

3.3. Leaves

The leaf blades exhibit variable shapes, ranging from elliptical to obovate or oblong-obovate, with typical dimensions of 5–13 × 4–6 cm. Both surfaces are glabrous or occasionally covered with a sparsely puberulent indumentum. The leaf margins are uniformly serrate, with teeth antrorsely curved toward the apex. The apex is acute, and the petiole measures 1.5–2.5 cm in length with a smooth, glossy surface (Figure 5) [16]–[18].

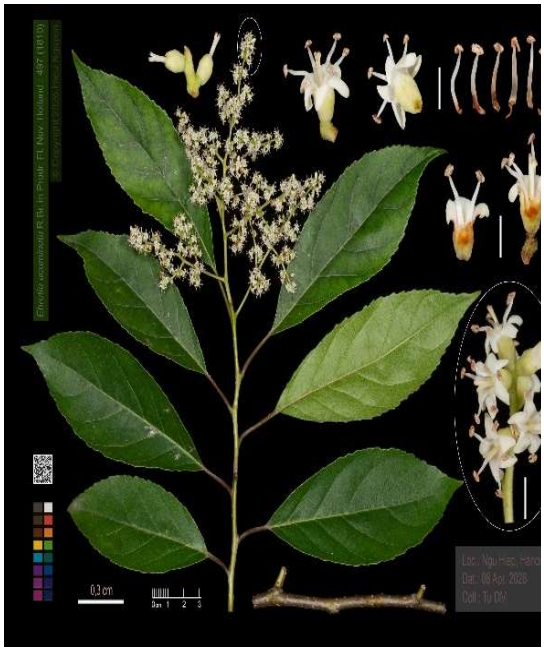

Figure 5. Branch with leaves and flowers (Source: Hieu Nguyen).

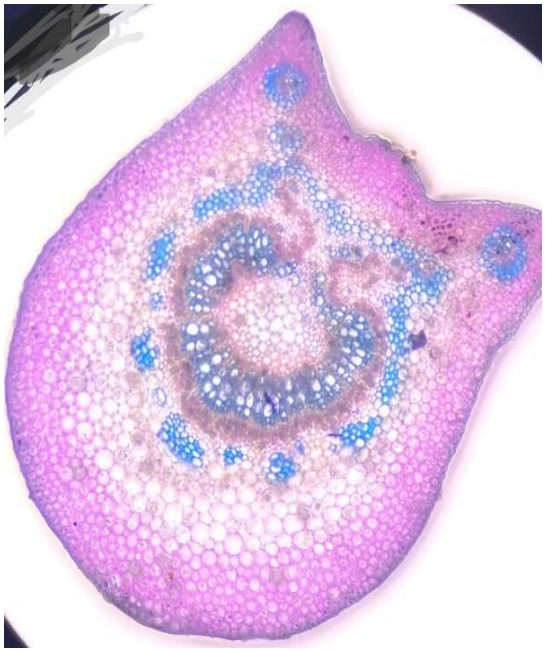

Figure 6. Transverse section of the petiole (Source: D.T.L. Huong).

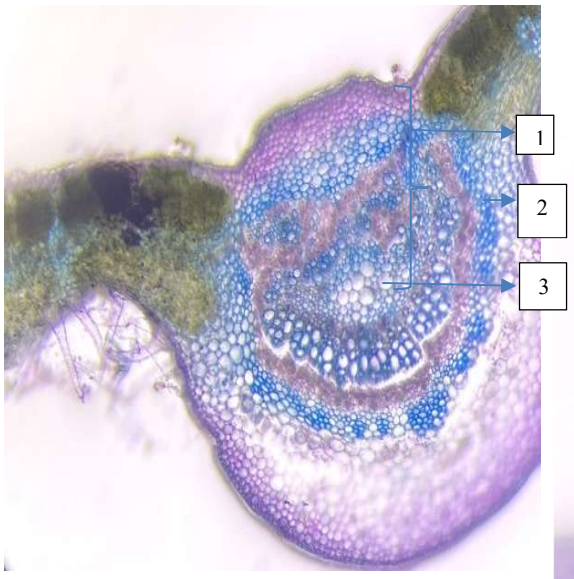

Figure 7. Transverse section of the midrib (Source: D.T.L. Huong);1.Vascular bundles; 2. Collenchyma; 3. Hard tissue.

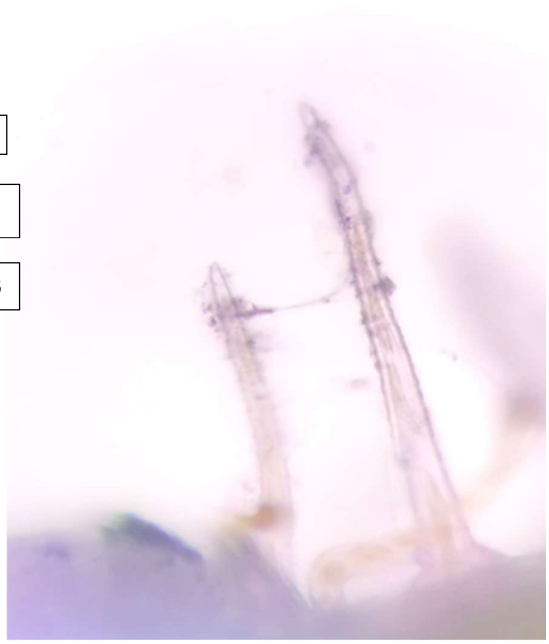

Figure 8. Trichomes (Source: D.T.L. Huong).

Petiole: The outermost structure of the petiole is defined by a layer of rectangular epidermal cells arranged in sequence, forming a resilient protective barrier. Notably, the differentiation of certain cells into multicellular trichomes (protective hairs) optimizes the organ's defense against environmental fluctuations and microbial invasion (Figure 8). Situated immediately beneath the epidermis is the angular

collenchyma (5–8 layers), primarily concentrated in the protruding ridges of the petiole–areas directly exposed to environmental stresses–providing essential mechanical support (Figure 6).

Sclerenchyma forms a discontinuous ring (3–5 layers) surrounding the vascular bundle (Figure 5, 6), providing mechanical support for the petiole and leaf blade. The petiole contains a single, arc-shaped collateral vascular bundle, with the phloem positioned exteriorly and the xylem interiorly. The xylem vessels are interconnected by a system of xylem parenchyma with uniform cell size.. Leaves had an average thickness of 305.00 ± 0.57 μm. Since the studied species is a heliophyte, the mesophyll was characteristically differentiated into palisade tissue and spongy tissue. The width of the palisade tissue was smaller than that of the spongy tissue, accounting for 24.63% and 54.57% [of the total leaf thickness], respectively. Situated beneath the upper epidermis is the palisade mesophyll, consisting of a single layer of elongated, cylindrical cells, closely packed and containing a high density of chloroplasts to optimize photosynthetic capacity. Adjacent to the lower epidermis, the spongy mesophyll is well-developed with large intercellular spaces, facilitating gas exchange through the dense system of stomata on the lower epidermis.

The structure of the leaf midrib is enveloped by well-developed vascular bundles (xylem and phloem), ensuring high efficiency in water and nutrient transport. The presence of a thick cuticle layer, combined with a high density of trichomes, not only creates the characteristic silvery-white appearance but also establishes a light-reflective mechanism. This mechanism minimizes surface heating and protects photosynthetic enzymes from photo-denaturation. These anatomical features serve as evidence for the species' effective survival strategy under high-intensity light conditions (Figure 7).

Vascular System: The vascular system is highly developed, increasing the contact surface area to form a dense network across the entire leaf blade. The presence of a thick cuticle layer, averaging 2.05 ± 0.32 μm in thickness, combined with a dense density of trichomes, not only creates the characteristic silvery-white appearance but also establishes a light-reflective mechanism, minimizing surface heating and protecting photosynthetic enzymes from photodegradation.

3.4. Flowers and Fruits

The inflorescence is a paniculate cyme, averaging 8–15 × 5–8 cm in size; the rachis is puberulent or subglabrous. Flowers are densely clustered and emit a characteristic fragrance. The calyx is 1.5–2 mm long, with ovate lobes featuring ciliate margins. The corolla is white, campanulate (bell-shaped), with a total length of 3–4 mm; the oblong corolla lobes are spreading and noticeably longer than the corolla tube. The stamens are exerted from the corolla throat; filaments are 2–3 mm long, adnate to the upper part of the corolla tube (approximately 0.5–1 mm from the base); anthers are ovate and roughly 1 mm in size. The style measures 1.4–2.5 mm in length, terminating in stigmatic branches approximately 0.5 mm long [17]–[20].

The fruit is a globose drupe, 3–4 mm in diameter, transitioning from yellow to orange upon maturity;

each pyrene contains two seeds.

Figure 12. Flowering branch
(Source: Hieu Nguyen).

Figure 11. Flower morphology
(Source: Hieu Nguyen).

Figure 13. Stamen morphology
(Source: Hieu Nguyen).

4. Conclusion

The morphological and anatomical characteristics of *E. acuminata* are consistent with the general diagnostic features of the genus *Ehretia*. These data contribute to the scientific framework for the classification and identification of the species.

Based on the analysis of *E. acuminata* specimens collected in Hanoi, this study has established a detailed dataset of its morphology and anatomy. The anatomical analysis of vegetative and reproductive organs (root, stem, leaf, flower, and fruit) reveals distinct adaptive traits typical of the Boraginaceae family. These anatomical structures reflect the characteristic features of a heliophilous (light-loving) plant species, predominantly distributed in secondary forests.

Acknowledgments

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