



Integrated Morphological, Anatomical, Biochemical and Molecular Characterization of *Uraria crinita* (Fabaceae) in Vietnam

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ABSTRACT

Background: *Uraria crinita* (L.) Desv. ex DC. (Fabaceae) is a medicinal legume traditionally used in Vietnam and other Asian regions. However, comprehensive studies combining morphology, anatomy, biochemistry and molecular data are scarce. Integrating biochemical profiling and DNA barcoding with classical taxonomy ensures accurate identification, conservation and utilization of this valuable species.

Methods: A specimen of *U. crinita* collected from Quang Tri Province (Vietnam) was examined for morphological, anatomical and biochemical characteristics. Microscopic sections were prepared from FAA-fixed tissues for structural observation. Total phenolic and flavonoid contents were determined by spectrophotometric assays. Genomic DNA was extracted using the CTAB protocol and two regions (*rbcL* and *ITS4-5*) were amplified, sequenced and compared through BLAST and phylogenetic analysis using MEGA X.

Result: The specimen exhibited characteristic features of Fabaceae, including imparipinnate compound leaves and purple racemose inflorescences. Anatomical observations confirmed dicotyledonous organization, particularly dense trichomes on the abaxial epidermis. Biochemical analysis revealed high total phenolic (22.19 ± 0.54 mg GAE/g DW) and flavonoid (23.37 ± 0.49 mg CE/g DW) contents, suggesting strong antioxidant potential. Molecular analysis showed that *ITS* (669 bp) and *rbcL* (540 bp) sequences shared 99-100% similarity with *U. crinita* accessions in GenBank (JN189714.1 and OQ885477.1), confirming the taxonomic identity. This integrative study combining morphological, anatomical, biochemical and molecular evidence provides comprehensive support for the identification of *U. crinita*. The biochemical richness of the species underlines its potential as a bioactive legume resource, contributing to its genetic conservation and pharmacological exploration.

Key words: Anatomy, Biochemical traits, DNA barcoding, Fabaceae, Morphology, *Uraria crinita*.

INTRODUCTION

The Fabaceae family comprises a wide range of economically and medicinally important species (Jahan and Rahman, 2022). They play crucial roles in global agriculture, pharmacology and ecosystem balance (Yen *et al.*, 2001; Dai *et al.*, 2021; Ali *et al.*, 2022). Numerous studies worldwide have highlighted the floristic diversity, medicinal importance and conservation concerns of Fabaceae species across tropical and subtropical regions, emphasizing the need for comprehensive taxonomic and biological investigations within this family (Hu *et al.*, 2023; Zhang and Cao, 2025).

Among these, *Uraria crinita* (L.) Desv. ex DC. is a traditional medicinal legume widely distributed in tropical Asia, including Vietnam (Thien *et al.*, 2021). The species has been used for centuries to treat arthritis, musculoskeletal pain, soft tissue injuries, respiratory disorders, diarrhea and general physical weakness (Dai *et al.*, 2021).

Previous phytochemical investigations have revealed that *U. crinita* contains diverse secondary metabolites such as isoflavanones, flavonoids, triterpenoids and saponins, which exhibit significant anti-inflammatory, antioxidant, hepatoprotective and anticancer activities (Mao *et al.*, 2014; Liu *et al.*, 2010; Yen *et al.*, 2001). These properties underscore its potential for pharmaceutical development and sustainable cultivation as a valuable leguminous medicinal crop in Vietnam (Thien *et al.*, 2021; Thien *et al.*, 2022).

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The genus *Uraria*, comprising more than 35 recognized species distributed mainly in tropical and subtropical regions of Asia, Africa and Oceania, includes several plants of ethnomedicinal importance (Chao *et al.*, 2017). Members of this genus are characterized by their rich phytochemical diversity and biological activities (Sharad *et al.*, 2024; Gurav *et al.*, 2008). However, most previous studies have primarily focused on phytochemical screening and pharmacological evaluation, while information regarding the morphological, anatomical, biochemical and genetic features of *U. crinita* remains limited. This lack of integrative data hinders precise taxonomic identification, comparative evaluation among populations and effective strategies for conservation and utilization.

Integrating biochemical profiling and DNA barcoding with classical morphological and anatomical analyses provides a more robust foundation for species authentication and genetic characterization (Chen *et al.*, 2023; Barykina and Kramina, 2006). Therefore, the present study was designed to conduct an integrated investigation of the morphological, anatomical, biochemical and molecular characteristics of *U. crinita* collected from Quang Tri Province, Vietnam. This work aims to establish comprehensive baseline data supporting accurate taxonomic identification, genetic conservation and potential biotechnological or pharmacological applications of this bioactive legume species.

MATERIALS AND METHODS

Plant materials

Specimens of *Uraria crinita* were collected from Lao Bao Commune, Quang Tri Province, Vietnam, in May 2025. Healthy, pest-free plants at the physiologically mature stage were selected for analysis. Young leaves for molecular identification were placed in Eppendorf tubes containing dry silica gel. Samples for anatomical study were fixed in FAA solution (Formalin-Acetic acid-Alcohol, 90:5:5 v/v). Morphological and anatomical studies were conducted at the Department of Biology, University of Education - Hue University, while molecular analyses were performed at the Institute of Biotechnology, Hue University.

Methods

Morphological characterization

Morphological characteristics were recorded using qualitative observations and quantitative measurements following the method of Nguyen (2008). For each trait, measurements were taken from 15-30 individuals and the mean values were used for analysis. The examined traits included plant height, compound leaf length, number of leaflets per leaf, size of the largest leaflet, hair density on stems and leaves, inflorescence length and width, petal length, number of pod segments and seed size. Descriptive statistics were calculated for all parameters (mean±SD).

Anatomical characterization

The anatomical structures of roots, stems and leaves were examined using the sectioning and staining method of Barykina and Kramina (2006). Fixed tissues were dehydrated, sectioned with a microtome, stained with safranin and fast green and mounted in glycerin jelly. Measurements were made using an ocular micrometer and stage micrometer following the method of Hajiboland *et al.* (2012), with 20 replicates per structure. Images were captured using a high-resolution digital microscope camera.

Molecular method

DNA extraction

Total genomic DNA was extracted from leaves of *U. crinita* using the FastPure Plant DNA Isolation Mini Kit (Vazyme, China) according to the manufacturer's protocol. DNA quality and integrity were verified by 1% agarose gel electrophoresis in 1× TAE buffer and visualized using GelRed staining (Biotium, USA).

PCR amplification and sequencing

Two gene regions, *rbcL* and *ITS4-5*, were amplified by PCR using specific primers (Table 1) (Hoang *et al.*, 2023; White *et al.*, 1990). The reaction mixture (50 µL total volume) contained 25 µL GoTaq® Green Master Mix (2×, Promega), 1 µL of each primer (10 pmol/µL, IDT, USA), 100 ng template DNA and sterile distilled water. PCR conditions included an initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 45 s, 56°C for 45 s, 72°C for 60 s; and a final extension at 72°C for 10 min.

PCR products were separated on 1% agarose gels, visualized under UV light and purified using the ISOLATE II PCR and Gel Kit (Bioline, UK). Purified amplicons were sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems, USA) at 1st BASE (Malaysia).

Sequence analysis

Raw sequences were edited using BioEdit and aligned using BLAST against the NCBI GenBank database for species confirmation. Phylogenetic relationships were inferred by the Maximum Likelihood method under the Tamura-Nei model (Tamura and Nei, 1993) implemented in MEGA X (Kumar *et al.*, 2018; Felsenstein, 1985).

Biochemical characterization

Biochemical analyses were conducted on the aerial parts (leaves and stems) of *Uraria crinita* to evaluate their primary and secondary metabolic constituents. All assays were performed in triplicate and results were expressed as mean±SD.

Moisture content was determined by the gravimetric method, in which samples were oven-dried at 105°C to constant weight. Total protein content was quantified using the Bradford method (Bradford, 1976) with bovine serum albumin as a standard. Total lipid content was estimated following Soxhlet extraction with petroleum ether. Reducing sugar content was determined according to the Bertrand titrimetric method, based on the reduction of cupric ions (Cu²⁺) to cuprous oxide (Cu₂O) in an alkaline medium, followed by titration with potassium permanganate (KMnO₄).

Table 1: Nucleotide sequences of primer pairs.

Primer	Nucleotide sequence (5' → 3')	References
<i>rbcLaF</i>	5'- ATGTCACCACAAACAGAGACTAAAGC-3'	Hoang <i>et al.</i> , 2023
<i>rbcLaR</i>	5'- GTAAAATCAAGTCCACCRCG -3'	
<i>ITS4</i>	5'-TCCTCCGCTTATTGATATGC-3'	FJ599761; White <i>et al.</i> , 1990
<i>ITS5</i>	5'-GGAAGGAGAAGTCGTAACAAGG-3'	

Vitamin C content was measured by iodometric titration using a standard iodine (I_2) solution and results were expressed as milligrams of ascorbic acid per gram of fresh weight ($mg\ g^{-1}\ FW$) (Trang *et al.*, 2024).

Total flavonoid content (TFC) was determined by the AlCl₃ colorimetric method described by Ribarova *et al.* (2005) with slight modifications. The reaction was carried out in an alkaline medium and absorbance was measured spectrophotometrically at 415 nm using catechin as the standard. Results were expressed as milligrams of catechin equivalents per gram of fresh weight ($mg\ CE\ g^{-1}\ FW$).

Total polyphenol content (TPC) was determined by the Folin-Ciocalteu method as described by Feduraev *et al.* (2019) with minor modifications. The absorbance of the blue complex was measured at 765 nm and results were expressed as milligrams of gallic acid equivalents per gram of fresh weight ($mg\ GAE\ g^{-1}\ FW$).

Statistical analysis

Morphological, anatomical and biochemical parameters were expressed as mean $\pm$ standard deviation (SD). Descriptive statistics and graphical summaries were prepared using Microsoft Excel 2017.

RESULTS AND DISCUSSION

Morphological characteristics

The morphological characteristics of the collected *Uraria crinita* specimens were analyzed and the results are summarized in Table 2. Quantitative data were obtained from 30 individuals per trait and expressed as mean $\pm$ standard deviation (SD).

Morphological observations showed that *U. crinita* (commonly known as Fox Tail or Dog Tail) is an erect, perennial shrub ranging from 100 to 150 cm in height, with an average stem height of 125.28 cm (Fig 1a). The stem is cylindrical and densely covered with short white trichomes.

Leaves are imparipinnate and compound, bearing 5-7 leaflets on lower branches and 9-11 leaflets on upper branches. Compound leaves are 30-42 cm long (average 34.62 cm) and both the petiole and rachis are densely pubescent (Fig 1b).

Table 2: Morphological characteristics of *Uraria crinita*.

Parameter	Unit	Value (Mean $\pm$ SD)
Stem height	Cm	125.28 $\pm$ 11.04
Length of compound leaf	Cm	34.62 $\pm$ 3.51
Length of the largest leaflet	Cm	14.81 $\pm$ 2.01
Width of the largest leaflet	Cm	3.12 $\pm$ 0.46
Inflorescence length	Cm	24.42 $\pm$ 4.55
Inflorescence width	Cm	2.49 $\pm$ 0.39
Length of representative petal	Mm	7.50 $\pm$ 1.14
Number of segments per pod	segments	4.10 $\pm$ 1.45
Seed size	Mm	2.06 $\pm$ 0.10

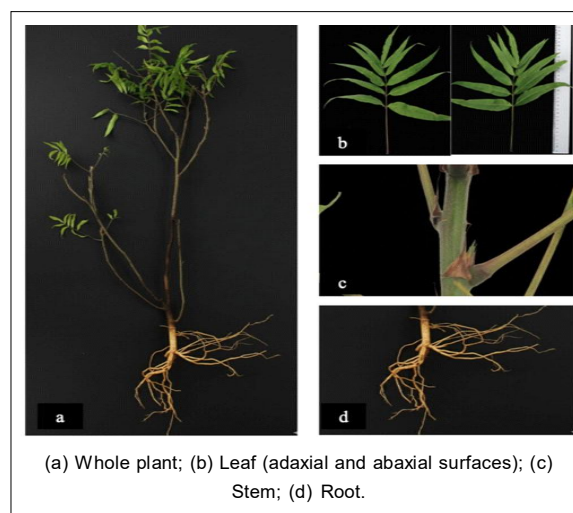


Fig 1: Morphology of *Uraria crinita*.

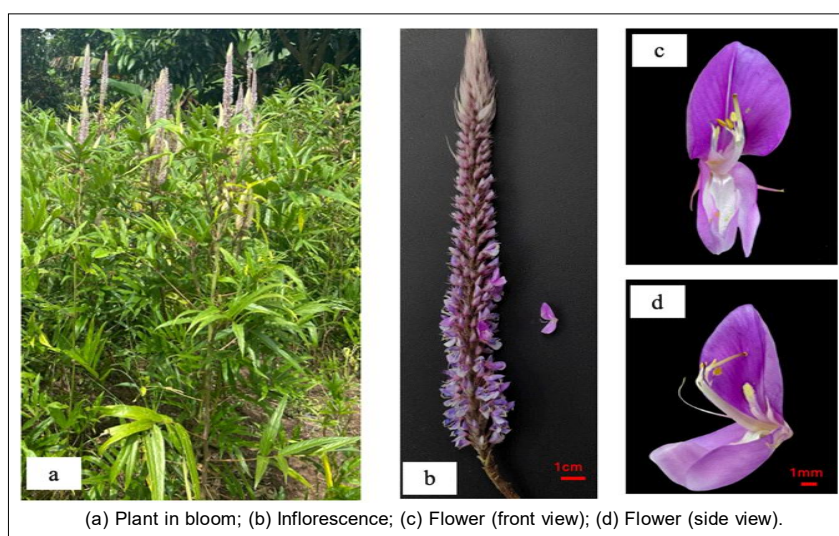


Fig 2: Flower morphology of *Uraria crinita*.

The leaflets are lanceolate, averaging 14.81×3.12 cm, with a smooth adaxial (upper) surface and a densely pubescent abaxial (lower) surface. These features are consistent with descriptions in the *Flora of Vietnam* (Pham, 1999) and The genus *Uraria* (Leguminosae) in China (Ohashi *et al.*, 2006), which identify dense indumentum and compound leaves as diagnostic traits of *U. crinita*.

The root system is of the taproot type, with a strong main root penetrating deeply into the soil and giving rise to numerous lateral roots that spread obliquely or horizontally (Fig 1d). The roots are light yellowish-brown, long and slender relative to their diameter. This root morphology is similar to that of other *Uraria* species where a well-developed lateral root system supports adaptation to well-drained soils (Wu and Raven, 2010; Pham, 1999; Deshmukh and Kothale, 2019).

The inflorescences are robust, terminal racemes with a distinct purple coloration (Fig 2). They measure 24.42×2.49 cm on average and the standard petals range from 6 to 9 mm in length (mean = 7.50 mm). The flowering period occurs from September to November under natural field conditions. The flowers are papilionaceous, with a diadelphous stamen arrangement (9 + 1) and a superior ovary - characteristics typical of the Fabaceae family.

The fruits are slightly pubescent pods, segmented into 2-6 constricted joints, each containing one seed (Fig 3a). Pods display distinct longitudinal veins and the seeds are dark brown, bean-shaped and range from 2.0 to 2.2 mm in diameter (mean = 2.06 mm) (Fig 3b).

Overall, the specimens collected in Quang Tri Province correspond closely to the morphological features of *U. crinita* described in earlier taxonomic references, including

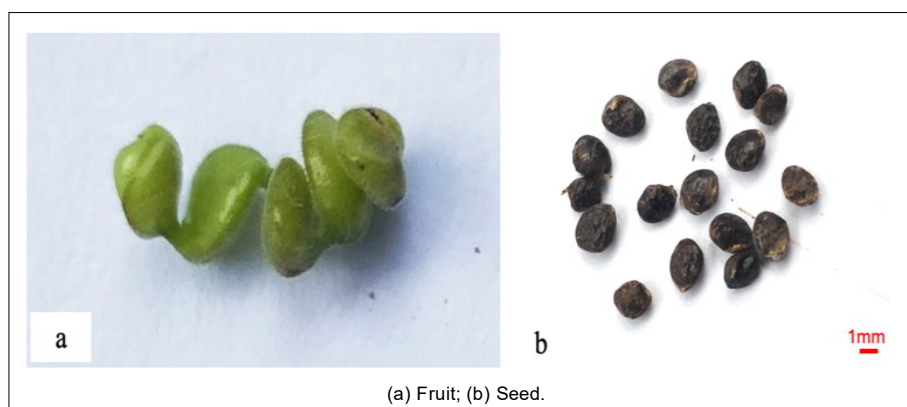


Fig 3: Fruit and seed morphology of *Uraria crinita*.

Table 3: Quantitative anatomical characteristics of vegetative organs of *Uraria crinita*.

Organ	Anatomical feature	Unit	Value (Mean $\pm$ SD)
Root	Cork layer thickness	μm	78.95 $\pm$ 5.34
	Cork layer thickness relative to root radius	%	5.31 $\pm$ 0.33
	Number of xylem vessels per mm^2	vessels/ mm^2	19.16 $\pm$ 1.18
	Diameter of large xylem vessels	μm	43.35 $\pm$ 4.37
	Diameter of small xylem vessels	μm	24.30 $\pm$ 3.99
Stem	Epidermal thickness	μm	46.13 $\pm$ 5.59
	Epidermal thickness relative to stem radius	%	2.54 $\pm$ 0.31
	Number of xylem vessels per mm^2	vessels/ mm^2	15.22 $\pm$ 0.84
	Diameter of large xylem vessels	μm	36.65 $\pm$ 3.39
Leaf midrib	Diameter of small xylem vessels	μm	16.55 $\pm$ 3.25
	Epidermal thickness of main vein	μm	9.70 $\pm$ 0.65
	Epidermal thickness relative to total midrib thickness	%	0.93 $\pm$ 0.07
	Number of xylem vessels	vessels	62.60 $\pm$ 6.01
Leaf - Lamina	Diameter of xylem vessels	μm	16.35 $\pm$ 3.31
	Epidermal thickness	μm	11.50 $\pm$ 2.09
	Epidermal thickness relative to total lamina thickness	%	12.02 $\pm$ 1.92
Petiole	Epidermal thickness	μm	21.95 $\pm$ 3.74
	Epidermal thickness relative to petiole radius	%	1.65 $\pm$ 0.31
	Number of xylem vessels per mm^2	vessels/ mm^2	24.59 $\pm$ 1.45
	Diameter of large xylem vessels	μm	27.65 $\pm$ 2.30
	Diameter of small xylem vessels	μm	16.70 $\pm$ 3.15

leaf arrangement, dense indumentum, racemose inflorescences and segmented pods (Pham, 1999; Ohashi *et al.*, 2006; Mudavath *et al.*, 2024). These diagnostic traits, together with anatomical and molecular evidence presented in subsequent sections, confirm the accurate identification of the studied specimens as *Uraria crinita* (Fabaceae).

Anatomical characteristics

Microscopic examinations and quantitative analyses of the anatomical features of *Uraria crinita* were performed under laboratory conditions. The quantitative characteristics of the root, stem and leaf tissues are summarized in Table 3.

Root

The transverse section of the root of *U. crinita* is circular or nearly circular in outline (Fig 4). The outermost layer is the cork (phellem), consisting of multiple rows of compact, dark-stained cells averaging 78.95 μm in thickness, corresponding to approximately 5.31% of the total root radius. This layer replaces the exfoliated epidermis and serves as a protective barrier against water loss and pathogen invasion.

Beneath the cork lies the cortex, composed of several layers of polygonal, thin-walled parenchyma cells with distinct intercellular spaces that facilitate aeration and storage of water and organic nutrients. The endodermis forms a clearly defined circular boundary between the

cortex and the stele, with thickened cell walls that regulate solute movement.

The vascular cylinder exhibits a typical radial arrangement, with alternating xylem and phloem strands. The phloem consists of small, thin-walled cells responsible for translocation of photosynthates, while the xylem is composed of vessels of varying sizes, oriented toward the center. The central region is dominated by large xylem vessels (37-50 μm in diameter; mean = 43.35 μm), interspersed with smaller vessels (20-30 μm ; mean = 24.30 μm). The average vessel density is 19.16 vessels per mm^2 and the central pith is poorly developed or nearly absent.

Stem

The transverse section of the stem of *Uraria crinita* is circular or nearly circular in outline (Fig 5). The overall anatomical organization follows the typical dicotyledonous stem structure, consisting of epidermis, cortex, vascular bundles and pith. Quantitative anatomical data are presented in Table 3.

The outermost epidermis is composed of a single layer of compact, polygonal cells with an average thickness of 46.13 μm , representing approximately 2.54% of the stem radius. The epidermal surface is covered with fine, uniseriate trichomes that provide mechanical protection and minimize transpiration. Beneath the epidermis lies a 2-3-layer collenchyma composed of irregularly shaped cells

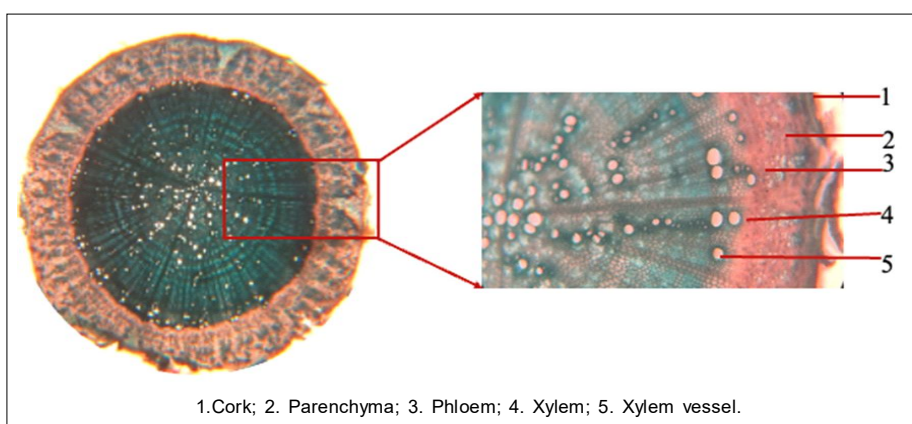


Fig 4: Transverse section of the root of *Uraria crinita*.

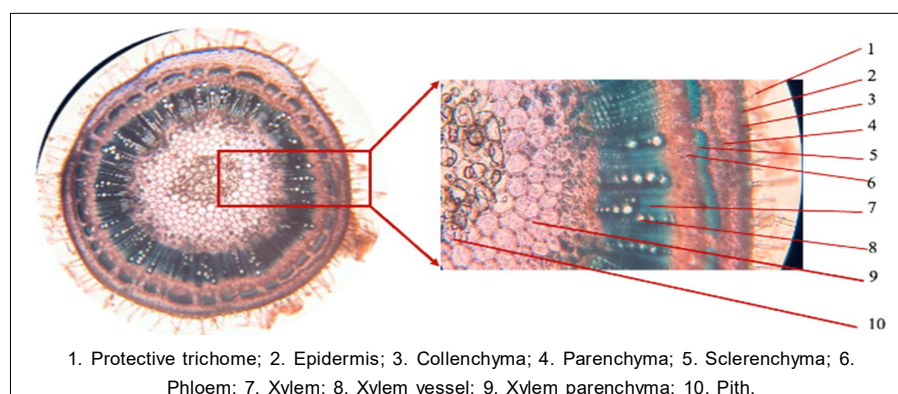


Fig 5: Transverse section of the stem of *Uraria crinita*.

with partially thickened walls, contributing to stem flexibility and mechanical support.

The subsequent parenchyma layer consists of 4-6 layers of thin-walled cells of variable sizes with large intercellular spaces, functioning in nutrient storage and aeration. Below the parenchyma, a sclerenchyma layer of 4-7 cell layers forms arc-shaped, lignified clusters situated above each phloem bundle. This tissue strengthens the stem and prevents collapse during secondary growth.

The vascular bundles are arranged in a continuous ring, with distinct xylem and phloem zones. The xylem region contains large vessels, xylem fibers and parenchyma cells, interspersed with well-defined radial wood rays. The average vessel density is 15.22 vessels/mm². Large xylem vessels (outer region) have diameters of 30-40 µm (mean = 36.65 µm), while smaller vessels (inner region) range from 13-20 µm (mean = 16.55 µm). The pith occupies the central region and is composed of thin-walled, irregularly arranged parenchyma cells of various sizes.

Leaf

The leaf anatomy of *Uraria crinita* was examined in both the midrib and lamina regions (Fig 6-7). The leaf exhibits typical dorsiventral structure, consistent with other members of the Fabaceae family (Pham, 1999; Al-Joboury *et al.*, 2017).

Midrib

The transverse section of the midrib is well developed, prominently convex on the lower surface and nearly flat on the upper surface (Fig 6). The epidermis consists of upper and lower layers, each formed by a single row of compact rectangular cells averaging 9.70 µm in thickness, accounting for approximately 0.93% of the total midrib thickness. The lower epidermis is densely covered with unicellular trichomes.

Beneath the upper epidermis lies the palisade parenchyma composed of 2-3 layers of small, tightly packed cells rich in chloroplasts. The spongy parenchyma occurs below the lower epidermis and consists of large, irregularly shaped cells with wide intercellular air spaces facilitating

gaseous exchange. Beneath these tissues, the collenchyma is formed by 3-5 layers of polygonal or rounded cells of varying sizes, providing mechanical support.

The phloem, located below the collenchyma, is organized in a curved band composed of 2-3 layers of nearly round cells with relatively uniform dimensions. The xylem vessels are circular, arranged in an arc within the central midrib

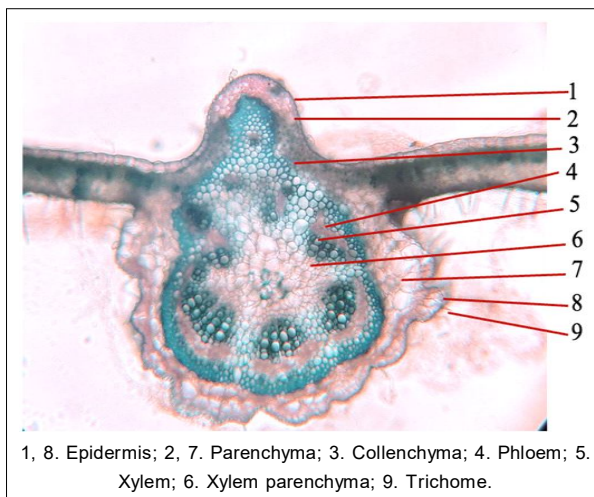


Fig 6: Transverse section of the leaf of *Uraria crinita*.

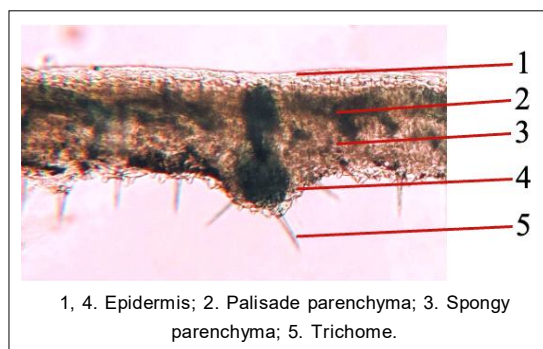


Fig 7: Transverse section of the leaf lamina of *Uraria crinita*.

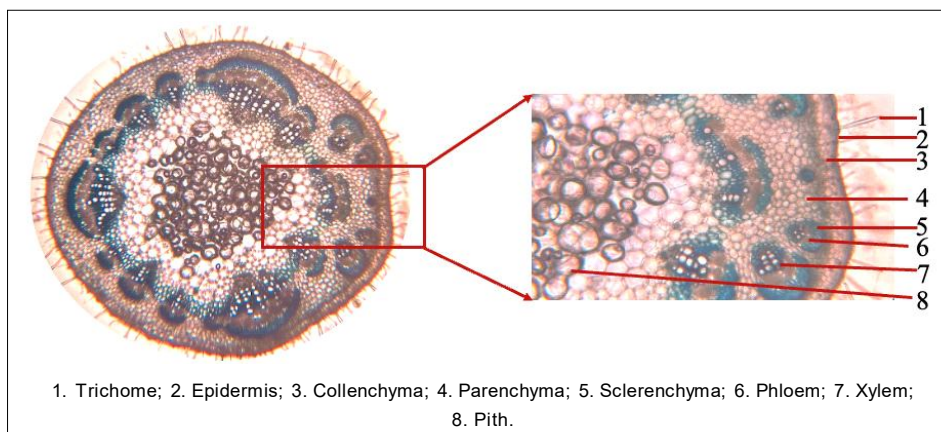


Fig 8: Transverse section of the petiole of *Uraria crinita*.

region. The number of xylem vessels ranges from 50 to 68, with an average of 62.6 vessels and an average diameter of 16.35 μm . The xylem parenchyma and fibers are interspersed among vessels, forming a continuous vascular strand.

These anatomical characteristics indicate a well-developed vascular system adapted for efficient water and nutrient transport. The presence of abundant trichomes on the abaxial surface suggests adaptation to reduce water loss and deter herbivory under tropical conditions.

Lamina

The lamina ranges from 170 to 220 μm in total thickness. It consists externally of an upper and a lower epidermis, each comprising a single layer of rectangular cells arranged compactly. The upper epidermis is slightly thicker (11.50 μm on average), accounting for approximately 10.02% of the total lamina thickness, while the lower epidermis is densely covered with trichomes (Fig 7).

The mesophyll is differentiated into palisade and spongy tissues. The palisade mesophyll is composed of a single layer of elongated, chloroplast-rich cells responsible for photosynthesis. The spongy mesophyll consists of irregularly shaped cells of variable size, loosely arranged to form large intercellular air spaces, facilitating gas exchange.

Petiole

The transverse section of the petiole shows a nearly circular outline with a slightly convex abaxial surface (Fig 8). The epidermis consists of a single layer of rectangular or polygonal cells arranged compactly, averaging 21.95 μm in thickness and accounting for approximately 1.65% of the petiole radius. The epidermal surface is externally covered with numerous unicellular protective trichomes, which likely reduce transpiration and deter herbivory.

Immediately beneath the epidermis lies a layer of angular collenchyma, particularly well-developed in the convex regions of the petiole, providing additional

mechanical support. Below the collenchyma is a multilayered parenchyma tissue composed of 3-5 layers of thin-walled polygonal cells with relatively uniform sizes.

The vascular system consists of numerous collateral vascular bundles arranged in an arc. Each vascular bundle comprises phloem on the outer side and xylem on the inner side. The phloem is made up of small, thin-walled cells responsible for translocating photosynthates, while the xylem contains large, round vessels with thickened walls facilitating water transport. The large xylem vessels have an average diameter of 27.65 μm , whereas the smaller ones average 16.70 μm . The mean vessel density is approximately 24.59 vessels mm^{-2} .

The vascular bundles are surrounded by sclerenchymatous tissue, which provides additional rigidity and protection. At the center of the petiole lies the pith, consisting of large, thin-walled polygonal or rounded parenchyma cells functioning in storage and structural maintenance.

Overall, the combination of anatomical features - including the dorsiventral leaf structure, arc-shaped sclerenchyma in the stem, radial xylem-phloem arrangement in the root and collateral vascular bundles in the petiole - represents a structural framework characteristic of the Fabaceae family (Pham, 1999; Silva *et al.*, 2012; Al-Joboury *et al.*, 2017). Together with the morphological and molecular evidence presented in the following sections, these diagnostic features provide strong confirmation that the examined specimens are correctly identified as *Urvia crinita* (Fabaceae).

Species identification by molecular biology

The scientific name of the Green-haired *Urvia* was re-identified based on the two gene regions *ITS4–5* in the nuclear genome and the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene region using specific primers as shown in Table 1. The results of analysis sequencing by the Sanger method showed that the obtained sizes of the *ITS4-5* and *rbcL* gene regions in

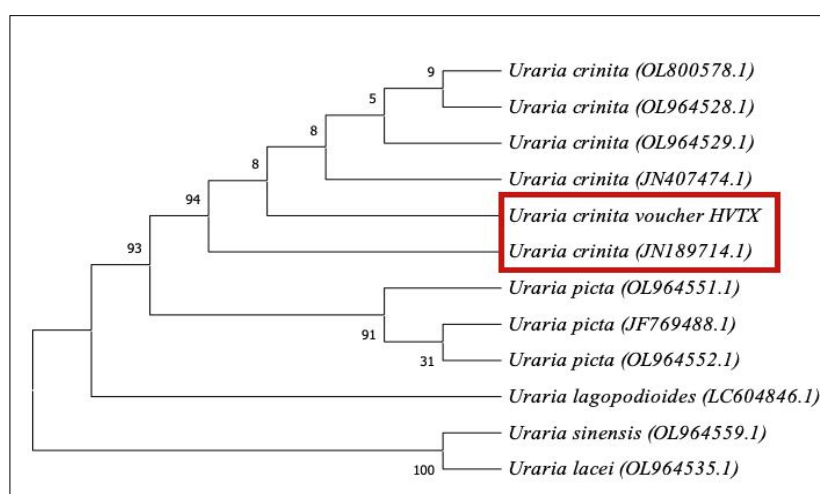


Fig 9: Phylogenetic tree based on the *ITS4–5* gene region of *Urvia crinita* voucher HVTX compared with reference sequences from GenBank.

the green-haired peacock were 669 bp (*ITS4-5*) and 540 bp (*rbcL*), respectively. The nucleotide sequences of these two gene regions were highly similar ($\leq 99\%$) to the corresponding gene region sequences published in Genbank of the species with the scientific names *Uraria crinita* (accession no. JN189714.1 of gene region *ITS4-5* and accession no. OQ885477.1 of gene region *rbcL*). The phylogenetic tree presentation in Fig 9 and 10 also shows that the Green-haired *Uraria* plant is in the same branch as the *Uraria crinita* species, with the corresponding Genbank registration codes as above. Thus, the Green-haired *Uraria* plant that we studied and collected in Lao Bao, Quang Tri, Vietnam, has the scientific name *Uraria crinita* and we named it *Uraria crinita* voucher HVTX. All nucleotide sequences of the gene region were deposited in GenBank with the corresponding reference codes PX495927 (*ITS4-5*) and PX776575 (*rbcL*).

The congruent phylogenetic topologies obtained from both nuclear and chloroplast markers confirmed that the examined specimen formed a monophyletic clade with previously reported *U. crinita* accessions, providing robust molecular evidence for its species identity. Consequently, the studied specimen was formally confirmed and registered under the certified name *Uraria crinita* voucher HVTX.

The use of two independent DNA barcoding loci significantly enhances taxonomic resolution and minimizes

potential misidentification arising from intraspecific variation or hybridization (Hebert *et al.*, 2003; Kress and Erickson, 2007). These molecular data establish a reliable reference for *U. crinita* populations in Vietnam and provide a foundation for future studies on biochemical characteristics, genetic diversity and phylogenetic relationships within the genus *Uraria*.

Biochemical characteristics

Biochemical profiling provides valuable insight into the nutritional and pharmacological potential of *Uraria crinita*. The analysis of the above-ground parts revealed the presence of several key biochemical constituents, including moisture content, lipids, proteins, reducing sugars, vitamin C, total flavonoids and total polyphenols. The quantitative data of these components are presented in Table 4.

The biochemical composition of the above-ground parts (stems and leaves) of *U. crinita* indicates a moderate moisture level ($8.80 \pm 0.20\%$), indicating good stability during storage. Lipids accounted for the highest proportion ($8.67 \pm 1.15\%$), followed by reducing sugars ($2.14 \pm 0.05\%$), whereas protein and vitamin C were present in relatively low amounts ($0.417 \pm 0.005\%$ and $0.140 \pm 0.005\%$, respectively) (Table 4).

Although *U. crinita* exhibited a relatively higher lipid proportion compared to some other Fabaceae species, this level is still considered low enough to be favorable for

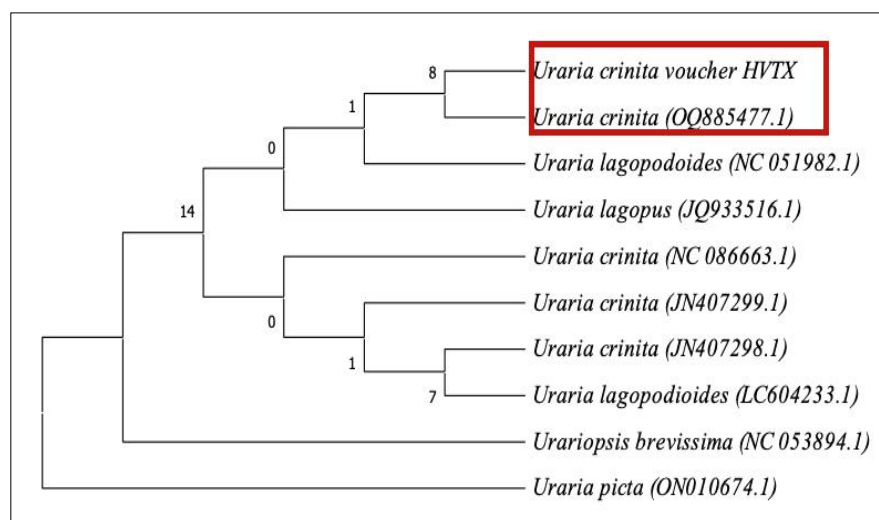


Fig 10: Phylogenetic tree based on the *rbcL* gene region of *Uraria crinita* voucher HVTX compared with reference sequences from GenBank.

Table 4: Some biochemical components of the above-ground parts of *Uraria crinita*.

Parameter	Unit	Value (Mean $\pm$ SD)
Moisture content	%	8.80 $\pm$ 0.20
Lipid	%	8.67 $\pm$ 1.15
Protein	mg/100 g	0.417 $\pm$ 0.005
Reducing sugars	mg/100 g	2.14 $\pm$ 0.05
Vitamin C	mg/100 g	0.140 $\pm$ 0.005
Total flavonoid content (TFC)	mg CE/g	23.37 $\pm$ 0.49
Total polyphenol content (TPC)	mg GAE/g	22.19 $\pm$ 0.54

the extraction of bioactive components. If the lipid content were excessively high, it could increase the viscosity of the extract, complicating filtration and purification processes. Moreover, lipid-rich extracts are often unstable and may develop undesirable odors during storage (Trang *et al.*, 2024). Therefore, the moderate lipid content of *U. crinita* is advantageous for obtaining stable extracts with high purity and potential biological activity.

The total flavonoid content (TFC) and total polyphenol content (TPC) were 23.37 ± 0.49 mg CE/g and 22.19 ± 0.54 mg GAE/g, respectively, demonstrating that the extract is rich in antioxidant phytochemicals (Table 4). These results suggest that the extract possesses considerable biochemical potential for pharmacological and nutraceutical applications. These values are notably higher than those reported for many other Fabaceae species. For instance, *Lathyrus* species exhibit TPC values of 0.17-5.10 mg GAE/g (Yazici *et al.*, 2019).

CONCLUSION

This study provides an integrative characterization of *Uraria crinita* (Fabaceae) collected from Quang Tri Province, Vietnam, through a combination of morphological, anatomical, molecular and biochemical analyses. Morphological observations confirmed that the specimens share key diagnostic traits with previously described *U. crinita* accessions, including densely pubescent stems and leaves, racemose purple inflorescences. Anatomical investigations further revealed typical Fabaceae structural features—such as a dorsiventral leaf, a dicotyledonous stem with arc-shaped sclerenchyma and a radial xylem-phloem arrangement in the root—supporting its taxonomic placement.

Molecular identification based on both nuclear (*ITS4-5*) and chloroplast (*rbcl*) DNA barcode regions confirmed a 99-100% sequence similarity with authenticated *U. crinita* accessions in GenBank. Phylogenetic analyses using the Maximum Likelihood method consistently placed the examined specimen within a strongly supported monophyletic clade of *U. crinita*, thus providing robust molecular evidence for accurate species identification.

Biochemical profiling of the above-ground parts (stems and leaves) revealed a balanced composition with moderate moisture and lipid levels, low protein, reducing sugars and vitamin C contents and notably high concentrations of total flavonoids and polyphenols. The high levels of antioxidant compounds highlight the pharmacological and nutraceutical potential of *U. crinita* and distinguish it from many other Fabaceae species.

Overall, the integration of morphological, anatomical, molecular and biochemical data establishes a comprehensive reference for *Uraria crinita* in Vietnam. These findings not only confirm the accurate identification of the species but also provide a scientific basis for future research on its phytochemical constituents, pharmacological properties and conservation strategies.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Consent for publication

All authors have read and approved the final version of the manuscript and consent to its submission to the Legume research Journal.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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