Botanical characteristics and *ITS* sequence of *Curcuma pierreana* Gagnep. – Zingiberaceae

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Abstract
Curcuma pierreana Gagnep. contains essential oils in all parts and rhizomes are used in traditional medicine for treating hyperthermia and irregular menstruation. Stigmasterol from the rhizome was found to inhibit ovarian and prostate cancer cells. However, botanical information regarding this species remains limited. Therefore, this study aimed to investigate detailed characteristics of Curcuma pierreana Gagnep. using samples collected from the nature reserve of Nam Khanh Hoa, Vietnam. The collected samples were determined to be Curcuma pierreana based on morphological characteristics: Leaf blades have red patches along the midrib and leaf sheaths are reddish brown and hairy. The rhizome is segmented, covered with thin scales, has yellow flesh and has an oval tuberous root. Inflorescences grow between the leaf sheaths and the tips of the bracts are red.

The L-shaped anthers have 2 filamentous spurs, the common crest is triangular and the dorsal surface of the anther has many white hairs. The ovary is hairy and the stigma is funnel-shaped. Anatomical characteristics include tetracellular stomata, many yellow oil cells, unicellular protective hairs, many vascular bundles in the cortex of the rhizome and an endodermis with a Casparian strip. Sequence analysis of the ITS gene segment, compared with data on GenBank, shows 99.3% similarity with the published Curcuma pierreana species sequence. The research results provide detailed images of morphology and ITS sequence, contributing to the accurate identification of Curcuma pierreana species in Vietnam.

Keywords: Anatomy, *Curcuma pierreana* Gagnep., *ITS* sequence, morphology

Introduction

Curcuma pierreana Gagnep. (*C. pierreana*), belonging to the Zingiberaceae, is a perennial rhizomatous herb native to Vietnam and Thailand[13,17]. Botanically, *C. pierreana* exhibits distinctive features typical of the genus *Curcuma* such as rhizomes, showy inflorescences and characteristic floral structures. These morphological and genetic features help to distinguish *C. pierreana* within the genus and contribute to its ecological adaptation and potential commercial value[12]. In addition to its botanical significance, *C. pierreana* is of interest in pharmaceutical and traditional medicine practices due to the presence of secondary metabolites and bioactive compounds[9,14]. The whole plant is rich in volatile oils, with more than 30 components reported from various plant parts such as the rhizome, stem, leaf and flower[10,11]. Preliminary evaluations of these compounds have demonstrated antibacterial and antifungal properties in extracts tested against strains of *E. coli*, *P. aeruginosa*, *S. aureus*, *S. faecalis* and *Candida albicans*[7]. This plant is widely cultivated in tropical and subtropical regions due to its high value as an ornamental and a pharmacologically important species[20]. Despite the initial botanical descriptions, comprehensive studies elucidating the genetic diversity, phytochemistry, ecological role and conservation status of *C. pierreana* remain limited. Like many ornamental flowering plants, *C. pierreana* can be identified through traditional methods when it bears flowers; however, reliable identification is difficult during non-flowering stages[22].

Furthermore, identification based on external morphology using traditional botanical taxonomic keys in the Ginger family (Zingiberaceae) is often challenging because of the high similarity in phenotypic characteristics across species. Accurate identification largely depends on the reproductive organs (flowers), which can be time-consuming and costly[2]. Nowadays, with the development of molecular biology techniques, DNA barcoding has emerged as an effective method for species identification by analyzing short DNA fragments, offering low costs and high reliability[4,8]. Ribosomal DNA (rDNA), particularly the internal transcribed spacer (*ITS*), a highly variable non-coding region, provides valuable information for systematics and helps to distinguish between *Curcuma* species[18]. Therefore, this study aims to describe in detail the morphological and anatomical characteristics of *Curcuma pierreana* Gagnep. and the role of the *ITS* gene in its classification and identification. Additionally, the powder characteristics of the leaves and rhizomes were analyzed. Our study could contribute to developing a database for the accurate identification and standardization of medicinal materials from this species in Vietnam.

Material and Methods

Plant materials: *Curcuma pierreana* fresh natural plants were identified and collected from the nature reserve of Nam Khanh Hoa, Khanh Hoa province, Vietnam. The samples

were washed with water and air-dried for subsequent characterizations.

Plant morphological characterization: The external morphology of plant organs was observed by the naked eye and described based on distinctive visual characteristics. Subsequently, the structure of each organ includes roots, rhizomes, leaves and flowers captured under a stereo microscope and characterized following the guidelines outlined in the Vietnamese Pharmacopoeia V[15]. To accurately determine the scientific name of *C. pierreana*, the morphology of the research samples was compared with the taxonomic key, images and descriptions in the references[19,24].

Anatomical structure analysis: Rhizome leaf petioles, leaf blades, rhizome (stems) and roots were cross-sectioned into thin slices using a microtome. Leaf petioles were cut in the middle. Leaf blades were sectioned at 1/3 from the base, including midribs and leaf blades. Rhizome stems were cut into cross-sections with diameters ranging from 0.4 to 0.6 cm. Roots were sliced into cross-sections with diameters of 0.1 to 0.2 cm. The sample was stained with a double stain of safranin and fast green. The upper and lower epidermis of the leaves were separated with a microtome and placed in water. Anatomical structure, epidermal structure and stomata were observed, photographed and described under an optical microscope.

Analysis of herbal powder characteristics: For powder microscopy analysis, fresh mature leaves and rhizomes were sliced into small pieces and dried in an oven at a temperature of 60°C until they reached a constant dry weight. The dried samples were coarsely powdered and shifted through a #32 sieve to obtain the herbal powder. The powder was taken randomly and placed over slides in distilled water. The details of the herbal powder constituents were observed, photographed and recorded under an optical microscope.

DNA extraction and *ITS* gene analysis: Fresh leaf samples were isolated and preserved at 4°C. DNA from the leaf samples was extracted using a DNA extraction kit (Gene JET Plant Genomic DNA Purification Mini Kit, Thermo Scientific, USA), following the manufacturer's protocol. The quantity and purity of DNA were determined by OD measurement (Thermo Scientific, USA). The *ITS* gene sequence was amplified by PCR with the primer pairs listed in table 1[5], with a primer annealing temperature of 54.5°C. The PCR products were checked for the presence of target DNA bands and sequenced by GeneLab. The decoded DNA sequences were analyzed and compared using BLAST tools in the gene bank for species identification and determination of the species with the highest similarity level.

Results and Discussion

Morphological characteristics: The perennial herb typically reaches a 20-50 cm height and bears 4-6 leaves (Figure 1A, 1C). The leaves are oval-shaped and green, measuring 12-18 cm in length and 4-6 cm in width. The adaxial surface of the leaves displays a dark purplish vein along the midrib (Figure 1A). The abaxial surface is light green with scattered short hairs. These leaf characteristics are similar to the leaves of *C. zedoaroides*. However, the leaves of *C. zedoaroides* are large 70–100×15–18 cm [3]. The leaf stalk is concave, about 6-10 cm long and green (Figure 1D); the leaf sheath is funnel-shaped, measuring 12-15 cm in length and brownish red (Figure 1E). The small ligule is 2 mm long and light brown (Figure 1E). The leaf stalk, leaf sheath and ligule have numerous short hairs on the underside.

The rhizome is round in cross-section, with a diameter of 0.5-1.0 cm, divided into several segments measuring 0.5-0.8 cm, covered by thin brown scales. The cross-section of the young rootstock appears light green (Figure 1B4). In contrast, the mature rhizome exhibits a pale-yellow bark region and a darker yellow core (Figure 1B2, B3). The internal color of the rhizomes of species in the genus *Curcuma* is also one of the important characteristics that helps to distinguish these species, for example, *C. longa* - orange yellow, *C. aeruginosa* - bluish green, *C. caesia* - grayish blue[1,2]. The roots are fibrous and white (Figure 1B1); occasionally the tip of the root swells into an oval-shaped tuber measuring 0.5-1 cm x 1.2-1.8 cm, with a greenish-white interior (Figure 1B5, 1B6).

The location of inflorescences of species in the genus *Curcuma* is different and is often divided into 3 groups. First, the lateral inflorescences directly from the rhizome are *C.aruna, C. newmanii, C. sahuynhensis...* Second, the terminal inflorescences are *C. siamensis, C. bicolor* and *C. cotuana.* The third group is inflorescences growing from both two positions such as *C. angustifolia*[21]. In this study, the inflorescence of *C. pierreana* is 4-6 cm long, bearing 3-4 flowers and grows from the middle of the leaf sheaths (terminal inflorescences). The flower cluster axis is cylindrical and white, measuring 5-7 cm long (Figure 1F). The bracts are pointed oval-shaped, carrying 2-3 flowers, red at the upper part and gradually turning into light greenish white at the lower part, 3-5 cm in length and 1.5-2 cm in width, overlapping each other (Figure 1G, 1H).

The calyx tube is white, 1-1.5 cm long, 0.5-0.7 cm wide, deeply split on one side, with the top divided into 3 lobes. The corolla tube is white, 3.5-4 cm long, covered in short hairs, gradually widening upwards into 3 white ovate lobes with uneven longitudinal veins. The large dorsal lobe has a slender, pointed tip in light red, while the two smaller lateral corolla lobes have rounded tips. The labellum is white, inverted egg-shaped, 1.5-2 cm in length and 0.4-0.6 cm in width, thicker in the middle and split into 3-5 mm into 2 lobes, with a dark yellow split area. The two large lateral staminodes (modified stamens) are white, with wide, pinkish-purple upper parts and narrowing lower parts, displaying numerous white secondary veins radiating from the midsection of the lamina (Figure 1G, 1I).

The pinkish-purple color at the tip of the staminodes is consistent with Saensouk's description of this species and this feature is also prominent and helps to distinguish this species from other species with purple at the base of staminodes namely *C. ecomata* and *C. corniculata* or are not purple at all *C. siamensis* and *C. rhomba*[21]. The L-shaped anther consists of 2 white oval-shaped sacs, longitudinally open, facing towards the inside of the flower. The dorsal surface has numerous fine white hairs. The base of the anther sac is rounded and curved upward, with 2 filamentous spurs measuring white in color and curving inwards. These spurs feature of anther match the description of Leong et al[13].

In the *Curcuma* genus, the spur feature of the anther is an important feature for distinguishing species from each other. *C. pierreana* has inward-curved thread-like spurs, distinct from *C. eburnea*, which has straight spurs[13]. Besides, the size of *C. pierreana* spurs is very short, less than 2 mm like the size of spurs at *C. arida*, *C. chantaranothaii*, *C. eburnea* and differs from the 3-4 mm long spurs of species *C. siamensis*, *C. longa* and *C. rangjued* [21].The common crest is triangular. The stigma is very short, forming a triangularly rounded hood, white in color, measuring 1-2 mm (Figure 1K). The pollen grains are white and round-shaped, measuring 50-55 µm (Figure 1L).

The ovary consists of 3 fused carpels, forming 3 chambers below, each chamber containing numerous ovules, attached to the central column. The ovary is ovoid, 0.5-0.8 cm long, white and covered in fine hairs; two epigynous glands are white, measuring 0.3-0.5 cm, attached at the top of the ovary. The style is filamentous, white, 1.2-1.6 cm long, with a white funnel-shaped stigma, measuring 0.2-0.3 cm, having white hairs extending from the middle between the two pollen chambers (Figures 1J, 1K, 1L,1M, 1N).

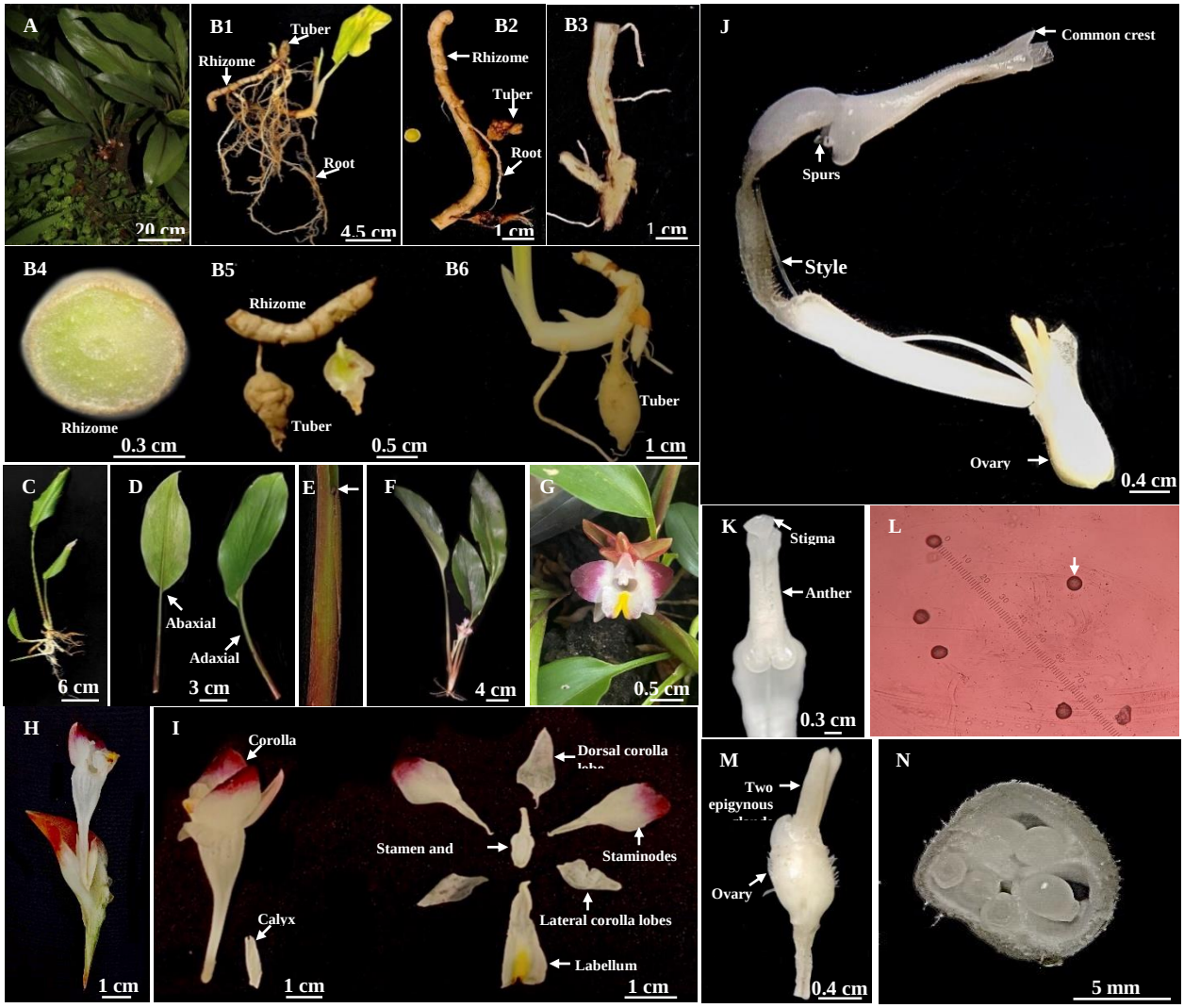

Figure 1: Morphological characteristics of *Curcuma pierreana*: (A) All parts of the plants; (B) Rhizome, root and tuber, the cross-section of the rhizomes and longitudinal section of the tuber; (C) Habit; (D) Leaves; (E) Ligule; (F) Terminal inflorescence in the leaf-sheaths; (G) Flower, front view; (H) Flower exserted from bracts and bracteoles; (I) Flower dissection; (J) Stamen and pistil, anthers with 2 filamentous spurs, dorsal surface with white hairs, the triangular common crest, filamentous style; (K) L-shaped anther and funnel-shaped stigma; (L) The round-shaped pollen grains; (M) Ovary-bearing epiginous glands and (N) Cross-section of an ovary.

Leaf: The leaf anatomy of *Curcuma pierreana* consists of the main vein and leaf blade. The petiole features a deeply concave upper surface and a rounded, convex lower surface, with two slender, pointed wings (Figure 2A). The microscopic shape of *C. pierreana's* petiole is open 'V' shaped which resembles that of *C. aromatica*, *C. vamana and C.zedoaria,* but is different from the open 'U' shape in *C. aeruginosa*, *C. amada*, *C. pseudomontana* and *C. zanthorrhiza*[6]. The anatomical structure is like that of the main vein of the leaf; however, there are 6-8 vascular bundles present in the middle of the petiole more than in the midrib of the leaf (Figure 2A). The anatomy of the leaf main vein has a concave upper surface and a rounded convex lower surface (Figure 2B).

The upper epidermal cells are polygonal in shape. Below the upper epidermal is the parenchyma, comprising of many layers of cells that are irregularly arranged and interspersed, including yellow oil cells (Figure 2B). The primary vascular bundles are located above the lower epidermis, with 3-4 smaller-sized bundles between the veins (Figure 2B). Each vascular bundle consists of the xylem above the phloem, with sclerenchyma consisting of 2-3 layers of polygonal cells arranged above the xylem and 3-7 layers below the phloem. The xylem comprises of 1-2 protoxylem and 1-2 metaxylem, nearly round, while the phloem includes 9-10 layers of small polygonal cells (Figure 2C). The large air cavity between vascular bundles is surrounded by 1-2 layers of parenchyma containing scattered small oval cells with chloroplasts.

In *C.pierreana*, the air cavities are often rectangular or squarish (Figure 2A, 2B), similar to those in the leaves and petiole of *C. vamana* and *C. zedoaria*. In *C.caesia*, *C. oligantha* and *C.pseudomontana*, these air cavities are oval or round[6]. The trichomes on the epidermis are also important in taxonomy[25]. In *C.pierreana*, the lower epidermal cells of the leaf and petiole are polygonal and have many unicellular trichomes (Figures 2A, 2B, 2E) different from trichomes consisting of 2 cells of *C. longa* and *C. zedoaria*[23]. In *C. aurantiaca* and *C. caesia*, trichome is absent[1]. The leaf blade anatomy has a uniform structure composed of oval-shaped parenchyma arranged within various-sized intercellular spaces (Figure 2D).

Most of the vascular bundles exhibit a structure resembling that of the bundles in the main vein. Between these bundles, there are numerous larger spaces surrounded by abundant small-sized oval parenchyma with many chloroplasts (Figure 2D). There are a lot of yellow oil cells in the parenchyma of the leaf and petiole (Figure 2F). The upper and lower epidermal cells are polygonal in shape with the presence of stomata (Figure 2G). Stomata are surrounded by 4 unevenly distributed tetracytic cells and are more prevalent on the lower epidermis (Figure 3). This type of stomatal arrangement is often found in species belonging to the Curcuma genus[25].

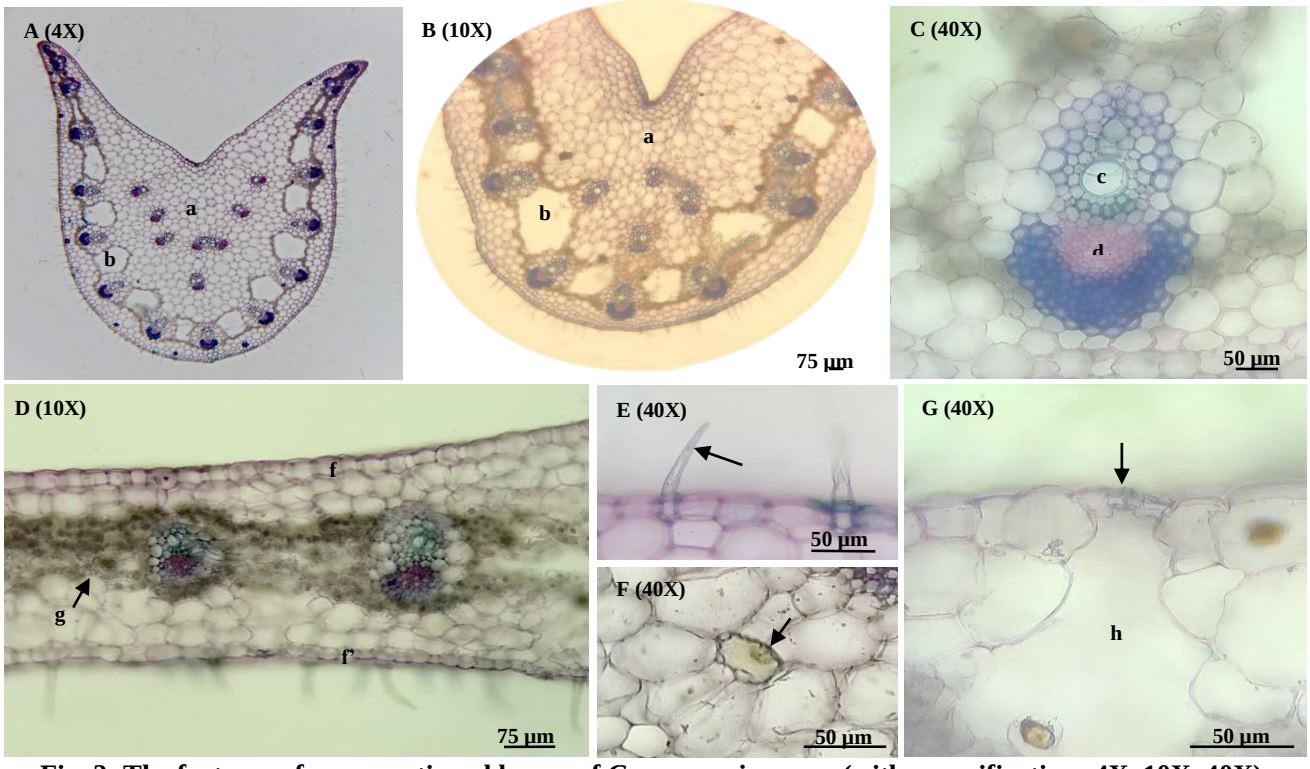

Fig. 2: The features of cross-sectioned leaves of *Curcuma pierreana* (with magnifications 4X, 10X, 40X): (A) Transverse section of mature petiole; (B) Midrib of leave; (C) Vascular bundles; (D) Leaf blade; (E) Unicellular trichomes; (F) Oil cell; (G) Stomata. (a) Parenchyma; (b) Air cavity; (c) Xylem; (d) Phloem; (e) Sclerenchyma; (f) Upper epidermis; (f') Lower epidermis; (g) Chloroplasts and (h) Sub-stomatal cavity.

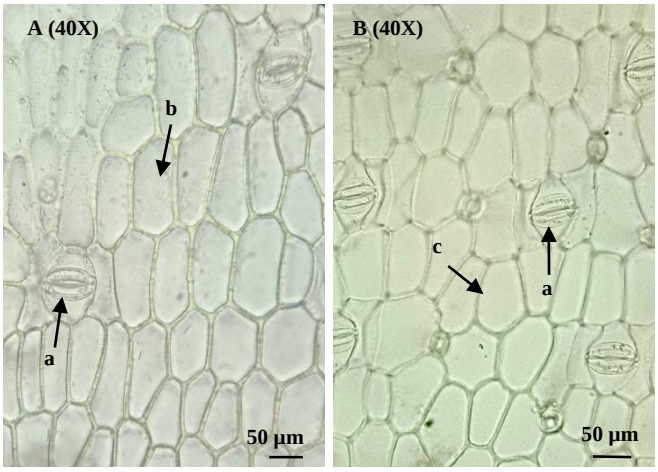

Fig. 3: Epidermis with stomata of *Curcuma pierreana* (with magnifications 40X): (A) Adaxial surface view of the epidermis with stoma open; (B) Abaxial surface view of the epidermis with stoma open. (a) Stomata; (b) Upper epidermal cells; (c) Lower epidermis cells.

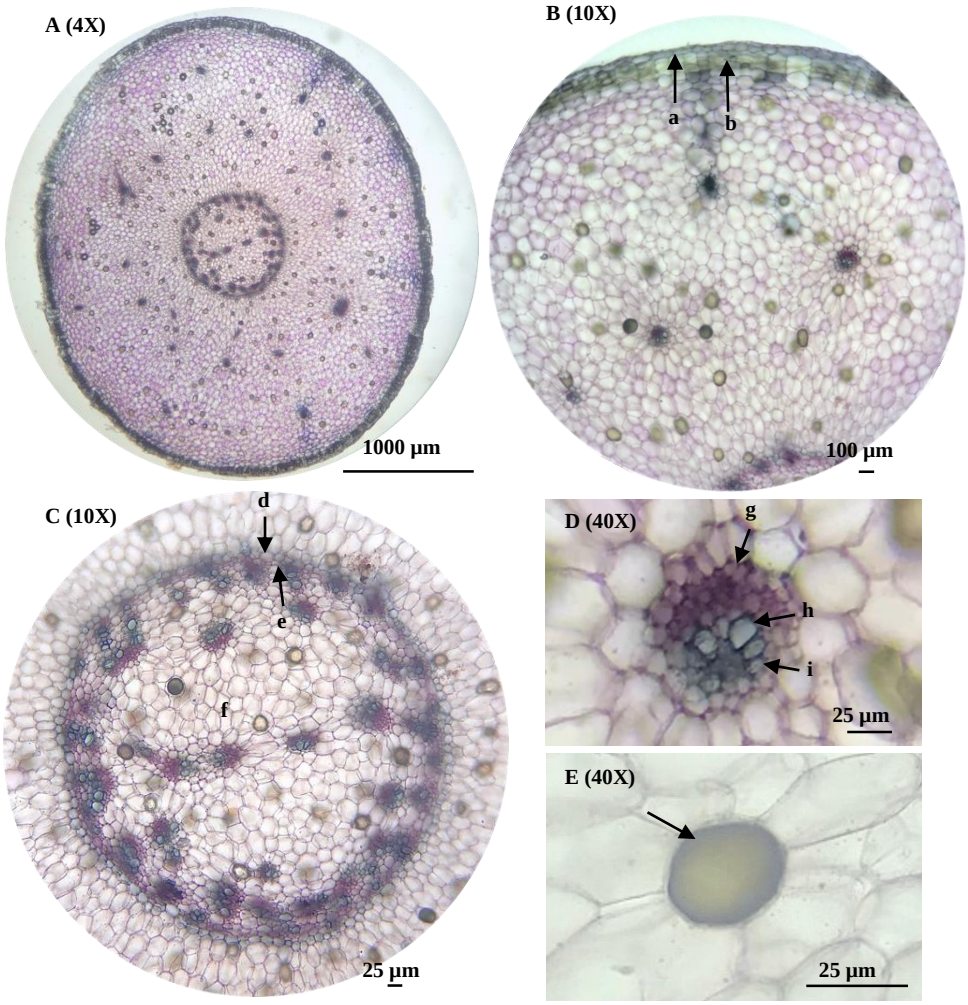

Fig. 4: The features of the cross-sectioned rhizome of *Curcuma pierreana* (with magnifications 4X, 10X, 40X):): (A) Transverse section of mature rhizome; (B) Cortex; (C) Stele; (D) Vascular bundles; (E) Oil cell. (a) Epidermis; (b) Multilayered hypodermis; (c) Parenchyma of cortex; (d) Endodermis with Casparian strips; (e) Pericycle; (f) Parenchymatous pith; (g) Phloem; (h) Metaxylem and (i) Protoxylem.

Rhizome: The cross-section of the rhizome exhibits an oval shape, with the cortex region occupying two-thirds of the radius of the root (Figure 4A). The epidermis consists of a layer of polygonal cells. The periderm comprises of 3-4 layers of rectangular cells arranged radially (Figure 4B). Below the epidermis are 4-9 layers of periderm cells, a

common feature of *Curcuma* species. However, under the same habitat, the number of periderm layers of different species is different such as *C. aeruginosa* (5-7 layers), *C. amada* (4-7 layers), *C.aromatica* (5-8 layers) and *C. caesia* (4-6 layers)[1]. Cortical parenchyma consists of irregular-shaped oval cells that vary in size (Figure 4B). In the cortex region, 10-20 primary vascular bundles are scattered with the phloem positioned above the xylem.

The xylem consists of 1-2 protoxylem and 1-2 metaxylem nearly round-shaped, while the phloem comprises of polygonal cells of small sizes (Figure 4D). The endodermis with Casparian strip comprises of a single layer of thin-walled polygonal cells (Figure 4C). The endodermis is continuous in *C. aeruginosa, C. amada*, *C.aromatica* and *C. caesia*, but discontinuous in *C. haritha, C. montana and C. zanthorrhiza*[1]. The central region contains many vascular bundles with a structure like that of the bundles in the cortex region (Figure 4C). The number of vascular bundles in the cortical region is small. This characteristic is similar to the species *C. aromatica and C. amada*[23].

The vascular bundles in the cortex and the central are closed type [25,26]. The number of yellow oil cells is present in the cortex more than in the central regions of the rhizome (Figure 4A, 4B, 4C). In *C.longa* and *C. zedoaria*, the number of curcumin cells is higher in the inner zone than in the outer zone[23]. In *C. pierreana*, these secretion cells are round (Figure 4E), similar to those in *C. aeruginosa, C. heyneana, C. mangga, C. soloensis, C. xanthorrhiza and C. zedoaria*, but different from the polyhedral cells in *C. domestica*[25].

Tuber and roots: The anatomical cross-section of the tuber is circular, with the cortex region occupying two-thirds of the radius of it (Figure 5A). The exodermis layer consists of polygonal cells with numerous root hairs. The suberized hypodermal cells consist of 3-4 layers of closely arranged polygonal cells. The cortical parenchyma comprises of oval-shaped cells with intercellular spaces (Figure 5B), containing scattered cells with yellow oil cells (Figure 5F). Cells near the central region contain a significant amount of starch (Figure 5D, 5E). The endodermis consists of a single layer of polygonal cells. Protoxylem bundles are arranged alternately with primary phloem bundles. The phloem comprises of irregularly arranged polygonal cells.

The protoxylem consists of 1-2 nearly round-shaped vessels that gradually increase toward the center of the root. The nearly round-shaped metaxylem vessels are located either under the protoxylem or between two bundles of protoxylem. The parenchyma tissue surrounding the metaxylem consists of 3-4 layers of polygonal cells with lignified cell walls. The innermost part of the tuber is composed of parenchyma with cellulose walls (Figure 5C). The root anatomy exhibits a structure like that of the tuberous root (Figure 6). However, the parenchyma of the roots does not contain starch observed in the tuber.

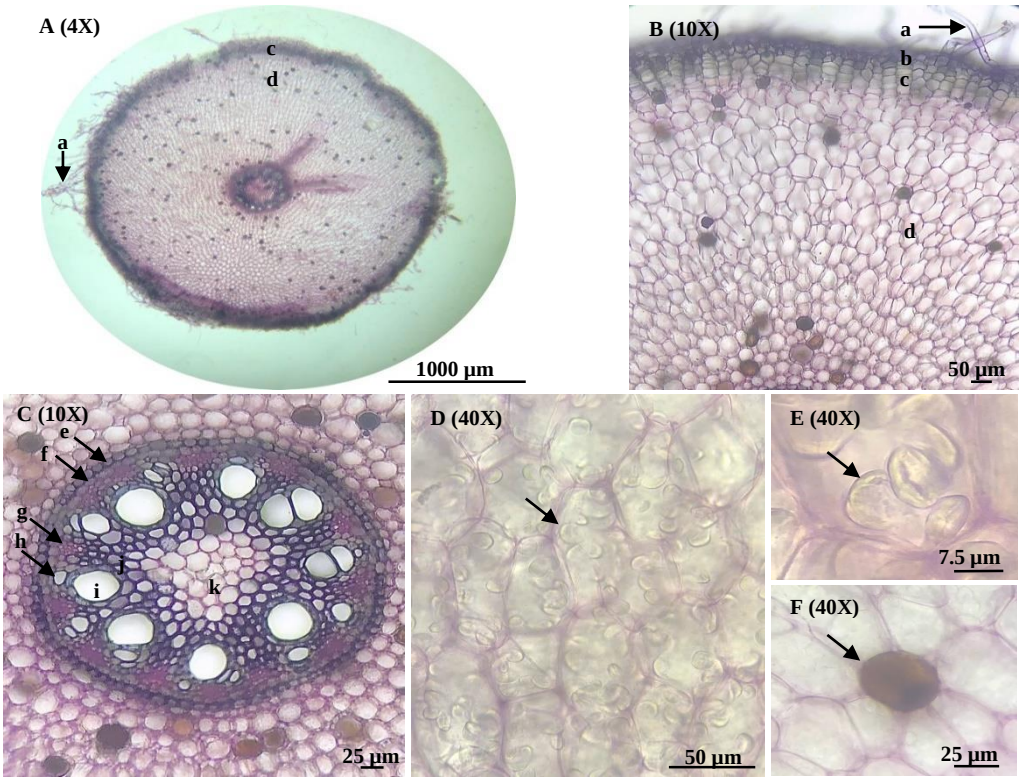

Fig. 5: The features of the cross-sectioned tuber of *Curcuma pierreana* (with magnifications 4X, 10X, 40X): (A) Transverse section of mature tuber; (B) Cortex; (C) Stele; (D) Starch grains; (E) Starch; (F) Oil cell. (a) Root hair; (b) Exodermis; (c) Suberized hypodermal cells; (d) Parenchyma of the cortex; (e) Endodermis, (f) Pericycle, (g) Phloem; (h) Protoxylem; (i) Metaxylem; (j) Sclerenchyma and (k) Parenchymatous pith.

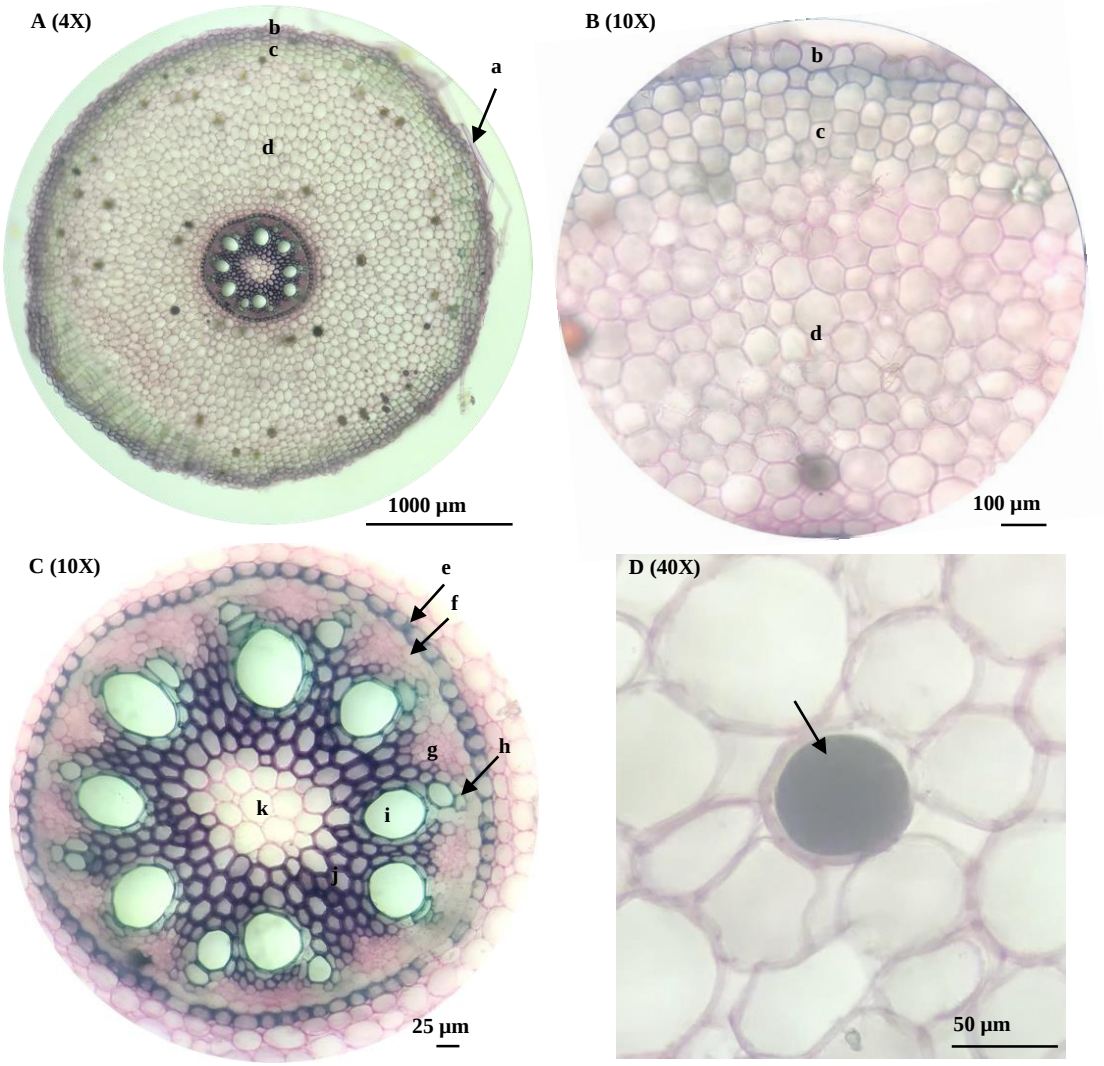

Fig. 6: The features of the cross-sectioned root of *Curcuma pierreana* (with magnifications 4X, 10X, 40X): (A) Transverse section of mature root; (B) Cortex; (C) Stele; (D) Oil cell. (a) Root hair; (b) Exodermis; (c) Suberized hypodermal cells; (d) Parenchyma of the cortex, (e) Endodermis; (f) Pericycle; (g) Phloem; (h) Protoxylem; (i) Metaxylem; (j) Sclerenchyma; (k) Parenchymatous pith; (D) Oil cell.

Characteristics of herbal powder: Anatomical and powder microscopy characters have an important role in raw drug standardization as well as in the identification the medicinal plants[16]. In this study, we investigated the anatomical characteristics of leaves, petioles, roots, tuberous roots and rhizomes. Besides, the powder characteristics of leaves and rhizomes were also analyzed. This description outlines the characteristics of the herbal powder derived from the leaves and rhizomes of *Curcuma pierreana*, highlighting their color, scent and observable structures when examined under a microscope. The leaf powder is green in color and emits a mild fragrance. When being observed under a microscope, various structures in leaf powder are visible including epidermis, stomata, fiber bundles, spiral xylem vessels, parenchyma and unicellular trichomes with thick cuticles which are also characteristic of this species (Figure 7).

The powder of the rhizomes is light brown-yellow and has a subtle fragrance. Upon microscopic examination, various structures can be observed: epidermal cells, bundle of fibers, sclerenchyma, parenchyma, oil cells and spiral xylem vessels. The oval-shaped starch grains with small circular hilum are usually present (Figure 8).

***ITS* gene analysis:** The PCR product of the *ITS* target gene from the total DNA extraction was assessed using electrophoresis, as shown in figure 9. The DNA sample was analyzed for the *ITS* sequence region and compared with published data in the genetic database, revealing the highest similarity with two species, *Curcuma rhomba* and *Curcuma pierreana* (Table 2).

However, upon surveying the morphological characteristics, we observed several differences between the studied sample and *Curcuma rhomba* (Table 3). Therefore, it can be concluded that the studied species is *Curcuma pierreana*. The *ITS* gene sequence also contributes to supplementing molecular data on *Curcuma pierreana* in Vietnam.

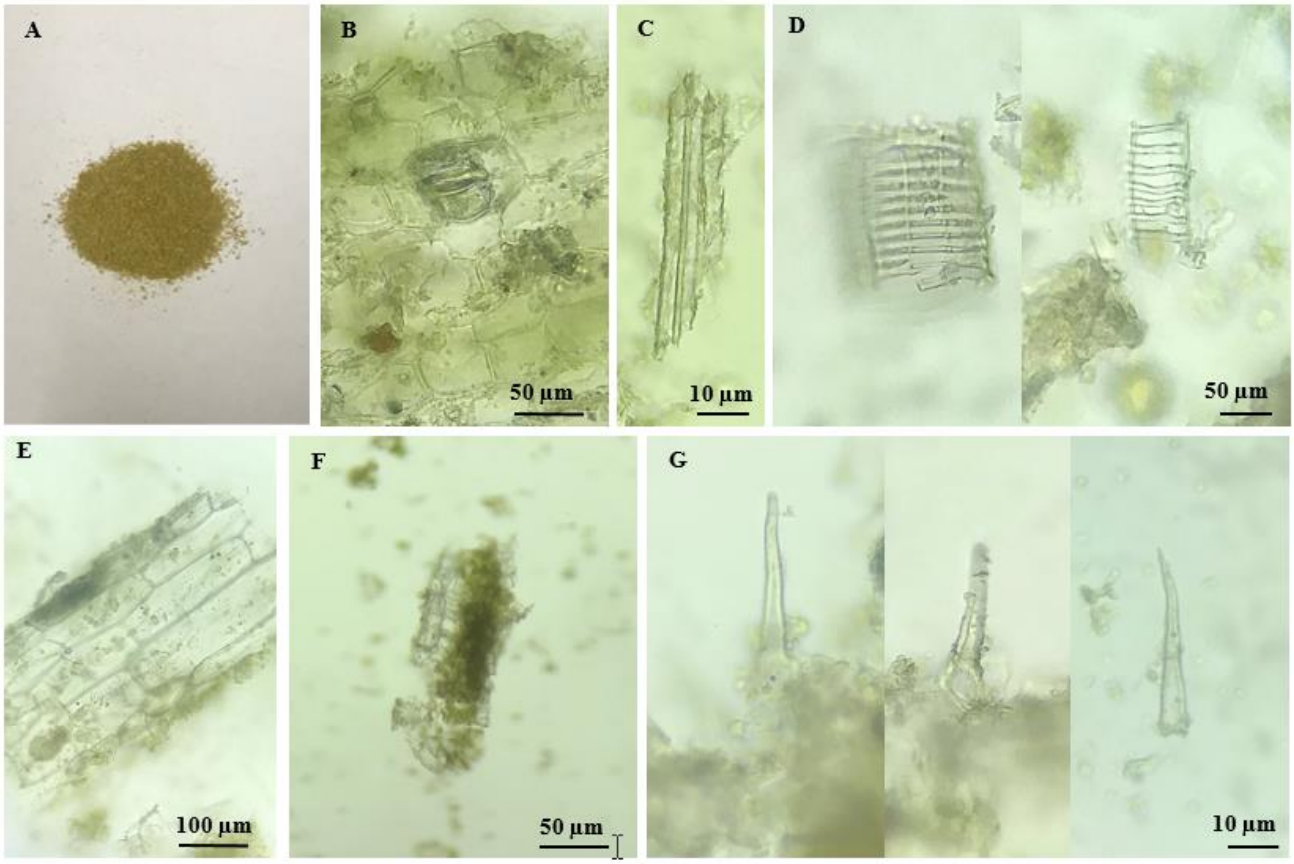

Fig. 7: The features of dried leaf powder of *Curcuma pierreana* (with magnifications 40X): (A) Leaf powder; (B) Surface view epidermis with stomata; (C) Bundle of fibers; (D) Spiral xylem vessel; (E) Parenchyma of the midrib; (F) Epidermis and parenchyma of the leaf blade and (G) Unicellular trichomes

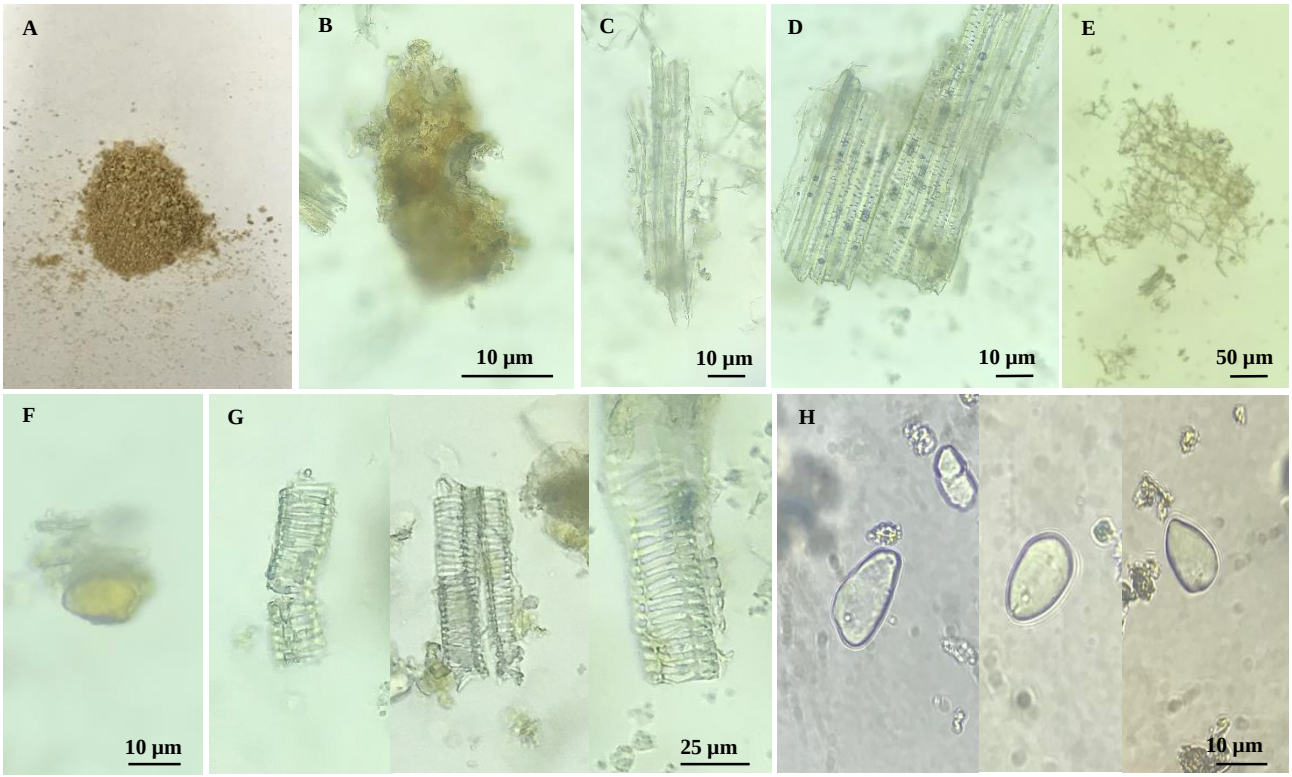

Fig. 8: The features of *Curcuma pierreana* rhizome powders (with magnifications 40X): (A) Rhizome powders; (B) Epidermal cells; (C) Bundle of fibers; (D) Sclerenchyma; (E) Parenchyma; (F) Oil cell; (G) Spiral xylem vessel and (H) Starch granules

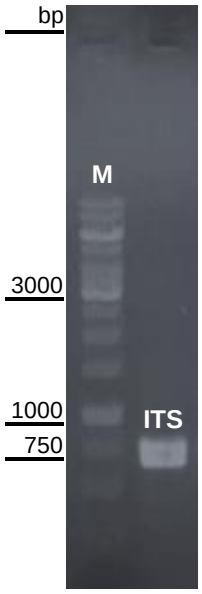

Fig. 9: Electrophoresis results of *ITS* gene PCR products of the collected samples (*C. pierreana*).

Table 2
The BLAST results of *ITS* gene sequences in collected *C. pierreana* samples.

Scientific name	Max Score	Total Score	Query Cover	Per. Ident	Acc. Len	Accession
Curcuma rhomba	1048	1048	95%	99,48%	575	JQ409880.1
Curcuma pierreana	1048	1048	95%	99,30%	575	JQ409879.1
Curcuma rhomba	1042	1042	95%	99,30%	575	JQ409878.1
Curcuma newmanii	1013	1013	95%	98,26%	575	JQ409876.1
Curcuma sp. CTL-2023a	1005	1005	95%	98,26%	571	OR139854.1

Table 3
Comparative morphological characteristics of *C. pierreana* and *C. rhomba*

Characters	*C. pierreana*		*C. rhomba*
Plant height	0.2-0.5 m		1.1 m or more
Ligules	2 mm long		1.5-1.7 cm long
Lamina	12-18 cm long, red line on the midrib. The underside of the leaves has many short hairs		29-46 long, There is no red line on the midrib. The underside of the leaf is glabrous.
Bracts	The top is red, the bottom is blue-white.		Pure red
Bracteoles	Present		Absent
Calyx	White		Pink
Corolla lobes	The dorsal lobe is white with a light red top		Semi-translucent red dorsal lobe
Lateral staminodes	The upper part is a wide, purple-pink oval and the lower part is white, tapering to a narrower shape		Rhomboid, orange-yellow, base dotted with dark red
Labellum	Inverted egg shape, white, yellow slit		Reniform, yellow
Anther	White, 2 filamentous spurs, dorsal surface with white hairs		Orange, without spurs

***ITS Curcuma pierreana* Salisb gene sequencing results (601 bp):** GATCATTGTTGAGAGAGCATAGAATGATG
GATGATTGTGAACGTGTGAACGTGACCCTTTCGTT
AGCCCACCCATGTTGGTGGGCGATTGACCGTAGCT
CGGTGCGATCGGCACTAAGGAACAATGAACTCGGA
AGCAGAGGGCCCCTCGCCGTGAGCGGGGAGCACA
ATGCATCGAAGATTCCTCGGAATCAAATGACTCTCG
GCAATGGATATCTCGGCTCTTGCATCGATGAAGAAC
GTAGTGAAATGCGATACTTGGTGTGAATTGCAGAA
TCTCGCGAACCATTGAGTCTTTGAACGCAAGTTGT
GCCCGAGGCCTTGTGGTCGAGGGCACGCCTGCTTG
GGTGTCATGGCATCGTCGCTTTTGCTCCATGCTTTT
TTGGCATTGAGCGCGCGCGGAAATTGGCCCCGTGT
GCCCTCGAGCACAGTCGGTCGAAGAGTGGGTAGT
CGGTRGTCGTCGAGCACGACGGATGTTGGTCGCCA
TGAGCGGGAACTGAACGTCGTCCTCGTCGTCTCGG

AACAAGTCCTCAAGAGACCCTATGTGATTGCGGAG TCGGACGAAAGTGCCGTGTCAATCATTTGTGGCCC CAAGTCAGGCG.

Conclusion

This study represents the comprehensive report characterizing the morphology, anatomy, herbal powder and DNA barcode of *Curcuma pierreana* Gagnep. Noteworthy morphological features of this species include red veins on the leaf blade and red leaf sheaths with numerous hairs. The segmented rhizome is covered by thin scales, with a yellow interior. The inflorescence emerges amid the leaf sheaths, with red-tipped bracts. The labellum is white, lobed, thickened in the center and yellow, while the lateral lobes are purplish pink. The pollen sacs possess elongated filaments, forming a rounded common crest. The ovary is hairy and the style forms a funnel shape. Anatomically, it exhibits tetracytic stomata, numerous yellow secretion cells, single protective hairs, numerous vascular bundles in the cortex and a Casparian strip in the endodermis of the rhizome.

The characteristic constituents of the herbal powder include a mild fragrance, egg-shaped starch grains with a small hilum, single protective hairs and secretion cells. The analysis of the ITS gene region of the studied sample, compared against genetic databases, indicates the highest similarity with *Curcuma pierreana* Gagnep. This detailed description covers various aspects of the plant's morphology, anatomy, herbal characteristics and ITS gene region, providing comprehensive insight into the identification and classification of Vietnam's *Curcuma pierreana* Gagnep.

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