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Comparative micromorphology of *Costus pictus* and *Hellenia speciosa* collected from Vietnam

NGUYEN THANH TRIET[1], TRAN VAN CHEN[1], DUONG NGUYEN XUAN LAM[2], NGUYEN THI THU HIEN[3], NGUYEN THI NGA[1], TRAN THI THUY QUYNH[1,♥]

[1]Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City. No. 217, Hong Bang Street, Ward 11, District 5, Ho Chi Minh City 700000, Vietnam. Tel./fax.: +84-28-38558411, ♥email: thuyquynhtran31@ump.edu.vn
[2]Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City. No. 217, Hong Bang Street, Ward 11, District 5, Ho Chi Minh City 700000, Vietnam
[3]Faculty of Pharmacy, Nguyen Tat Thanh University. No. 300A, Nguyen Tat Thanh Street, Ward 13, District 4, Ho Chi Minh City 700000, Vietnam

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Abstract. *Triet NT, Chen TV, Lam DNX, Hien NTT, Nga NT, Quynh TTT. 2025. Comparative micromorphology of* Costus pictus *and* Hellenia speciosa *collected from Vietnam. Biodiversitas 26: 407-417. Costus pictus* and *Hellenia speciosa* are two herbs belonging to the family Costaceae that have been used in traditional medicine to treat diabetes and are also used as daily food. The comparative anatomical characteristics between *C. pictus* and *H. speciosa* from Vietnam have not been systematically noted. This study aimed to develop microanatomical monographs of *C. pictus* and *H. speciosa* from Vietnam. Six fresh plants each were randomly collected for use in this study. Anatomical structures of rhizomes, roots, stems, and leaves were carried out using the iodine green-carmine staining method. The anatomical structural features of plant parts were analyzed and recorded through optical microscopy. The anatomical structural features of the tested samples are similar to those of *Costus* species. Notably, the anatomical structure of *H. speciosa* rhizome is similar to its stem, but different from that of *C. pictus* rhizome. The microstructures of their roots and leaves easily distinguish these species from each other. Additionally, smaller unicellular trichomes were observed in *C. pictus* leaves, while sub-air cavities, larger unicellular stout trichomes, and peltate glandular trichomes were found in *H. speciosa* leaves. The present evidence highlights the main structural features for distinguishing and identifying Vietnamese *C. pictus* and *H. speciosa*.

Keywords: Anatomy, *Costus pictus*, *Hellenia speciosa*, micromorphology, structural feature

INTRODUCTION

The genus *Costus* L. was previously classified in the family Zingiberaceae, but later, with the inclusion of many novel floras, a separate family, Costaceae Nakai, was created from the family Zingiberaceae. The uniqueness in leaf and root anatomy and the monostichous spiral phyllotaxy feature supported Costaceae in leaping a new family and easily distinguishing it from other families in the order Zingiberales (Selvakumarasamy et al. 2021; Böhmová et al. 2023; Li et al. 2024). The current phylogenetic analysis of the family Costaceae has shown that the family consists of eight genera with 162 species (POWO 2024a). Among them, *Costus* is the largest genus in the family Costaceae, mainly native to the tropical and subtropical regions of South America, Africa, and Southeast Asia (POWO 2024a, b). *Costus pictus* D.Don was introduced to Vietnam in particular from America as an herbal cure for diabetes. In 2024, *C. speciosus* was proposed as a synonym of *Hellenia speciosa* (J.Koenig) S.R.Dutta based on the reported phylogenetic relationship results, according to the study of Li et al. (2024).

Costus pictus (fiery costus or insulin plant) and *H. speciosa* (crepe ginger) are rhizomatous perennial herbs (Shaikh et al. 2022). *Costus pictus* is purple toward the base stems and has spiral light green leaves, bright yellow flowers with orange-red stripes, and large green, ovate bracts (Shaikh et al. 2022). *Hellenia speciosa* is an erect, apex-branched herb and slightly woody toward the base stems and terminal ellipsoid or ovoid-like inflorescences with white flowers (Shaikh et al. 2022; FOC 2024). Both species are two herbs that have been used in traditional medicine to treat diabetes and are also used as plant food in Southeast Asia (Waisundara et al. 2015; Selvakumarasamy et al. 2021; Sivalingam et al. 2021).

One of the factors that contribute to the effective treatment of diseases is the correct use of herbal medicines. There are many factors related to the quality of drugs, such as the growth stage of the plant, the distribution area, climatic conditions, and the origin of the medicinal species. Therefore, the first step in using medicinal plants in evidence-based medicine is accurately identifying the plant species (WHO 2011; Ministry of Health 2017). The micromorphological method plays an important role in certain taxonomic identification issues, because these microanatomical features relate to elements of plant structure. In addition, the identification of medicinal plants and standardization of crude medicinal herbs are highly dependent on micromorphological features (Anu et al. 2020; Chen et al. 2024). Therefore, taxonomists still use micromorphological analysis to contribute to the accurate identification of plant species.

The anatomical characteristics of different parts of several *Costus* species have been reported, such as *C. afer*

Ker Gawl. leaves and stems (Tomlinson 1956; Edeoga and Okoli 1998; Sonibare et al. 2023), *C. rumphianus* Valeton ex K.Heyne leaves and stems, *C. elegans* Veitch ex J.Dix leaves and stems, *C. lucanusianus* J.Braun & K.Schum. leaves and stems (Tomlinson 1956; Edeoga and Okoli 1998), *C. pictus* leaves (Aruna et al. 2014), *H. speciosa* rhizomes (Kumar et al. 2020) and *Chamaecostus cuspidatus* (Nees & Mart.) C.D.Specht & D.W.Stev. leaves were also studied in the family Costaceae (Hegde et al. 2016). Although the anatomical characteristics of *C. pictus* leaves and *H. speciosa* rhizomes have been reported, the anatomical characteristics of the roots and rhizomes from *C. pictus* as well as the roots and leaves from *H. speciosa* have not been studied. Moreover, the comparative anatomical characteristics between *C. pictus* and *H. speciosa* cultivated in Vietnam have not been systematically examined for their anatomical characteristics. The current study aimed to investigate and compare the detailed micromorphological characteristics of *C. pictus* and *H. speciosa* from Vietnam, serving as reference standards for identification, authentication, and distinction of these plants from potentially adulterated plants. This anatomical information could be useful in providing micromorphological monographs for quality control and standardization of raw medicinal materials.

MATERIALS AND METHODS

Plant materials

Six fresh plants were randomly gathered from the cultivated habitat in Tam Vu Town, Chau Thanh District, Long An Province, Vietnam, in October 2023 (10°25'58.0" N, 106°28'02.6" E). The scientific name of the research sample was determined by comparing standard morphology with photographs accessed from https://powo.science.kew.org/ (POWO 2024c, d) and descriptions in reference documents (Thomas and Palni 2016; FOC 2024). The two specimens' scientific names were identified as *C. pictus* and *H. speciosa* (Figure 1). The voucher specimens (collector's numbers: CP-01.23 and CSp-02.23) were kept at the Department of Traditional Pharmacy, Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam. The experimental samples were cleaned thoroughly under tap water for anatomical feature analysis.

Figure 1. A and B. Morphology of *Costus pictus* collected in Vietnam; C and D. Morphology *Hellenia speciosa* collected in Vietnam

Procedures

The anatomical characteristics of the experimental samples were carried out using the iodine green-carmine double staining method followed the Vietnamese Pharmacopoeia V (Ministry of Health 2017). In brief, the different parts (leaves, roots, rhizomes, and stems) of the experimental samples were cut into appropriate sections or pieces and then cut into thin slices (about 10-20 µm thick) using a razor blade. These thin cross-sections were cleaned and discolored with 5.0% (w/v) chloramine-T and 50% (v/v) chloral hydrate for 10 min, respectively. These slices were then neutralized with 1.0% (w/v) acetic acid for 2 min until the samples became clearer. Finally, the standard slices were double stained with 0.3% (w/v) iodine green (for 5 s) and 1.0% (w/v) carmine (for 10 s), respectively. After each step, the excess chemicals were removed with double-distilled water (ddw). The standard slice samples were placed on glass slides with 1-2 drops of glycerin-water mixture (50/50, v/v) available and covered with a coverslip. The slices were observed and photographed via an optical microscope (Labomed, USA) at 4x, 10x, and 40x magnifications (Ministry of Health 2017; Chen et al. 2022). Moreover, the leaf epidermis was also peeled and analyzed according to the procedure of the Vietnamese Pharmacopoeia V (Ministry of Health 2017; Chen et al. 2024). As a result, the anatomical features of the specimens were noted.

Data analysis

A standard ruler and an eyepiece micrometer scale (Olympus, Japan) were used to identify the dimensions of sample parts (including roots, rhizomes, leaves, and stems) and cell structure components, respectively. All results were recorded and analyzed using Microsoft Excel 2023 software (WHO 2011, 2018; Chen et al. 2024).

RESULTS AND DISCUSSION

The anatomical characteristics of different parts (i.e., roots, rhizomes, stems, and leaves) of *C. pictus* and *H. speciosa* collected from Vietnam were systematically reported in the present study. The comparative microscopic observations of cross-sectioned roots, rhizomes, stems, and leaves from two study samples, revealed that many of these micromorphological features are homologous. In particular, the present results demonstrated structural similarities of rhizomes and stems only in *H. speciosa*.

Roots

The cross-sections of *C. pictus* and *H. speciosa* roots are nearly circular. From the outside to the inside, comprising the two regions: 1) the cortex and 2) the stele (Figure 2).

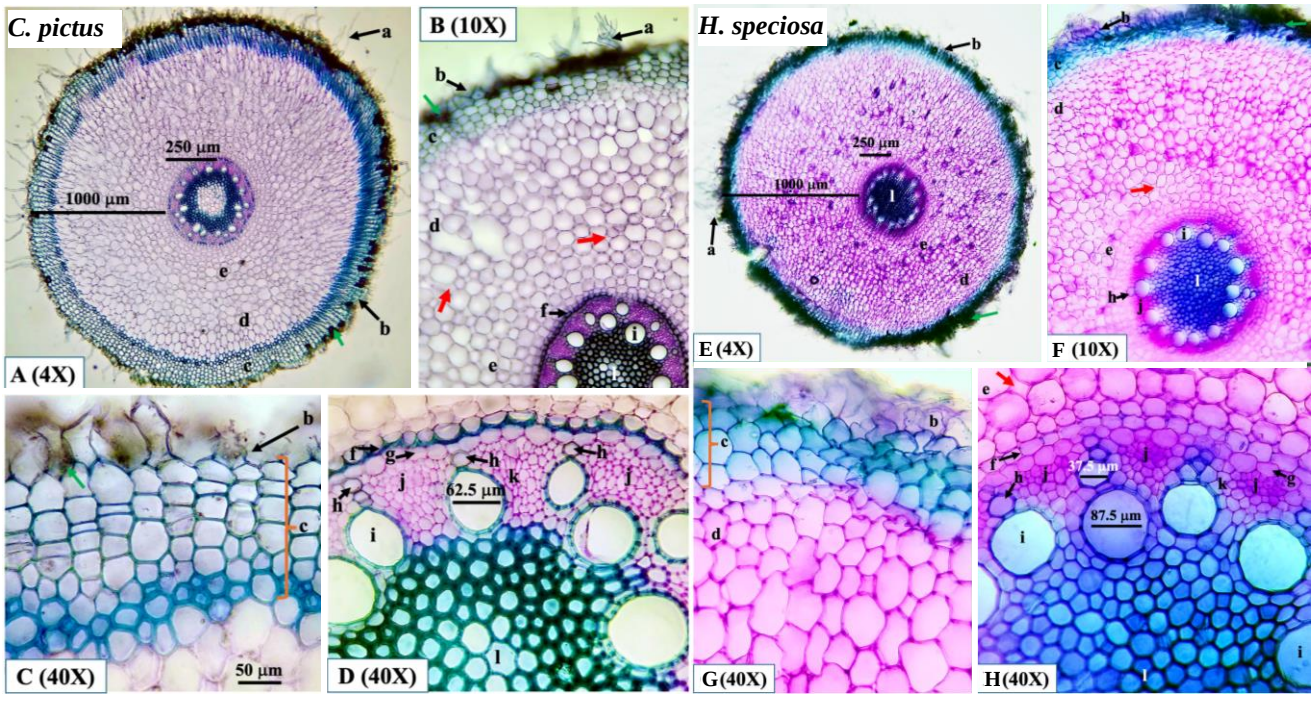

Figure 2. The features of the cross-sectioned root of *C. pictus* (A-D) and *H. speciosa* (E-H) (with magnifications: 4X, 10X, and 40X): (a, a') hair-root layer; (b, b') long root hairs; (c, c') suberized hypodermal cells with cork-impregnated walls (green arrow); (d, d') the outer cortex parenchymatous cells; and (e, e'), the inner cortex parenchymatous cells with triangular or polygonal intercellular spaces (red arrow); (f) u-shaped thickening in the endodermis; (f') casparian strip in the endodermis; (g, g') pericycle; (h, h') protoxylem bundles and protoxylem vessels; (i, i') metaxylem bundles and metaxylem vessels; (j, j') phloem; (k, k') parenchymatous ray; (l, l') parenchymatous pith

The cortex region

The outermost layer, called the hair-root layer (Figure. 2.b, b'), consists of a single epidermis layer, polygonal cells, and cellulose walls, along with long root hairs (Figures 2.a, a'). The next section is a suberized (suberoide)-impregnated layer consisting of 7-9 layers for mature *C. pictus* roots (Figures 2.Ac, Cc) (meanwhile, young roots have about 3-4 layers of cells (Figure 2.Bc)) and 4-5 layers for *H. speciosa* (Figure 2.c'), polygonal cells, and cork-impregnated walls (Figure 2, green arrow), arranged radially or randomly. The continuous arrangement of the cortex parenchyma region includes many layers of round polygonal cells and cellulose walls, arranged randomly (outer cortex parenchyma; Figures 2.d, d') or arranged in radial rows, and concentric rings (inner cortex parenchyma; Figures 2.e and e') with triangular or polygonal intercellular spaces (Figure 2, red arrow).

The stele region

U-shaped endodermis, called casparian strip, with a layer of polygonal cells for *C. pictus* (Figure 2.f), but this layer is not clear for *H. speciosa* (Figure 2.f'). The next layer, named pericycle, has a single layer of polygonal cells and cellulose walls in both species (Figures 2.g, g'). The vascular bundle system in *C. pictus* has about 16 phloem bundles arranged alternately with 16 protoxylem bundles on the same ring, while *H. speciosa* has 14 phloems and 14 protoxylem bundles. The phloem is composed of polygonal cells with slightly wavy cellulose walls and is arranged randomly (Figures 2.j, j'). Each protoxylem bundle has 1-3 vessels; the protoxylem vessels are roundly polygonal, lignified walls, and differentiated centripetally (Figures 2.h, h'). The metaxylem bundles consist of approximately 16-17 vessels for *C. pictus* (Figure 2.i) and 14-15 vessels for *H. speciosa* (Figure 2.i'), with round polygonal cells and lignified cell walls. The ray parenchyma comprises 1-2 rows of oblong polygonal cells with cellulose walls (Figures 2.k, k'). The pith parenchyma is multi-layered, with polygonal cells, lignify-impregnated cell walls, and random arrangement without intercellular spaces in both species (Figures 2.l, l').

Rhizomes

The cross-sections of the rhizomes of *C. pictus* and *H. speciosa* are almost circular. From outside to inside, it consists of the epidermis layer, collenchyma tissues or parenchymatous tissues, sclerenchymatous bundle sheath, and vascular bundle systems (Figures 3 and 4).

The anatomical features of *C. pictus* rhizomes are as follows: the outermost layer (i.e., the epidermis layer) consists of a single layer of polygonal cells with cellulose walls and is covered by a thin and flat cuticle (Figure 3.a). The cortical parenchyma has many layers of polygonal cells with cellulose walls, randomly arranged and scattered with vascular bundles observed in this region (Figures 3.b, b'). The endodermis has a casparian strip with a layer of polygonal cells (Figure 3.c). The pericycle consists of a layer of polygonal cells with cellulose walls (Figure 3.d). The vascular bundle system (Figure 3: red and green circles) consists of numerous vascular bundles of irregular sizes, randomly arranged in many rings from the pericycle inwards. Each vascular bundle is surrounded by a sclerenchymatous bundle sheath (Figure 3.h) with phloem (Figure 3.e), xylem (1-2 vessels) (Figure 3.f), and xylem tissues (Figure 3.g). The phloem consists of polygonal cells with cellulose walls, randomly arranged (Figure 3.e), while the xylem is polygonal cells with lignified walls (Figure 3.f). The xylem tissue is composed of polygonal cells with cellulose walls (Figure 3.g). The sclerenchymatous bundle sheath has polygonal cells with lignified walls. The pith parenchyma has many parenchymatous layers of polygonal cells with cellulose walls and many parenchyma cells with intercellular spaces (Figure 3.i) containing mucus (Figure 3.j).

The anatomical features of *H. speciosa* rhizomes are as follows: the outermost epidermis has a layer of polygonal cells with cellulose walls, covered by a thin, flat cuticle (Figure 4.a). Several cortical stomata with air cavities (green arrow) are observed (Figure 4.d). These cortical stomata are formed because the rhizomes can grow near the ground (Figure 4.d). The next layers are the outer cortical angular collenchyma (3-4 layers) with polygonal cells and cellulose walls, randomly arranged (Figure 4.b), followed by the inner cortical parenchyma (6-7 layers) with polygonal cells and cellulose walls, randomly arranged to form triangular or polygonal intercellular spaces (Figure 4.c). The irregular-sized vascular bundles covered by the sclerenchymatous bundle sheath are observed in the cortex region (Figures 4.B, C). The sclerenchymatous bundle sheath ring has 2-3 layers of polygonal cells and lignify-impregnated walls (Figure 4.e). The vascular bundle system consists of many irregular-sized vascular bundles, randomly arranged in many rings from the sclerenchymatous sheath into the pith region. Each vascular bundle comprises the phloem (Figure 4.f), xylem (1-5 vessels) (Figure 4.g), xylem tissue/xylem parenchyma (Figure 4.i), and an outer sclerenchymatous bundle sheath (Figure 4.h). Notably, each vascular bundle in the pith region (Figures 4.D, E) has a similar structure to that in the cortex region, but the sclerenchymatous bundle sheath is only present above the phloem and below the xylem (Figure 4.E); that is, it does not form a sclerenchymatous bundle sheath ring. The phloem has polygonal cells with cellulose walls and is randomly arranged (Figure 4.f). The xylem has polygonal cells and lignified cell walls (Figure 4.g). The xylem parenchyma has polygonal cells and cellulose walls (Figure 4.i). The stele parenchyma has polygonal cells with cellulose walls and many polygonal intercellular spaces (Figure 4.j).

Stems

The anatomical characteristics of the *C. pictus* stem are different from those of the *H. speciosa* stem. The cross-section of the *C. pictus* stems is nearly circular (Figure 5.A). The outermost layer is the epidermis, covered by a thin and flat cuticle, comprising a layer of polygonal cells with cellulose walls (Figure 5.a). The next layer is the parenchyma cell with 4-6 layers of polygonal cells, cellulose walls, and randomly arranged (Figure 5.b).

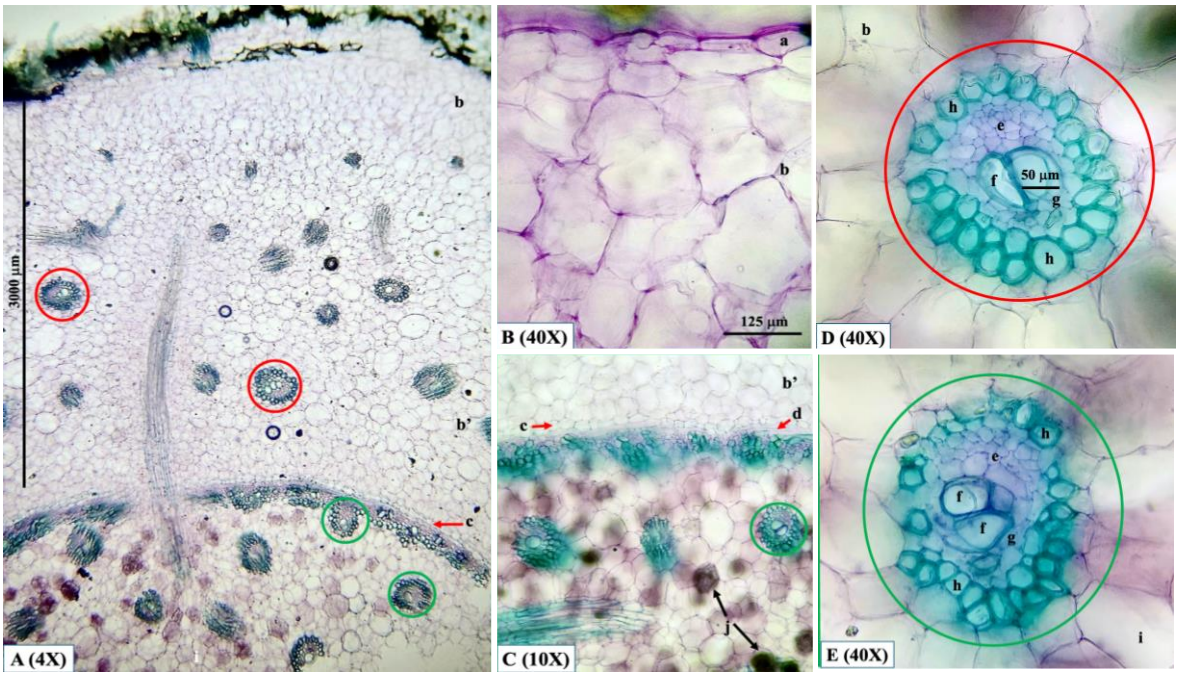

Figure 3. The features of the cross-sectioned rhizome of *C. pictus* (with magnifications A. 4X, C. 10X, and B-D-E. 40X): (a) epidermis with flat and thin cutin layer; (b, b') parenchymatous cells of the cortex region with vascular bundles (red circle) and vascular bundles of the pith region (green circle); (c) casparian strip in the endodermis; (d) pericycle; (e) phloem; (f) xylem and xylem vessels; (g) xylem parenchyma; (h) sclerenchymatous bundle sheath; (i) parenchymatous cells with intercellular spaces of the pith region; (j) mucus

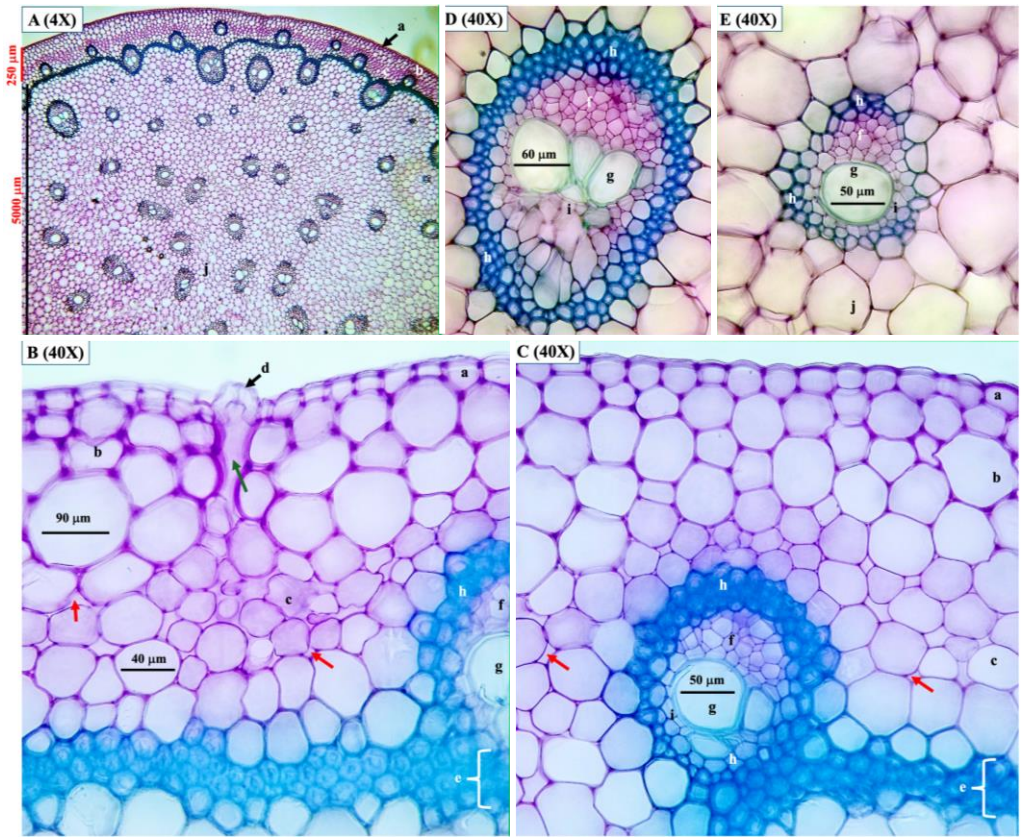

Figure 4. The features of the cross-sectioned rhizome of *H. speciosa* (with magnifications A. 4X and B-E. 40X): (a) epidermis with flat and thin cutin layer; (b) the outer cortical angular collenchymatous cells; (c) the inner cortical parenchymatous cells with triangular or polygonal intercellular spaces (red arrow); (d) the cortical stomata with air cavities (green arrow); (e) sclerenchymatous bundle sheath ring; (f) phloem; (g) xylem and xylem vessels; (h) sclerenchymatous bundle sheath; (i) xylem parenchyma; (j) parenchymatous cells with intercellular spaces of the pith region

The sclerenchymatous sheath ring of the cortex region consists of 1-2 layers of polygonal cells and lignify-impregnated walls with vascular bundles of this cortex region (red circle). The vascular bundles system consists of many vascular bundles of irregular sizes, randomly arranged in many rings from the sclerenchymatous sheath ring (Figure 5.g) and gradually distributed inwards. Each vascular bundle in the sclerenchymatous sheath ring (where the cortex and medulla meet) consists of the phloem (Figure 5.c), 1-2 xylem vessels (Figure 5.d), xylem parenchyma (Figure 5.e), and an outer sclerenchymatous bundle sheath (Figure 5.f). Each vascular bundle in the pith region has a similar structure to that in the cortex region, but the sclerenchymatous bundle sheath is only present at the two ends above the phloem and below the xylem (Figure 5.D). The phloem has polygonal cells with cellulose walls, randomly arranged (Figure 5.c). The xylem vessel has polygonal and lignify-impregnated walls (Figure 5.d). The xylem parenchyma has polygonal cells and cellulose walls (Figure 5.e). The sclerenchymatous bundle sheath has polygonal cells and lignify-impregnated walls (Figure 5.f). The pith parenchyma consists of many layers of polygonal cells with cellulose walls (Figure 5.h).

The cross-section of the *H. speciosa* stems (Figures 5.E-H) has the same shape and structural characteristics as its rhizomes (Figure 4). However, the structural differences between the *H. speciosa* stems and the rhizomes are shown as follows: The angular collenchymatous tissue is absent in the cortex region. The xylem in each vascular bundle in the stems consists of 1-2 xylem vessels (Figures 5.E, F: green circle), while 1-5 xylem vessels are observed in the rhizomes.

The anatomical structures of the Vietnamese *C. pictus* and *H. speciosa* roots, rhizomes, and stems are similar to those of Indian *C. pictus* (Aruna et al. 2014; Shaikh et al. 2022) and *H. speciosa* (=*C. speciosus*) (i.e., based on the histochemical analysis of leaves, roots, stems, and rhizomes that we observed through cross-sectional images in previously reported studies) (Kumar et al. 2020; Shaikh et al. 2022) and other species of the family Costaceae, such as *C. afer* (Sonibare et al. 2023) and *C. spectabilis* (Shehu et al. 2021), mainly the epidermis, cortex and pith regions, and vascular bundle systems. Our results, however, demonstrated the structural similarity of rhizomes and stems only in *H. speciosa* species, which was different from *C. pictus* species in this study. These characteristics are evidence to identify or differentiate Vietnamese *C. pictus* and *H. speciosa* from other species.

Silica was completely absent in the roots of all *Costus* spp. (Tomlinson 1956), consistent with the observation in the root samples of both *C. pictus* and *H. speciosa* (=*C. speciosus*) roots from Vietnam. Oil cells, a prominent feature of Zingiberoideae, are completely absent in *Costus* spp. (Tomlinson 1956) and were observed to be absent in tissue specimens of both *C. pictus* and *H. speciosa* (=*C. speciosus*) from Vietnam. It is therefore reasonable to assume that they are absent from the entire subfamily Costoideae. However, the presence of oil cells is difficult to detect in some species from the family Costaceae and may be easily overlooked; therefore, further studies are needed to systematically re-evaluate this component of the family Costaceae.

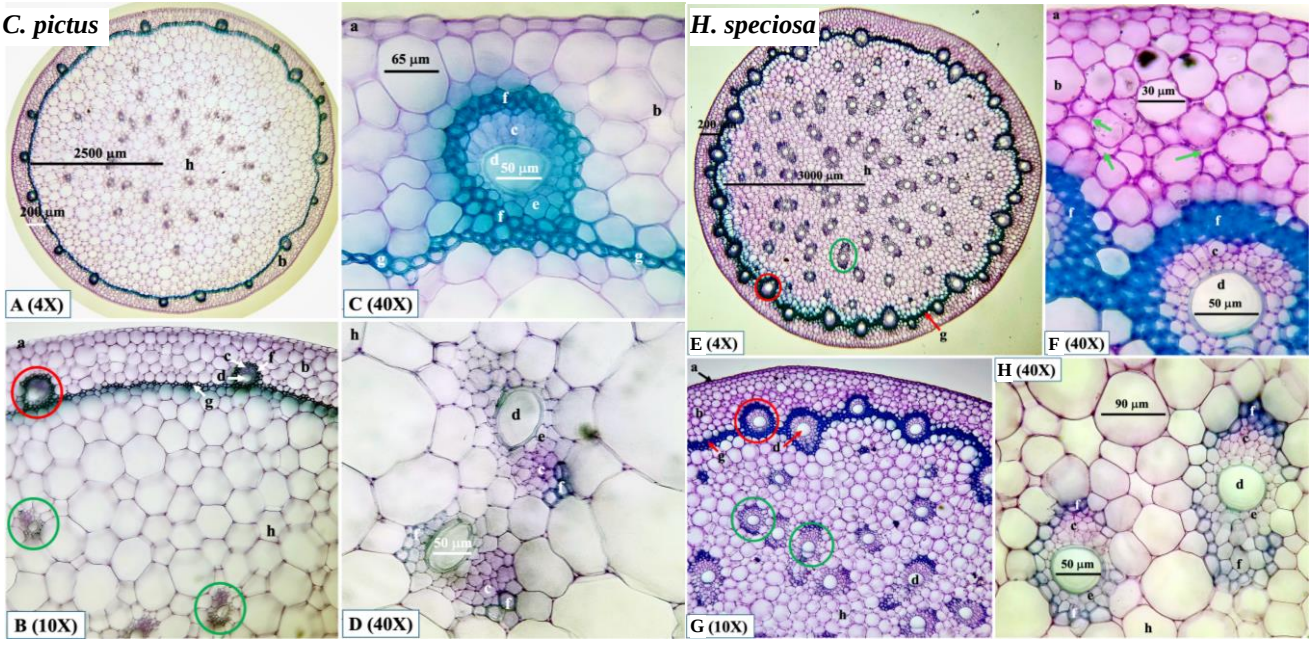

Figure 5. The features of cross-sectioned stem of *C. pictus* (A-D) and *H. speciosa* (E-H) (with magnifications: 4X, 10X, and 40X): (a, a') Epidermis with flat and thin cutin layer; (b) parenchymatous cells of the cortex region; (b') parenchymatous cells with triangular or polygonal intercellular spaces (green arrow) of the cortex region; (c, c') phloem; (d, d') xylem and xylem vessels; (e, e') xylem parenchyma; (f, f') sclerenchymatous bundle sheaths of the cortex region (red circle) and the inner region (green circle); (g, g') sclerenchymatous sheath ring; (h, h') parenchymatous cells of the pith region

Leaves

The midrib regions (1) of *C. pictus* and *H. speciosa* leaves are 3-4 times thicker than the leaf blade regions (2). The anatomical features of *C. pictus* leaves are as follows (Figures 6.A-E): 1) The midrib region (Figures 6.A, B, and E): The upper and lower epidermis surfaces are concave and convex, respectively (Figure 6.A). The structural features of the upper and lower epidermis are similar, consisting of a layer of polygonal cells with cellulose walls and a covered thin and flat cutin layer (Figures 6.a, b). The unicellular trichomes are concentrated in the lower epidermis (Figure 6.c). The angular collenchyma is above the lower epidermis, with 1-2 layers of polygonal cells and thick cellulose walls (Figure 6.d). Parenchyma has many layers of cells, polygonal shape, cellulose wall, randomly arranged, scattered (Figure 6.e) with silica or sand-like calcium oxalate-containing cells (Figure 6.g) (around the vascular bundles, the parenchyma cells are often small in size and contain many chloroplasts (Figure 6.h). The vascular bundles system consists of many vascular bundles arranged in rows from the midrib to both sides of the leaf blade region (Figure 6.i; red circle) (scattered with a few vascular bundles randomly with closed sclerenchymatous sheath arranged near the lower epidermis (Figure 6.j; green circle)). Each vascular bundle in the sclerenchymatous ring consists of the phloem (Figure 6.n), 2-3 xylem vessels (Figure 6.l), xylem parenchyma (Figure 6.m), and sclerenchymatous bundle sheath above the xylem and below the phloem (Figure 6.n). The phloem has polygonal cells with cellulose walls and are randomly arranged (Figure 6.k). The xylem has polygonal cells and lignify-impregnated walls (Figure 6.l). Moreover, the xylem parenchyma has polygonal cells and cellulose walls (Figure 6.m). The sclerenchymatous bundle sheath has polygonal cells and lignify-impregnated walls (Figure 6.n). 2) Leaf blade region (Figure 6.C): The structural features of the upper and lower epidermis are similar, consisting of a layer of polygonal cells with cellulose walls, covered by a thin and flat cutin layer. Hypodermis cells (2-3 layers) present under the adaxial and abaxial epidermis cells (Figure 6.f). Notably, stomata are only concentrated in the lower epidermis (Figure 6.o). Parenchyma has many layers of polygonal cells with cellulose walls, randomly arranged (around the vascular bundles, parenchyma cells are often small in size and contain many chloroplasts (Figure 6.h). Many vascular bundles are arranged in rows with a structure similar to the vascular bundles of the midrib region.

The anatomical features of *H. speciosa* leaves are as follows (Figures 6.F-J): The structural characteristics of *H. speciosa* leaves are similar to those of *C. pictus* leaves. However, the structure of *H. speciosa* leaves has several different features: in the midrib region, the upper and lower angular collenchyma consist of 3-4 layers and 4-5 layers, respectively, with polygonal cells and thick cellulose walls (Figure 6.e'). The number of xylem vessels is greater, with 1-4 vessels. Silica or sand-like calcium oxalate is absent in this region. Additionally, the presence of stomata (Figure 6.o') with sub-air cavity (Figure 6.p') and longer unicellular stout trichomes (the swollen trichome base (red arrow)) (Figure 6.c') along with the peltate glandular trichomes (Figure 6.d') is concentrated in the lower epidermis of the leaf blade region. Hypodermis cells (2-5 layers) present under the adaxial and abaxial epidermis cells in this region (Figure 6.g').

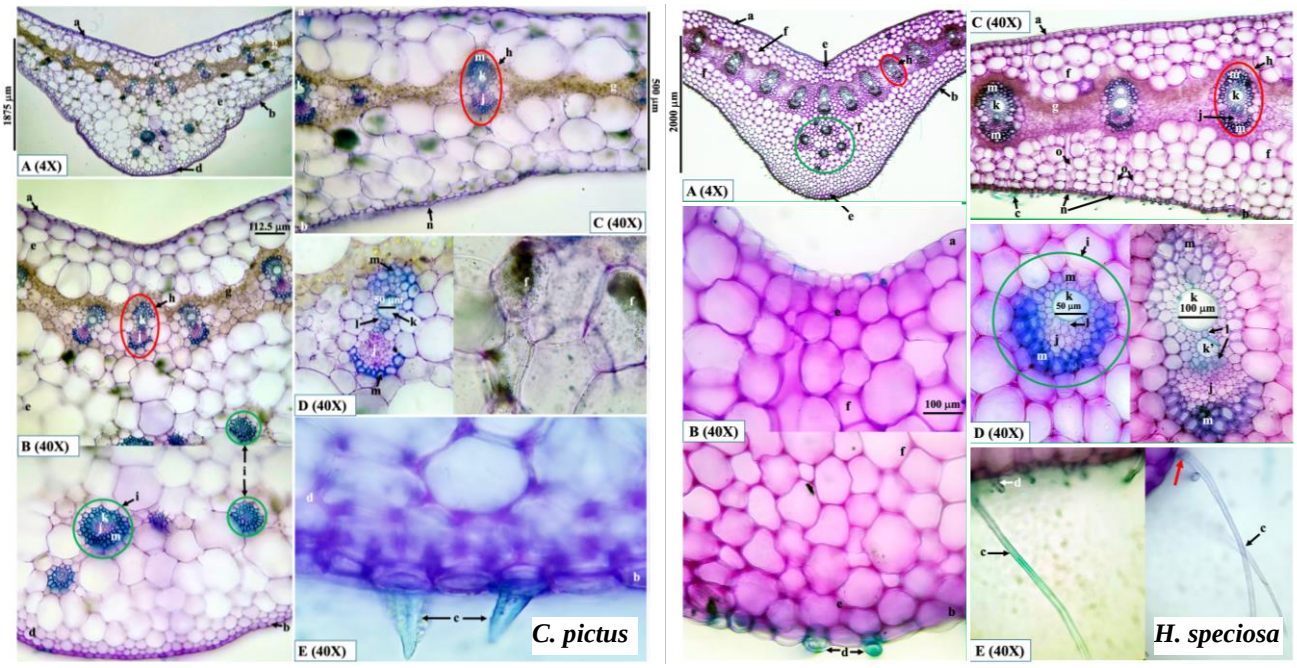

Figure 6. The features of cross-sectioned of *C. pictus* (A-E) and *H. speciosa* leaves (F-J) (with magnifications: 4X and 40X): (a, a') upper epidermis; (b, b') lower epidermis; (c) the unicellular trichomes; (c') the unicellular stout trichomes (red arrow); (d') the peltate glandular trichomes; (d, e') collenchymatous cells; (e, f') parenchymatous cells; (f, g') hypodermis cells; (g) silica or sand-like calcium oxalate; (h, h') chloroplasts; (i, i') the vascular bundles system and vascular bundles (red circle); (j, j') the vascular bundles system and closed vascular bundles (green circle); (k, k') phloem; (l, l') xylem and xylem vessels; (m, m') xylem parenchyma; (n, n') sclerenchymatous bundle sheath; (o, o') lower stomata; (p') sub-air cavity

Approximately 3-4 and 5-6 closed vascular bundles were observed in *C. pictus* and *H. speciosa* samples from Vietnam, respectively, and this feature was similar to the number of closed vascular bundles from *C. pictus* and *H. speciosa* (=*C. speciosus*) (Kerala, India) (Shaikh et al. 2022). However, the results of approximately 1-2 closed vascular bundles in the midrib region of *C. pictus* species in a study by Aruna et al. (2014) were less than those of the present results and a study by Shaikh et al. (2022). In addition, based on the number of closed vascular bundles of samples (3-6 closed vascular bundles) from Vietnam, it is possible to distinguish them from several other species, such as *C. afer*, *C. elegans* (=*C. malortieanus*), *C. lucanusianus*, *C. rumphianus* (approximately 1-2 closed vascular bundles) (Tomlinson 1956). The features of the cross-sectioned blade leaf from the *Costus*, *Chamaecostus*, and *Hellenia* species are so distinct. The most characteristic feature is a well-developed hypodermal layer; in contrast, the epidermis is very narrow and covered by a flat and thin cutin layer. Overall, there is one or more than one hypodermal layer below each surface of *Costus*, *Chamaecostus*, and *Hellenia* species, such as *C. afer* (only one layer below in both surfaces), *C. malortieanus* (2-layered adaxially, 2 to 3-layered abaxially), *C. rumphianus* (2-layered adaxially, 2-layered abaxially) (Tomlinson 1956), *Ch. cuspidatus* (=*C. igneus*) (1-layered adaxially, 2-layered abaxially) (Pazhanichamy et al. 2010), *C. pictus* (2-layered adaxially, 2 to 3-layered abaxially) (Aruna et al. 2014), compared with the current study, namely *C. pictus* (2-layered adaxially, 3-layered abaxially) and *H. speciosa* (=*C. speciosus*) (2 to 4-layered adaxially, 3 to 5-layered abaxially). Notably, the characteristics of the sub-air cavity in the lower epidermis were recorded in *C. malortieanus* (Tomlinson 1956), *C. igneus* (Hegde et al. 2016), and Vietnamese *H. speciosa* species.

Epidermal cells and stomata

The stomatal apparatus, which occurs in only the lower leaf epidermis in two samples studied, was of the hexacytic type (with two pairs of lateral subsidiary cells and one pair of polar/terminal subsidiary cells) (green circle) and tetracytic type (with four subsidiary cells) (orange circle) for *C. pictus* (Figure 7.B), whereas the tetracytic and paracytic types were found in *H. speciosa* (Figure 7.D). The epidermal cells in both species are rectangular or polygonal with curved or anticlinal walls, arranged randomly with irregular sizes between cells (Figures 7.A, B, C, and D). However, the lower epidermal cells of *H. speciosa* are more elongated in size, with irregular sizes between cells (Figure 7.D). In addition, cylindrical calcium oxalate crystals or silica particles on the upper epidermal surface (Figures 7.C, f) and unicellular stout trichomes (Figure 7.e) along with the swollen trichome base (red circle) on the lower epidermal surface were also observed in *H. speciosa* (Figure 7.D), whereas silica particles were observed in *C. pictus* (Figures 7.B, f).

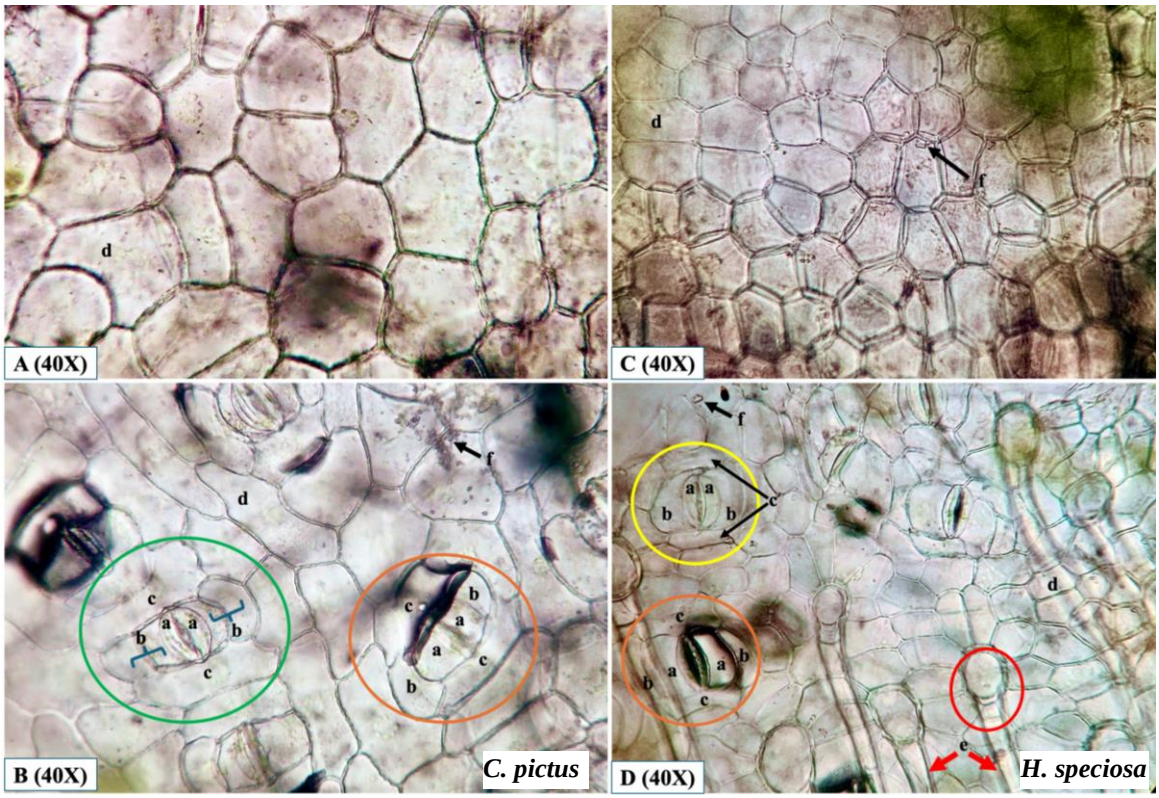

Figure 7. Epidermis with stomata of *C. pictus* (A, B) and *H. speciosa* (C, D) (with magnifications 40X): (A) upper surface view of the epidermis; (B) lower surface view of the epidermis with stoma; (C) upper surface view of the epidermis with stoma; (D) lower surface view of the epidermis with stoma; (a) guard cells; (b) lateral subsidiary cells; (c) polar/terminal subsidiary cells; (d) epidermal cells; (e) the unicellular stout trichomes; (f) cylindrical calcium oxalate crystals or silica particles

Table 1. Micromorphological comparison between *Costus pictus* and *Hellenia speciosa*

Characteristics	*Costus pictus*	*Hellenia speciosa*
Roots		
Suberized hypodermal cells	7-9 layers	4-5 layers
Casparian Endodermis	U-shaped	unclearly
Vascular bundles	16 phloem and 16 protoxylem bundles 16-17 metaxylem vessels	14 phloems and 14 protoxylem bundles 14-15 metaxylem vessels
Rhizomes		
The cortex region	the cortex region > stele region parenchymatous cells	the stele region > cortex region angular collenchymatous cells; parenchymatous cells
The stele region bundle	< 2 xylem vessels	< 5 xylem vessels
Pericycle	+	-
Sclerenchymatous sheath ring	-	+
Stems		
The cortex region	4-6 layer parenchymatous cells	3-4 layer angular collenchymatous cells; 6-7 layer parenchymatous cells
	cell size: < 65 μm	cell size: < 30 μm
	~ 20 vascular bundles	~ 32 vascular bundles
Sclerenchymatous sheath ring	< 2 layers	< 3 layers
The stele region	2500 μm	3000 μm
	~ 35 vascular bundles	~ 65-70 vascular bundles
Leaves		
The midrib region	1-2 layer collenchymatous cells	3-5 layer collenchymatous cells
	~ 4 vascular bundles	~ 6 vascular bundles
	< 2 xylem vessels	< 4 xylem vessels
	xylem size: < 35 μm	xylem size: < 50 μm
The leaf blade region	shorter/smaller sub-air cavity	longer/larger sub-air cavity
	xylem size: < 50 μm	xylem size: < 100 μm
	hypodermal layer: 2-layered adaxially, 3-layered abaxially	hypodermal layer: 2 to 4-layered adaxially, 3 to 5-layered abaxially
Trichomes	shorter unicellular trichomes	longer unicellular trichomes
	-	peltate glandular trichomes
Silica/sand-like calcium oxalate	+	-
Epidermal cells and stomata		
The unicellular stout trichomes with the swollen trichome base	-	+
Stomatal apparatus	hexacytic and tetracytic types	paracytic and tetracytic types

Note: +: Present; -: Absent

The main features of the two experimental leaves from *C. pictus* and *H. speciosa* in the current study, including the upper and lower epidermis, collenchymatous and parenchymatous cells, mesophyll tissues, stomata, and sub-air cavity, had similar structural features to the *C. pictus* specimens collected from Tamil Nadu, India by Aruna et al. (2014) and the *H. speciosa* (=*C. speciosus*) specimens collected from Kerala, India by Shaikh et al. (2022). However, the absence of trichomes on both leaf surfaces from Kerala *C. pictus* (Shaikh et al. 2022) and Tamil Nadu *C. pictus* (India) (Aruna et al. 2014) and trichomes only in the lower leaf epidermis was observed in Long An *C. pictus* (Vietnam). Moreover, the presence of the unicellular stout trichomes only in the lower leaf epidermis was found in both samples from *H. speciosa* (=*C. speciosus*) (Vietnam) and *H. speciosa* (=*C. speciosus*) (India) (Shaikh et al. 2022). Particularly, the multicellular glandular with a pointed apex was observed in the epidermis surface leaf of *C. elegans* (=*C. malortieanus*) (Tomlinson 1956) and *H. speciosa* (=*C. speciosus*) (Shaikh et al. 2022), but not in the Vietnamese sample. Stomata were diffusely distributed and were found only in the lower leaf epidermis of the tested samples. This feature is consistent with previous studies (Aruna et al. 2014; Kumar et al. 2020; Shaikh et al. 2022). Notably, only one type of hexacytic stomata was found in the *C. pictus* (India) sample (Aruna et al. 2014), while hexacytic and tetracytic stomata types were observed in the *C. pictus* (Vietnam) sample. Sonibare et al. (2023) observed that the stomata type on the abaxial surface of *C. afer* is characterized by paracytic stomata and no trichomes on this surface. Additionally, it can be suggested that one of the characteristics to distinguish the *H. speciosa* (=*C. speciosus*) (Vietnam) plant from other species is based on the two types of tetracytic and paracytic stomata. The different characteristics of *C. pictus* and *H. speciosa* are presented in Table 1.

The first step in herbal medicine quality control is ensuring the correctness of the desired species for the intended use. There are various techniques, such as macroscopic and microscopic morphological identification and chemical analysis, especially the characteristics of plant tissues observed through a microscope to identify the

appropriate plant species while they are still in their unextracted form (WHO 2011, 2018; Osman et al. 2019). The observation of tissue-level morphology or anatomy is a main aid for the authentication of herbal drugs (Muyumba et al. 2021). Along with the morphological method, the anatomical method has been used for a long time to accurately identify some plants because it is thought to be useful and accurate (WHO 2011, 2018; Alamgir 2017; Srivastava and Misra 2018; Osman et al. 2019). Taxonomists can properly determine plant specimens and detect any possible confusion by observing the typical anatomical features of plants. Additionally, the microscope is a quick and easy tool for identifying plants anatomically (i.e., characteristics of plant cells) (Chen et al. 2024). Previous studies observed that species of the family Costaceae could be delimited based on their anatomical structural features (i.e., leaves, roots, rhizomes, and stems), such as *C. afer* (Tomlinson 1956; Edeoga and Okoli 1998; Sonibare et al. 2023), *C. rumphianus*, *C. elegans* (=*C. malortieanus* H. Wendl.), *C. lucanusianus* (Tomlinson 1956; Edeoga and Okoli 1998), *C. pictus*, *H. speciosa* (=*C. speciosus*) (Aruna et al. 2014; Kumar et al. 2020; Shaikh et al. 2022), *C. cuspidatus* (=*C. igneus*) (Hegde et al. 2016), *C. dubius*, *C. laevis*, *C. woodsonii* (Smisha and Sabu 2018), *C. spectabilis* (Shehu et al. 2021). The important anatomical features of rhizomes, roots, stems, and leaves of *C. pictus* and *H. speciosa* were recorded in detail via optical microscopy in the present study. However, the literature on the anatomical structure characteristics of some species of the *Costus* and *Hellenia* is still limited. More in-depth investigations are needed to obtain more information about this genera's anatomical structure.

In conclusion, a micromorphological comparison between *C. pictus* and *H. speciosa* from Vietnam on the anatomical structural characteristics of their roots, rhizomes, stems, and leaves are the highlights of the current study. Notably, *C. pictus* and *H. speciosa* are easily distinguished from each other micromorphologically by their root and leaf microstructures. Briefly, the root microstructure of *C. pictus* consists of 8-9 suberized hypodermal cell layers, U-shaped casparian endodermis, and 16 phloem bundles arranged alternately with 16 protoxylem bundles on the same ring, followed by leaf microstructure with hexacytic and tetracytic types of stomata of the lower epidermis, along with shorter unicellular trichomes. Meanwhile, *H. speciosa* root microstructure is distinguished by features such as 4-5 suberized hypodermal cell layers, casparian endodermis unclearly, 14 phloems arranged alternately with 14 protoxylem bundles on the same ring, followed by leaf microstructure with tetracytic and paracytic types of stomata of the lower epidermis, along with sub-air cavity, longer unicellular stout trichomes, and peltate glandular trichomes. This specific information can be used in a micromorphological monograph to accurately identify these species, namely *C. pictus* and *H. speciosa*.

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