BIODIVERSITAS
Volume 23, Number 8, August 2022
Pages: 3907-3920

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230807

Morphological characters, pharmacognostical parameters, and preliminary phytochemical screening of *Curcuma sahuynhensis* Škorničk. & N.S.Lý in Quang Ngai Province, Vietnam

TRAN VAN CHEN[1], DUONG NGUYEN XUAN LAM[1], CAO LY TAN THONG[1], DANH DUC NGUYEN[2], NGUYEN THANH TO NHI[3,♥], NGUYEN THANH TRIET[4,♥♥]

[1]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

[2]Institute of Applied Technology, Thu Dau Mot University. No. 06, Tran Van On Street, Phu Hoa Ward, Thu Dau Mot City 751000, Binh Duong Province, 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. ♥email: nttnhi@nttu.edu.vn

[4]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. ♥♥email: nguyenthanhtriet1702@ump.edu.vn

Manuscript received: 20 June 2022. Revision accepted: 17 July 2022.

Abstract. *Chen TV, Lam DNX, Thong CLT, Nguyen DD, Nhi NTT, Triet NT. 2022. Morphological characters, pharmacognostical parameters, and preliminary phytochemical screening of* Curcuma sahuynhensis *Škorničk. & N.S.Lý in Quang Ngai Province, Vietnam. Biodiversitas 23: 3907-3920. Curcuma sahuynhensis* (Vegetable Turmeric or Rau Nghe) has been used as a flavor and a spice in Vietnamese cuisines. Information on micromorphological characters, pharmacognostic parameters, and phytochemical screening of *C. sahuynhensis* has not been reported in the literature. Therefore, the accurate verification of plants requires further research to determine their quality and safety in order to make optimal use of them as raw materials for medicines and food. In this work, macro-and micromorphological features, pharmacognostic parameters, and phytochemical screening were performed according to the Vietnamese Pharmacopoeia V guidelines and other published data. Macromorphologically, it can grow up to 75 ± 5 cm tall and up to 10 per pseudostem. The inflorescence consists of 10–24 bracts. Rhizome branched, and remote tubers were present. The flower was yellow with a warm yellow midrib band, and an incision up to 0.6 cm long on the apex labellum. Anthers are L-shaped, stalked with no knobs below the thecae. Micromorphologically, there are anatomical similarities between the species in the genus *Curcuma*, particularly in terms of leaves, roots, and rhizomes. Under the lower epidermis of the leaf midrib are small vascular bundles (8–10 bundles). The stomatal index (SI) was 8.97 in abaxial and 1.06 in adaxial of the leaf. Prismatic calcium oxalate crystals were found in the leaf but they were absent in the root. Starch grains were also found in leaf powder (11–12.5 µm) and rhizome powder (15–20 µm). Additionally, the presence of oleoresin (in leaves and rhizomes) and the absence of crystals in the cortex region of the rhizome was found to be significant features in identifying *C. sahuynhensis*. Phytochemical screening found that its rhizome and inflorescence contain essential oil, amino acids, flavonoids, tannins, coumarins, triterpenoids, polyuronides, and reducing compounds. Pharmacognostically, rhizome powders' moisture content, total ash value, and acid-insoluble ash value were 11.79 ± 0.20%, 7.32 ± 0.60%, and 0.24 ± 0.08%, respectively.

Keywords: *Curcuma sahuynhensis*, macro-and micromorphological features, physico-chemical parameters, phytoconstituents, powder characteristics

Abbreviations: ddw: double distilled water, SI: stomatal index

INTRODUCTION

Globally, about 130 species in the genus (Plants of the World Online 2022), *Curcuma* L. is the third-largest genus of the Zingiberaceae family (Záveská et al. 2012), and they are well-known for various medicinal applications. Among different habitats in the world, the Indochinese region is supposed to be within top-abundant reservoirs for Zingiberaceae (Leong-Škorničková et al. 2010). Consequently, dozens of *Curcuma* species have been discovered in Vietnam over decades. Among them, *Curcuma sahuynhensis* was reported in 2015 (Leong-Škorničková et al. 2015). *C. sahuynhensis* is actually a perennial rhizomatous herb, an endemic species found in Vietnam. The rhizomes, young shoots, and inflorescences of *C. sahuynhensis* are extensively used as a flavor and a spice in Vietnamese cuisines. It is a familiar component in the cuisine of the local people of Quang Ngai Province, and thus it is also called Vegetable Turmeric or Rau Nghe (Sam et al. 2020).

Sam et al. (2020) reported the rhizome essential oil of *C. sahuynhensis* is against *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Candida albicans*. The major components in the essential oil that showed good activity were *β*-pinene (52.7%), *β*-caryophyllene (11.1%), *α*-pinene (8.6%), caryophyllene oxide (6.5%), and (*Z*)-*β*-farnesene (5.9%), etc (Sam et al. 2020). However, except for essential oil, the other chemical constituents of the plant have not yet been investigated.

Macromorphologically, *C. sahuynhensis* has been described in detail by Leong-Škorničková et al. (2015) and

Sam et al. (2020). However, there has been no published research on detailed descriptions of its micromorphological features. Thus, the micromorphological feature's identification of *C. sahuynhensis* becomes extremely necessary.

Previous studies have shown that the genus *Curcuma* L. is a taxonomically difficult species due to its short flowering period and nearly identical vegetative parts (Leong-Škorničková et al. 2008). Due to extensive polyploidization and hybridization, distinctive levels of genetic and morphological variation among *Curcuma* species were reported, implying great diversity of this genus (Záveská et al. 2012). Additionally, the macromorphological similarity of several species creates difficulties in their identification (Leong-Škorničková et al. 2008; Anu et al. 2020). Accurate species identification is important for searching and targeting their different biological activity potentials. Traditionally, macromorphological characteristics description is the primitive method for taxonomy. In the case of morphological classification of the genus *Curcuma* when they do not have all the aerial parts (e.g., in the non-flowering stage) that it is very difficult to identify them precisely.

Therefore, in many cases, the external morphology of the rhizomes and leaves is the part used for species identification; however, using only their outer form is often difficult. Micromorphologically, morpho-anatomical (the plant body's structure), histochemical, and powder microscopic features were related to their structure and composition, so it plays an important role in taxonomic identification problems. Anu et al. (2020) reported that the micro-morphological features have an important role in the standardization of raw medicinal herbs as well as in the accurate identification of medicinal plants (Anu et al. 2020). There is increasing evidence that scientists have been used micromorphological methods to draw taxonomical conclusions (Remashree and Balachandran 2006; Uma et al. 2014; Amel 2015; Wijayasiriwardene et al. 2016; Yee et al. 2019; Anu et al. 2020; Liu et al. 2020; Van Chen et al. 2022a). In addition, species identification by the micromorphological method is a useful tool in overcoming the limitations of the macromorphological taxonomy for closely related species whose characteristics may still not have enough based to distinguish. In other words, the micromorphological method has been used to support macromorphological taxonomy, as well as has been used to identify medicinal plants.

In order to provide detailed and precise scientific evidence of the botanical features, this study combined macro-and micromorphological analysis to accurately identify *C. sahuynhensis* (in Quang Ngai Province, Vietnam). Additionally, this study conducted phytochemical screening to identify different chemical components in Vegetable Turmeric and standardize the pharmacognostical parameters of medicinal herbs. The results obtained in this work would establish criteria for identifying *C. sahuynhensis* and select the correct species for medicinal use. In particular, it will also encourage further experimental attempts for high-degree isolation and more in-depth biological effects to develop *C. sahuynhensis* into a valuable medicinal plant.

MATERIALS AND METHODS

Plant material

Curcuma sahuynhensis fresh plants were harvested from Sa Huynh village, Duc Pho district, Quang Ngai province, Vietnam. The samples were washed free of soil before being air-dried for pharmacognostic, preliminary phytochemical screening, and macro-and micromorphological analyses.

Procedures of morphological study

Macromorphological characteristics

To determine the scientific name of *C. sahuynhensis*, the morphological comparison method was used according to the guidelines of the Vietnamese Pharmacopoeia V (Ministry of Health 2017) and the samples were compared with the taxonomic key (Nguyen et al. 2022), images, and description in the references (Sam et al. 2020) to identify the species name.

Micromorphological characteristics

Through the iodine green-carmine double staining method of the Vietnamese Pharmacopoeia V (Ministry of Health 2017) as a guideline, the micromorphological characteristics of the samples were identified. First of all, the representative specimens (leaves, roots, rhizomes) were trimmed into small pieces. Then, the specimens were cut by hand-cut into thin slices (about 10–20 μm) with a razor blade. Next, thin-complete transverse sections were used for staining. These samples were cleared in 50% (v/v) chloral hydrate reagent for 10 minutes after being cleansed in 5.0% (w/v) chloramine-T. They are subsequently acidulated in 1.0% (w/v) acetic acid for two minutes. Those slices were dyed in 0.3% (w/v) Iodine Green (about less than five seconds) and 1.0% (w/v) Carmine (until the slices had a clearer color). The excess reagents (chloramine T, chloral hydrate, acetic acid, iodine green, and carmine) were eliminated step by step using double-distilled water (ddw). Further, the sections were mounted in mixed glycerin-water (50-50) and covered by a coverslip. Then, the prepared slides were observed under a light microscope at 4X, 10X, and 40X magnifications (HumaScope, Germany).

Besides, by using the protocol described in the Vietnamese Pharmacopoeia V (Ministry of Health 2017) the features of the leaf powder and rhizome powder were discovered. The steps are followed: a small amount of powder was taken on a glass slide and mixed with drop water, and covered by a coverslip. The histochemical study was also done by using iodine-potassium iodide reagents to detect starch granules. All slides were observed under a light microscope at 10X, and 40X magnifications (HumaScope, Germany).

The characteristics of the upper and the lower surface leaf epidermis, and the stomata were observed under the microscope with magnification (40X; Labomed, USA)

(Zahara 2020). In addition, for general observations on the distribution of stomata in both epidermal surfaces, the slide was prepared for the determination of the number of stomata for the stomatal index (SI) with 20 replicates. It was observed by the field of view under the microscope with 40X magnification for this species with the following formulas (Ministry of Health 2017):

Determination of stomatal index (SI):

$$SI\ (\%) = (S/(S+E)).100$$

Where, S and E were the number of stomatal cells and epidermal cells (including trichome) in the microscopic field of view, respectively.

Procedures of phytochemical study

Pharmacognostic evaluation

C. sahuynhensis rhizomes were cleaned of impurities, then cut into small pieces, and air-dried. The drying process stops when the sample can maintain the water content in the raw material by about 13% (This moisture content for most fungi can not grow). The material was dried and pulverized. After that, the moisture content, total ash value, and acid-insoluble ash value of the powder sample must all meet the requirements of the Vietnam Pharmacopoeia for raw medicinal materials. These pharmacognostic parameters of rhizome powder were determined according to methods described in the Vietnamese Pharmacopoeia V (Ministry of Health 2017).

Preparation of vegetable turmeric extracts and preliminary phytochemical screening

The preliminary phytochemical study of secondary metabolites was performed by the Cuiley method (Cuiley 1984) with slight modification. The powdered samples (rhizome, inflorescence**)** were extracted using diethyl ether then with ethanol, and finally with distilled water so that extracts were obtained. The rhizome and inflorescence extracts of *C. sahuynhensis* were then screened for the presence of phytochemical constituents. The phytochemical constituents such as carbohydrates (reducing sugars), essential oil, fats, amino acids, sterols, tannins, flavonoids, alkaloids, coumarins, cardiac glycosides, saponins, and polyuronides were analyzed qualitatively using the standard protocol. The assays were repeated thrice under the same conditions.

Data analysis

Morphological features

Morphological data were analyzed according to Van Chen et al. (2022a). Macromorphologically, the size of the six sample parts was measured using a standard ruler. Micromorphologically, all values of cell size and cell components were measured by eyepiece micrometers (Olympus, Japan). However, the stomatal number and stomatal index (SI) were counted and determined with 20 replicates (magnification 40X; Labomed, USA). All results (including maximum and minimum values) were presented as mean values ± SD (Standard Deviation) by using Microsoft Excel 2016.

Phytochemical analysis

The experiment of the phytochemical study was in triplicate and the results were expressed as mean values ± SD (Standard Deviation). The data results were calculated and analyzed using Microsoft Excel 2016.

RESULTS AND DISCUSSION

Morphological characters

Macromorphological features

Curcuma sahuynhensis is a perennial rhizomatous herb composed of underground parts (roots, rhizomes, tubers) and overground parts (leafy shoots, pseudostems, petioles, leaf blades, and inflorescences).

Aerial shoot, rhizome, and root tuber: Vegetable Turmeric's aerial shoot 27–75 ± 5 cm tall (includes leaf shoots, leaf sheaths, petiole, leaf, and inflorescence) (Figure 1.A). The rhizome is cylindrical to oval, 1.4–4.0 x 0.5–1.0 cm, light brown outside, creamy white to pale yellow when broken or cut, fragrant and spicy taste (Figure 1.D and 1J). The rhizome branched and tuberous roots 7.0–16.0 cm long, grown deep into the ground, distant from the main rhizome. Root tubers 3.0–9.0 x 0.8–2.0 cm, outside light brown and rough, inside (the cross-section) spongy white at outer turning pale white at the center (Figure 1.I).

Leaves: up to 10 per pseudostem. Lamina elliptic to lanceolate, lightly plicate, 25.0–34.0 x 12.5–14.0 cm in size (measured in the stage of flowering), apex attenuate to acuminate, base obtuse to round bulb, adaxially dark-green and glabrous, abaxially light-green and puberulent (Figure 1.A). Petiole canaliculate, 3.0–24.0 cm long, petiole of first leaf shortest, innermost leaf tallest, green, and glabrous; leaf sheaths trough-shaped, glabrous, 3.0–14.0 cm long, white to light-green at the base turning dark-green towards the apex with 5–7 sheaths hugging each other to form a pseudostem (Figure 1.A). The ligule is translucent, hyaline up to 0.3–0.5 cm long, greenish-white, and glabrous (Figure 1.B).

Inflorescence: lateral (sometimes terminal), 18.0–25.0 cm long (Figure 1.C); peduncle 5.0-16.0 cm long and 6.0–8.0 mm in diameter, pale green, with 1 small sheath of light-green and up to 10 cm long, embracing the peduncle; spike 7.0–15.0 cm tall and consists of 10–24 bracts, with no obvious coma (Figure 1.E); bracts 3.5–4.5 x 1.5–2.0 cm, broadly ovate to narrowly rhombic (the bracts at the base are larger than the others at the apex of the inflorescence, whitish at the base tinged green or pale pink to coral-red at the apex, both sides glabrous (Figure 1.F).

Flowers: it is 4.0–5.5 cm long; calyx 1.5–1.8 cm long, with irregular 3–lobed, semi-translucent, white tinged pink; corolla tube 1.8–1.9 cm dorsal corolla lobe grown upright, 2.0 x 0.7 cm, triangular, pink, glabrous; lateral corolla lobe twisted (in blooming), 1.8 x 0.6 cm in size, triangular, pink, glabrous; labellum 1.6 x 1.7 cm, obovate, yellow at apex turning white at the base with warm yellow midrib band, apex emarginated with an incision up to 0.6 cm long; lateral staminode 1.5–1.7 x 1.1–1.3 cm, irregular ovoid, dark yellow and fades towards the base (Figure 1.H and 1H'). Stamens 1.6–1.7 cm long; filaments 4.0–5.0 mm long

joined to stigma, with cream-white to light yellow; Anther L-shaped, stalked, 1.2 cm long, pale yellow-orange, containing glandular hair; pollen is ovate-shaped, white (Figure 1.G). Epigynous glands two, 5-6 mm long. Stigma is 0.1 cm wide, creamy white, and ostiole facing forward (Figure 1.G). The tyle of the ovary is the lower ovary, 0.4–0.5 x 0.2–0.4 cm in size, trilocular, and creamy white. The ovary's cross-section has 3 chambers, each chamber containing many ovules (Figure 1.K).

Fruits: there are simple capsules, globose, three-celled, with brown bulges on older fruit, 1.4 cm in diameter, white and glabrous; each fruit has about 18 seeds. The seed is irregular oblong ovoid, 0.4–0.5 x 0.2–0.3 cm, light brown, glossy (Figure 1.H").

The blooming season is mainly from May to August when the lateral inflorescence of *C. sahuynhensis* produced. Some individual produce terminal inflorescence making the flowering time extends to November. Fruiting is from June to November.

Taxonomic note

The morphological similarities between species also contribute to taxonomic difficulties in the genus *Curcuma*. Therefore, the current study of *C. sahuynhensis* was carried out on various plant parts in terms of its morphology. *C. sahuynhensis* in Quang Ngai Province has macromorphological characteristics almost identical to the ones in the protologue reported by Leong-Škorničková et al. (2015). The diagnosis in this type description showed that *C. sahuynhensis* is almost similar to *C. xanthella* but differs in characteristics of anther, labellum, inflorescence and bract (Leong-Škorničková et al. 2015; Sam et al. 2020). However, the two species which are described later, *C. cotuana* Luu, Škorničk. & H.Đ.Trần and *C. vinhlinhensis* D.D. Nguyen & T.A. Le more resembles at first glance *C. sahuynhensis*. It is most similar to *C. vinhlinhensis* (Nguyen et al. 2022) in the general view of habit, warm yellow to orange flowers, and the L-shaped anther but distinguished from the latter by the longer spike, the apex of the floral tube pale yellow (vs. tinged pinkish red) internally, midrib band of labellum absent lateral red lines (vs. present two lateral red lines), anther L-shaped with no the knobs below the thecae (vs. with two small blunt knobs below the thecae). It is also similar to *C. cotuana*. The differences were discussed in the prior description of the latter (Lu'u et al. 2017). A detailed comparison of *C. sahuynhensis* with two allies is presented in Table 1.

Table 1. Comparison of the morphologically vegetative and reproductive characteristics of *Curcuma sahuynhensis* with two allies. Details showing the difference between each species with the host plant are given in bold

Character		***C. sahuynhensis***	***C. vinhlinhensis***	***C. cotuana***
Inflores-cence	Position	Lateral and terminal	Lateral only	Terminal only
	Spike	6–15 cm	**10–20 cm long**	5–9 cm long
Calyx		14–19 mm	10–15 mm	20–22 mm
Floral tube		1.8–2.8 cm, internally white, turning pale yellow towards the apical part	2.1–2.3 cm, **internally white in basal half turning deep purple in apical half**	**Ca. 3.5 mm**, internally white throughout
Lateral staminodes		15–22 × 10–14 mm, unequally ovate to obovate to rhomboid - Warm yellow to Orange throughout	17–18 × 11–13 mm, elliptic, apex round **- Warm yellow to Orange throughout with a red spot at the base**	**19–21 × 9.5–10.5 mm, unequally ovate to rhomboid** - Warm yellow to Orange throughout
Labellum	Shape	Obovate, apex emarginated, incision up to 7 mm long	Obovate, apex emarginated with incision up to 3 mm long,	Rhomboid, apex emarginate, incision up to 6 mm
	Color	Dark yellow to orange with a bright median band without any dark red lines at the base	Dark yellow to orange, **midrid band yellow with two lateral dark red lines at the base**	Midrid band without any dark red lines at the base
	Size (mm)	15–23 × 12–18	18–19 × 14–15	21–23 x 15–16
Filament		6–7 mm long	**3–4 mm long**	7 mm long
Anther		8–9 mm long, without knobs below the thecae	8–9 mm long **with two small blunt knobs below the thecae**	**12.5 mm long with two blunt knobs below the thecae**
Anther spurs		3.5–5 mm long, outward-facing	**2–3 mm long**, conical, outward-facing	3.5–4 mm long, outward-facing
Anther crest		Less than 1mm long, apex emarginate	Reduced, up to 0.5 mm long, apex emarginate	**1.0–1.2 mm, bi-lobed**

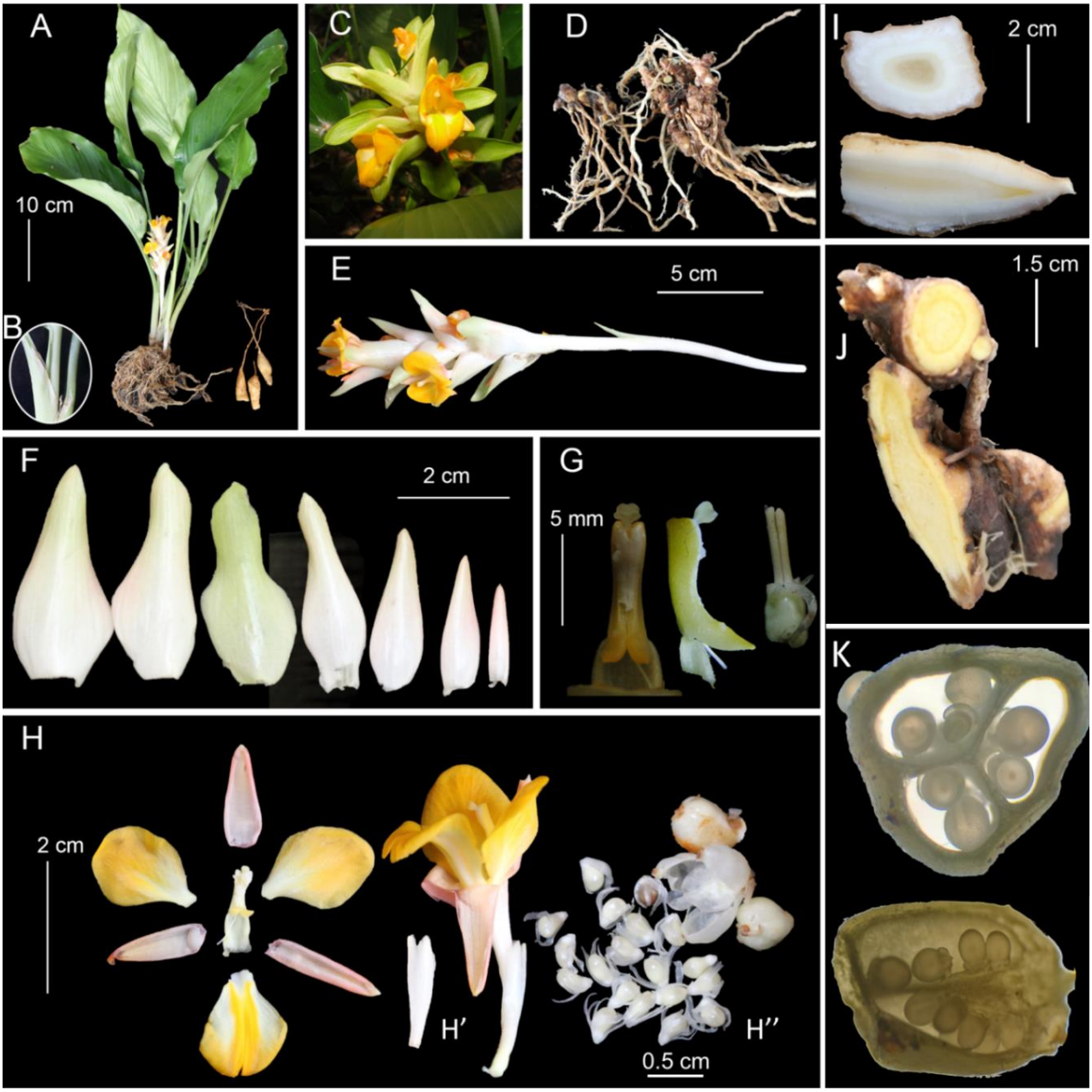

Figure 1. Morphological characteristics of *Curcuma sahuynhensis*: A. All parts of the plants; B. Ligule; C. Habit; D. Root and rhizome; E. Inflorescence; F. Bracts from the base to the top of an inflorescence; G. Anther (with L-shaped), ovary bearing epiginous glands; H. Fresh flower dissection; H'. Single flower with calyx tube; H". Fruits and seeds; I. Cross-section of the tuber; J. Cross-section of the rhizomes; K. Ovary's cross-section has 3 chambers containing many ovules. Photos A, B, D–K by Tran Van Chen, Photos C & G taken by Danh Duc Nguyen.

Micromorphological features

Leaves

Midrib and vascular bundles

A transverse section of the leaf is symmetrical, which is comprised of midrib and leaf blade (Figures 2.A and 2.C). The upper surface of the midrib is marginally concave forming an acute angle, while the lower one is significantly convex imitating a parabolic shape (Figures 2.A and 2.B). The adaxial and abaxial epidermis is a single-layer tissue consisting of polygon-shaped cells, whose walls vary in size and are totally soaked with cellulose. The upper angular collenchyma consists of polygon-shaped and diverse-magnitude cells, which are arranged tightly into 5–15 layers (Figure 2.B). The aerenchyma is immense and is formed through a tight arrangement of about 30–40 circular-shaped and unequal-size cells, which create enormous pores alternating large vascular bundles (Figure 2.c). Parenchyma cells constructing the aerenchyma also contained a lot of chloroplasts, which formed a greenish boundary between the collenchyma and the vascular bundles. Nearby the abaxial epidermis, the arc of large vascular bundles mimicked the curve of the below midrib

that contained 10–13 heterogenous bundles interspersed with air cavity on a parabolic curve (Figures 2.B and 2.E). In this each vascular bundle (Figure 2.E), the xylem is located in the superior position while the phloem is located in the inferior site. It is covered by a ring of the irregularly-sized sclerenchymatous sheath, which included 3–5 layers of lignified polygonal cells (Figure 2.G). Xylem included 1–3 primary veins and 2–4 secondary veins, which are nearly round-shaped, lignified, and randomly distributed (Figure 2.e). Phloem is larger than xylems in every bundle and consists of cellulose-covered polygonal cells, which are made up of 7–10 layers (Figure 2.F). However, the central midrib has 4–6 smaller phloem-xylem bundles scattered in the upper collenchyma whose structure resembled the ones' in the arc of large vascular bundles, but sometimes contained phloem that is randomly organized above the xylem (Figure 2.A). The structure of the lower collenchyma resembled that of the upper one, but the number of layers was just 3–5. In addition, oil cells are scattered throughout the leaf parenchyma (Figure 2.h). The abaxial epidermis took after the adaxial epidermis but further obtained several non-glandular unicellular trichomes (Figure 2.l).

Leaf blade

A transverse section through the leaf blade is composed of 5–7 layers of collenchymatous cells (Figure 2.C). The adaxial and abaxial epidermis consisted of a single layer, polygonal-slightly elongated cells. It is covered with a moderately thin cuticle (Figures 2a and 2a'). The number of stomata in the abaxial epidermis has more than that of the adaxial epidermis. The stomata are paracytic type (Figure 2.i), and it is connected to the sub-stomatal cavity (Figure 2.j). The spongy parenchyma consisted of 3–5 layers, with irregular shapes. The middle cell layer contained vascular bundles (Figure 2.C), many chloroplasts (Figure 2.d), and prismatic calcium oxalate crystals (Figure 2.k).

Stomata

The stomata are paracytic with the guard cells (Figure 2.a) (containing chloroplasts (Figure 2.b)) lying parallel to the stomatal pore opening forming an elliptical (Figures 2A and 2B). In addition, a pair of terminal cells is presented, and the stomatal pore (Figure 2.d) is located perpendicularly to the elongated-hexagonal epidermal cells (Figure 2.c). The stomata are found on both sides of the leaf, but the number of them is less on the adaxial surface than those on the abaxial surface of the leaf.

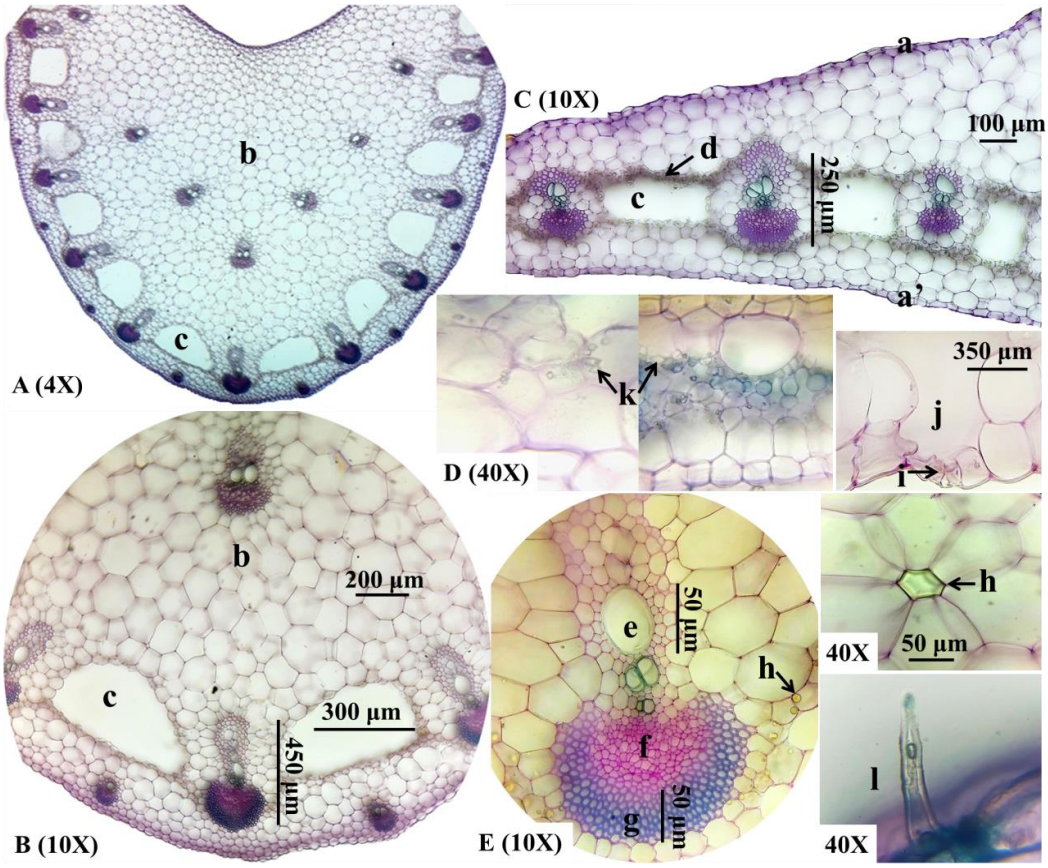

Figure 2. The features of cross-sectioned leaves of *Curcuma sahuynhensis* (with magnifications 4X, 10X, 40X): (A, B) midrib, (C, D) leaf blade with prismatic calcium oxalate crystals and stomata, (E) vascular bundles and collenchymatous cellupper, oil cell. (a) upper epidermis; (a') lower epidermis; (b) parenchyma tissue; (c) air cavity; (d) chloroplasts; (e) xylem; (f) phloem; (g) sclerenchymatous bundle sheath; (h) oil cell; (i) stomata; (j) sub-stomatal cavity; (k) prismatic calcium oxalate crystals; (l) non-glandular unicellular trichomes

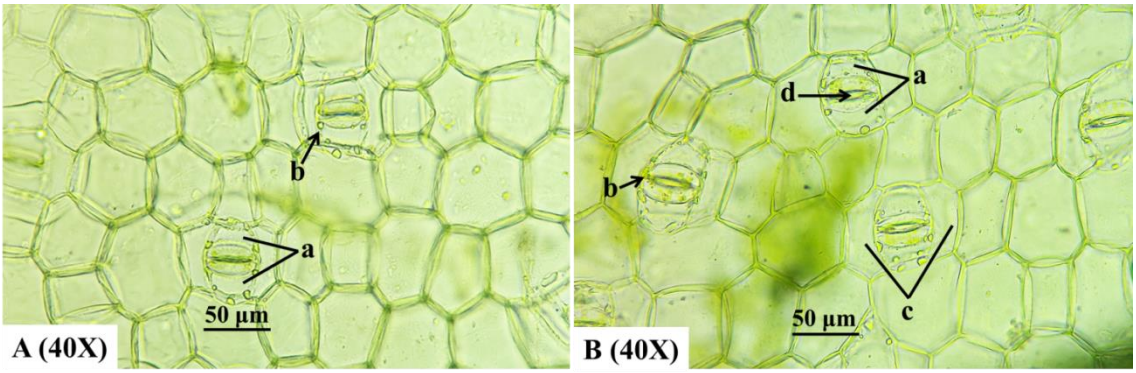

Figure 3. Epidermis with stomata of *Curcuma sahuynhensis* (with magnifications 40X): (A) adaxial surface view of the epidermis with stoma closed; (B) abaxial surface view of the epidermis with stoma open; (a) guard cells; (b) guard cell chloroplasts; (c) epidermal cells; (d) stomatal pore

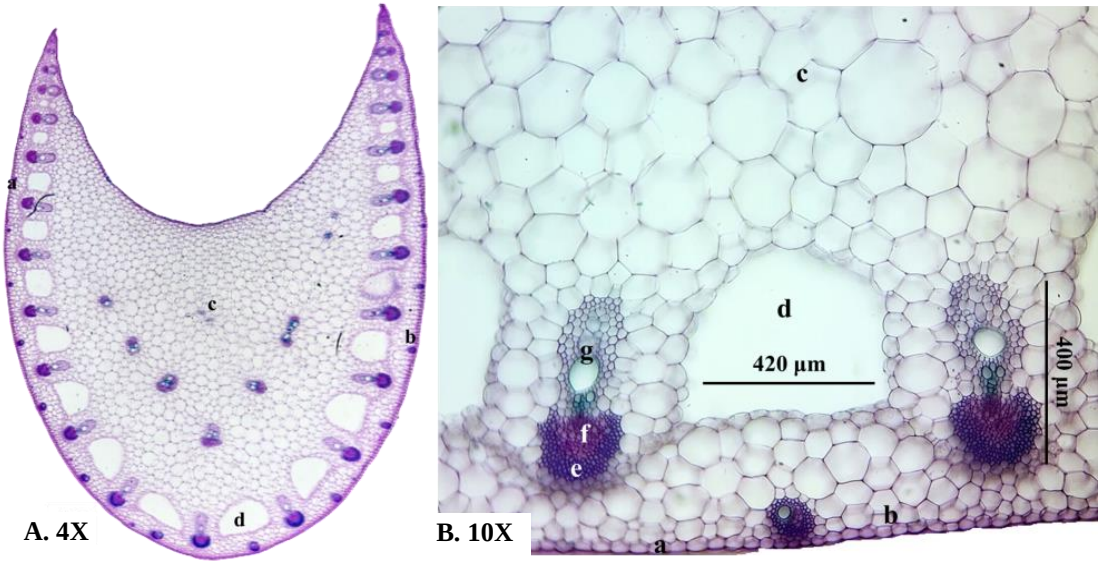

Figure 4. A-B. The features of cross-sectioned petiole of *Curcuma sahuynhensis* (with magnifications 4X, 10X): (a) epidermis; (b) hypodermis; (c) parenchyma tissue; (d) air cavity; (e) sclerenchymatous bundle sheath; (g) xylem; (f) phloem.

Stomatal index

The results obtained showed that the stomatal index (with magnification 40X) is different on each surface of the epidermal cell. The highest stomatal index on abaxial and the lowest one on adaxial found in *C. sahuynhensis* are 8.97 and 1.06, respectively.

Petiole

A transverse section of the petiole is a symmetrical, concave ovate shape, and its structure resembles that of the leaf (Figure 4). The structure of the petiole is included the upper epidermis, the lower epidermis (Figure 4.a), hypodermis (Figure 4.b), parenchymatous (Figure 4.c), air cavity (15–17 cavities) (Figure 4.d), and vascular bundles (35–40 bundles (–45)) (Figures 4.e-f). However, the number of parenchymal cells and vascular bundles of the petiole is more than that of the leaf.

Similar to other genera of Zingiberaceae, trichomes on the leaf surfaces of *C. sahuynhensis* were unicellular. Liang et al. (2020) and Zhao et al. (2022) showed that stout trichomes were only found in the genus *Curcuma*, *Alpinia*, *Amomum*, and *Elettaria*, while the genus *Kaempferia*, *Hedychium*, and *Zingiber* have both delicate and stout trichomes types. Additionally, delicate trichomes were only found on the epidermis of the genus *Boesenbergia*, *Globba* (Liang et al. 2020; Zhao et al. 2022).

Under the lower epidermis of *C. sahuynhensis* leaf midrib are small vascular bundles (8–10 bundles); however, these vascular bundles were not observed in *C. albiflora* (Wijayasiriwardene et al. 2016), *C. comosa* (Yee et al. 2019). Small vascular bundles in leaf midrib are also found in *C. sahuynhensis* petiole (13–15 bundles). This is a feature that distinguishes *C. sahuynhensis* from other *Curcuma* species.

Calcium oxalate crystals of various shapes and sizes in some plant tissues have been reported to be common ingredients in the plant kingdom and occur in more than 215 plant families (McNair 1932; Franceschi and Horner

1980). In reality, the function of these crystals is not completely clear but has been related to several proven functions, such as calcium regulation and other minerals, as well as plants protection against herbivores, pathogens, and heavy metals neutralization (Franceschi and Horner 1980; Volk et al. 2002; Korth et al. 2006). In the current study, prismatic calcium oxalate crystals which present in leaves of *C. sahuynhensis* as in *C. albiflora* (Wijayasiriwardene et al. 2016) were not found in *C. sahuynhensis* rhizome, which was a component commonly found in the rhizomes of *Curcuma* species (e.g., C. zedoaria) (Amel 2015). In studies of many researchers showed that many crystals were commonly found in the epidermal cells on both leaf surfaces in some Zingiberaceae genera studied (such as *Globba*, *Alpinia*, *Amomum*, *Elettaria*, and *Zingiber*) (Tomlinson 1956; Kajornjit et al. 2018; Salasiah and Meekiong 2018; Zhao et al. 2022). To our knowledge, crystals only occur in the epidermis of *C. sahuynhensis*, but in both epidermal layers of other *Curcuma* species, showing that crystals are absent. This crystal is present in *C. sahuynhensis* leaves and is regarded as a typical micromorphology character. Further investigation is needed to determine whether this crystal is still present in the leaves of other *Curcuma* species. Thus, crystals in the leaf epidermis can also have taxonomic significance for *C. sahuynhensis*.

The common presence of silica in the roots and leaves of the family Zingiberaceae was reported by Tomlinson (1956), Uma et al. (2014), and Liu et al. (2020). However, the current study reported the absence of silica in the roots and leaves of *C. sahuynhensis*. This characteristic difference is regarded as a potentially informative taxonomic character and might be used to distinguish this species. Especially, starch granules found in the leaf are another distinctive character from the other *Curcuma* species.

Zahara (2020) reported that stomatal characterization is significant in plant identification and taxonomy (Zahara 2020). In earlier studies, paracytic typed stomata were discovered in many species of the Zingiberaceae family, such as *Zingiber officinale*, *Curcuma domestica*, *Alpinia galangal*, *Etlingeraelatior* (Rudall et al. 2017; Zahara 2020) and *Distichochlamys citrea* (Van Chen et al. 2022a), and was also present in *C. sahuynhensis*. However, tetracytic typed stomata are present in *C. comosa* (Yee et al. 2019).

In pharmacognosy, the number of stomata and the stomatal index are usually relatively constant quantities for a species. In many cases, the number of stomata has been shown to be significant for taxonomic delimitation in the family (Ministry of Health 2017). The density of stomata in leaves is related to the transpiration, gas exchange, and photosynthesis rate of plant leaves. Previous studies have shown that the higher the stomatal density is, the more stomata can be opened to absorb CO_2. Simultaneously, leaf stomatal density was positively correlated with stomatal conductance, photosynthetic rate, and gas exchange rate. Similarly, the number of stomata is inversely related to the rate of transpiration Shiva et al. 2017; Zahara 2020). This proved that the number of stomata of the upper epidermis is less than that of the lower epidermis of *C. sahuynhensis* leaves.

Root and rhizome

Root

The transverse section of a root is approximately round and it is divided into 2 distinct areas, namely the cortex and the pith. The cortex zone located beneath the root hair-bearing exodermis (Figures 5.a and 5b) occupies roundly 2/3 of the section. The suberized hypodermal cells consist of 5–6 layers of polygonal cells arranged tightly together and their walls are suberized (Figure 5.c). The parenchymatous cells of the outer cortex are arranged randomly, forming several layers of polygon-shaped cells with cellulose-impregnated and curved walls (Figure 5.d). The parenchymatous tissue of the inner cortex comprises 4–5 layers of rectangular cells positioned into concentric rings and radial lines, and their walls are impregnated with cellulose and intercellular spaces (Figure 5.e). The U-shaped thickening endodermis has polygonal cells arranged side-by-side on one layer (Figure 5.f). The pith dominates 1/3 of the root section radius. The pericycle is a polygonal cell layer with cellulose-impregnated walls, and each cell of the pericycle is placed side-by-side with an endodermis cell (Figure 5.g). Every protoxylem bundle consists of 3–4 vessels resembling polygonal cylinders, which are lignified and positioned centripetally, each protoxylem bundle interlaces with one primary phloem bundle, making up to 15–16 pairs laying on a circle (Figure 5.i). The primary phloem has polyhedral cells, which are cellulose-impregnated and arranged into clusters (Figure 5.h). Metaxylem consists of 12–13 lignified vessels (Figure 5.i) and their magnitudes are 4–5 times larger than protoxylem (Figure 5.j). Besides, the parenchymatous pith is included two regions (the outer domain and the inner domain). The outer domain consists of many polygonal cell layers with lignified walls and the cells are positioned randomly resembling sclerenchymatous tissue (Figure 5.k). This part tends to invade the stele region. The inner domain comprises a few layers of circular cells, intercellular spaces, with cellulose-impregnated walls and arranged resembling angular parenchymatous tissue (Figure 5.l). Oil cells are scattered throughout the cortex region (Figure 5.m).

In general, the layered cells of the exodermis, the suberized hypodermal cells, and the outer cortex region are similar to *C. zeodaria*, *C. aromatica*, *C. longa*, and *C. amada*. However, cells of the inner cortex of *C. sahuynhensis* are characterized by linear intercellular spaces (similar to *C. zeodaria*, *C. aromatica*, and *C. longa*) but different from *C. amada* (triangular intercellular spaces). The endodermis with 'U'-shaped thickened walls of *C. sahuynhensis* are similar to *C. longa*, *C. zeodaria*, and *C. amada* but except for thin-walled in *C. aromatica*. Similar to *C. longa*, *C. zeodaria*, and *C. aromatica*, the pith region of *C. sahuynhensis* are polygonal parenchymatous cells and lacks intercellular spaces but the feature is absent from *C. amada* (Uma and Muthukumar 2014).

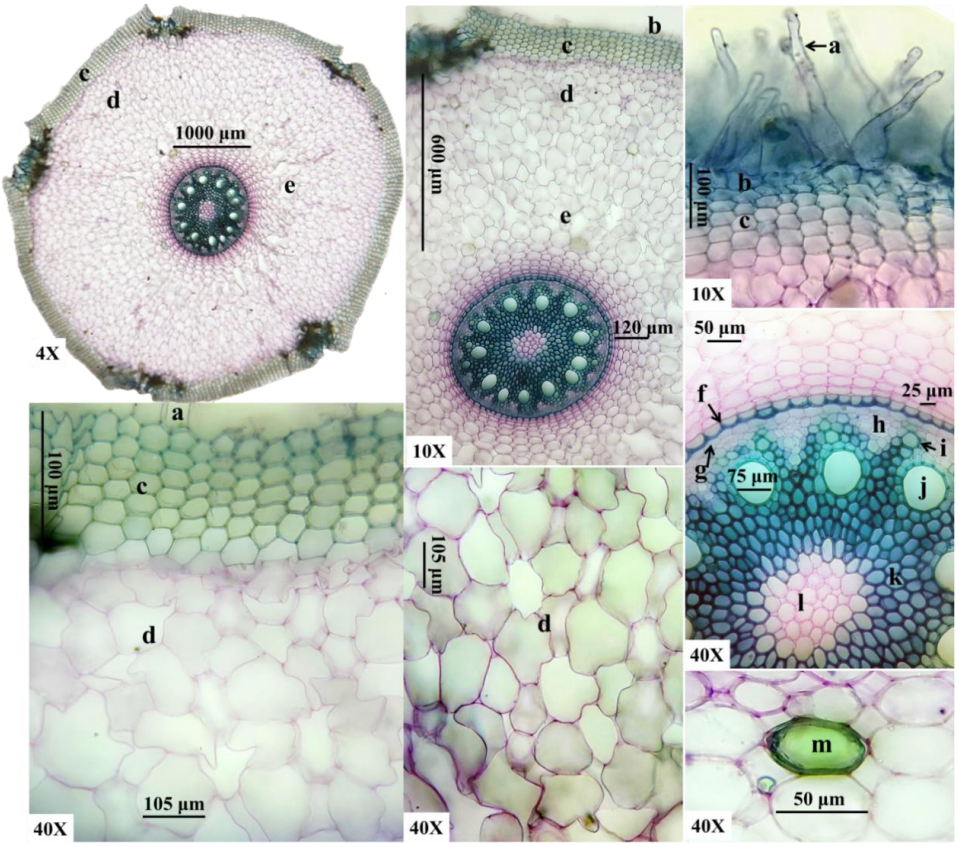

Figure 5. The features of the cross-sectioned root of *C. sahuynhensis* (with magnifications 4X, 10X, 40X): (a) root hair; (b) exodermis; (c) suberized hypodermal cells; (d) parenchymatous cells of the outer cortex; (e) parenchymatous cells of the inner cortex with intercellular spaces; (f) "U"-shape thickening in endodermis; (g) pericycle; (h) phloem; (i) protoxylem; (j) metaxylem; (k) sclerenchymatous conjunctive tissues; (l) parenchymatous pith; (m) oil cell

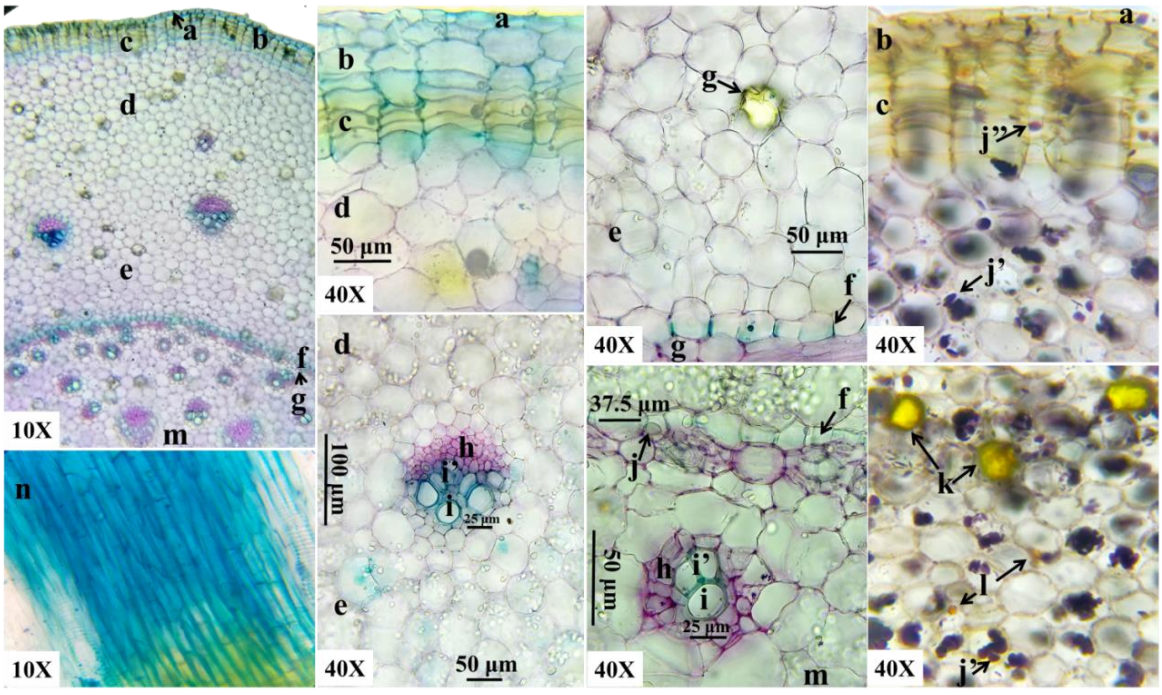

Figure 6. The features of cross-sectioned rhizome of *C. sahuynhensis* (with magnifications 4X, 10X, 40X): (a) epidermis; (b) exodermis; (c) multilayered hypodermis; (d) parenchymatous cells of outer cortex; (e) parenchymatous cells of inner cortex; (f) casparian strips in endodermis; (g) pericycle; (h) phloem; (i) metaxylem and (i') protoxylem; (j) starch granules; (j', j'') starch granules (after reacting with iodine-potassium iodide reagent); (k) oil cell; (l) oleoresin mass; (m) parenchymatous pith with triangular intercellar spaces; (n) lateral rhizome formation.

Rhizome

Anatomically, the transverse section of the rhizome is almost circular in outline (Figure 6). The outer layer is a slightly cutinized epidermis, which is built by a single rectangular or polyhedral cell layer (Figure 6.a). The exodermis (suberized tissue) is located under the epidermis, consisting of 2–3 layers of polygonal cells, scramble (Figure 6.b). The next layer is a multilayered hypodermis consisting of 3–4 rectangular or polygonal layers (Figure 6.c). The parenchymatous tissue of the cortex consists of nearly-circular cellulose-impregnated cells and is randomly placed. The triangular or polygonal intracellular spaces between those cells are tiny (Figures 6.d and 6e). Vascular bundles vary in magnitude and scatter within the cortex and stele regions. Casparian strips in the endodermis consist of one polyhedral cell layer (Figure 6.f). Between the endodermis and the vascular bundles (from the stele region) is a polygonal pericycle with discontinuous cells (Figure 6.g). Vascular bundles are placed onto several orbits, whose magnitudes rise from the endodermis. Each bundle has primary phloem locating upper (Figure 6.h) and primary xylem placing lower position (Figure 6.i). The primary phloem has polyhedral cells with curved cellulose walls and is arranged into clusters (Figure 6.h). Metaxylem consists of 1–2(–4) lignified polygonal vessels and their magnitudes are 1–2 times larger than protoxylem. Meanwhile, the protoxylem consists of 2–3(–5) lignified polygonal vessels (Figure 6.i). The parenchymatous pith is characterized by many polygonal cell layers with triangular intercellular spaces, which are randomly placed (Figure 6.m). Starch granules (Figures 6.j–j''), oil cells (Figure 6.k), and oleoresin masses (Figure 6.l) are scattered or densely clustered in the parenchymatous cortex and parenchymatous pith. Lateral formation arises from the pericycle of the rhizome stele region, consisting of many bundles of fibers (dividing near the xylem elements of the rhizome), dark blue (Figure 6.n). This pericycle creates lateral root development and enables secondary root growth.

It is similar to other *Curcuma* species (e.g., *C. aeruginosa, C. albiflora, C. amada, C. aromatica, C. aurantiaca, C. caesia, C. haritha, C. montana, C. zedoaria, C. longa, C. zanthorrhiza*, and *C. Comosa*), the cortex and the stele regions contain many starch granules, and oil cells (Amel 2015; Wijayasiriwardene et al. 2016; Sajwan et al. 2020; Anu et al. 2020). Parenchyma cells of *C. sahuynhensis* contained oleoresin masses which is similar to *C. albiflora* (Wijayasiriwardene et al. 2016).

In this study, the roots and rhizomes of micromorphology and anatomy of *C. sahuynhensis* have been described for the first time. There are anatomical similarities between the roots and rhizomes, particularly in terms of typical characteristics of the micromorphological (from the outside to the inside: the epidermis, the cortex region, and the stele region). However, the cortical regions of the *C. sahuynhensis* roots are wider than that of the rhizomes, and the width of their stele regions is opposite.

It can be insisted that further micromorphological studies in group can understand the ecophysiology changes as well as the structure of *Curcuma* species.

Powder

Root and rhizome powder

The powder is dark-brown, light spicy taste, and aroma specific odor (Figure 7.a). On microscopic observation, there is the presence of numerous fragments of lignified, lemon-yellow epidermis cells with polygonal, irregular in shape (Figure 7.b) and elongated-polygonal fragments of epidermises (Figures 7.c and 7d). Spiral vessels (Figure 7.e) and scalariform vessels (Figure 7.f) are fragments of xylem vessels thickening. In addition, red-brown oleoresin masses are also found (Figure 7.g). Numerous starch granules are ovoid or oval in shape (15–20 µm in diameter) (Figure 7.h). The generally branched hilum of starch granule punctate or short streaked (hilum located at the small end of the seed is ovoid), formed well-defined concentric lines or absent (Figure 7.h). They are commonly present in the observation field.

Leaf powder

The dried powder leaf is dark-green, and has an aroma odor, a light spicy taste (Figure 8.a). Microscopically, the leaf powder is characterized by the presence of the polygonal fragments of epidermises, and contains the paracytic stomata (Figure 8.b). The numerous fragments of green parenchyma with thin walls (Figure 8.c), fragments of parenchyma contain clusters of green chloroplasts and red-brown oleoresin (Figure 8.d), parenchymatous cells with oil cells and calcium oxalate crystals (rhomboid-shaped) (Figure 8.e), parenchymatous cells with thick red-brown fiber (Figure 8.f), fragments of thick scalariform xylem vessel (Figures 8.g and 8h), fragments of thick spiral xylem vessel (Figure 8.i), the thick-walled bundle of fibers with nearly rounded tips (Figure 8.k), and starch granules (11-12.5 µm in diameter) (Figure 8.l) are also present in leaf powder. Overall, there were similarities in composition found in fresh leaves and leaf powder. However, it is difficult to locate components such as upper epidermis, lower epidermis, and hypodermic cells in the leaf powder.

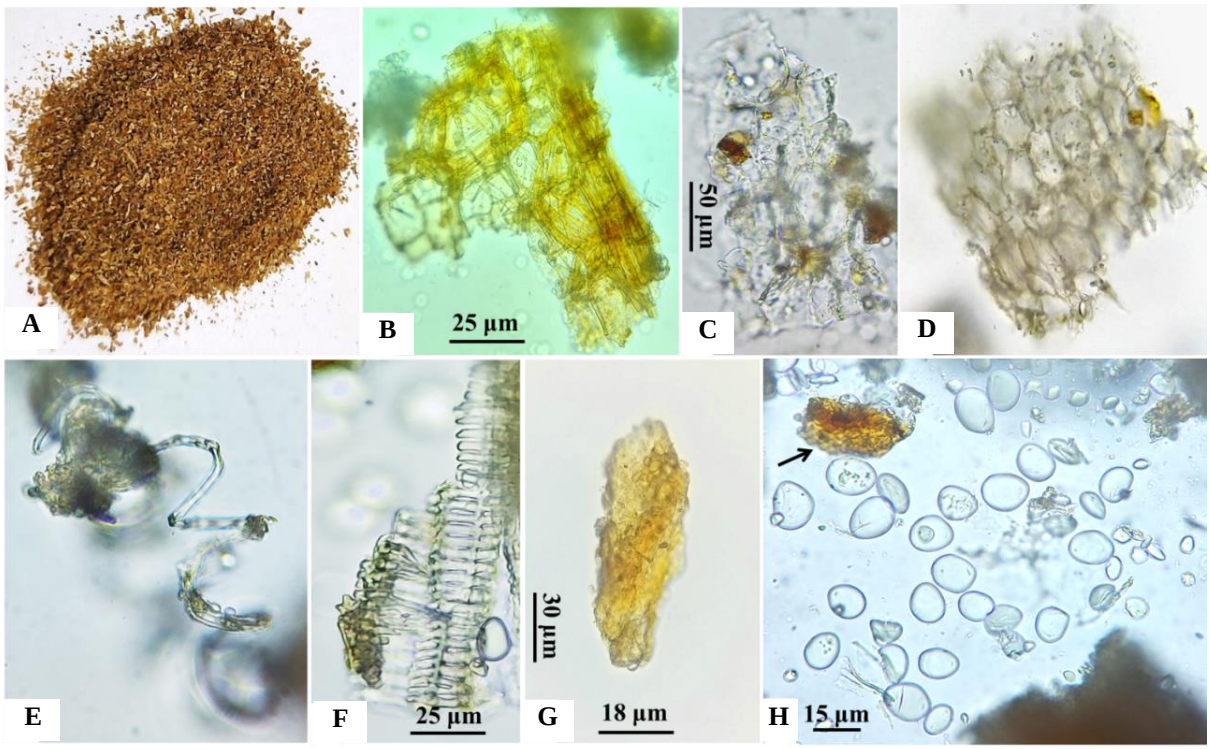

Figure 7. The features of *C. sahuynhensis* root and rhizome powders: (A) root and rhizome powders; (B) epidermal cells; (C, D) parenchymatous cells; (E) spiral xylem vessel; (F) scalariform xylem vessel; (G) red-brown oleoresin masses (arrow); (H) starch granules

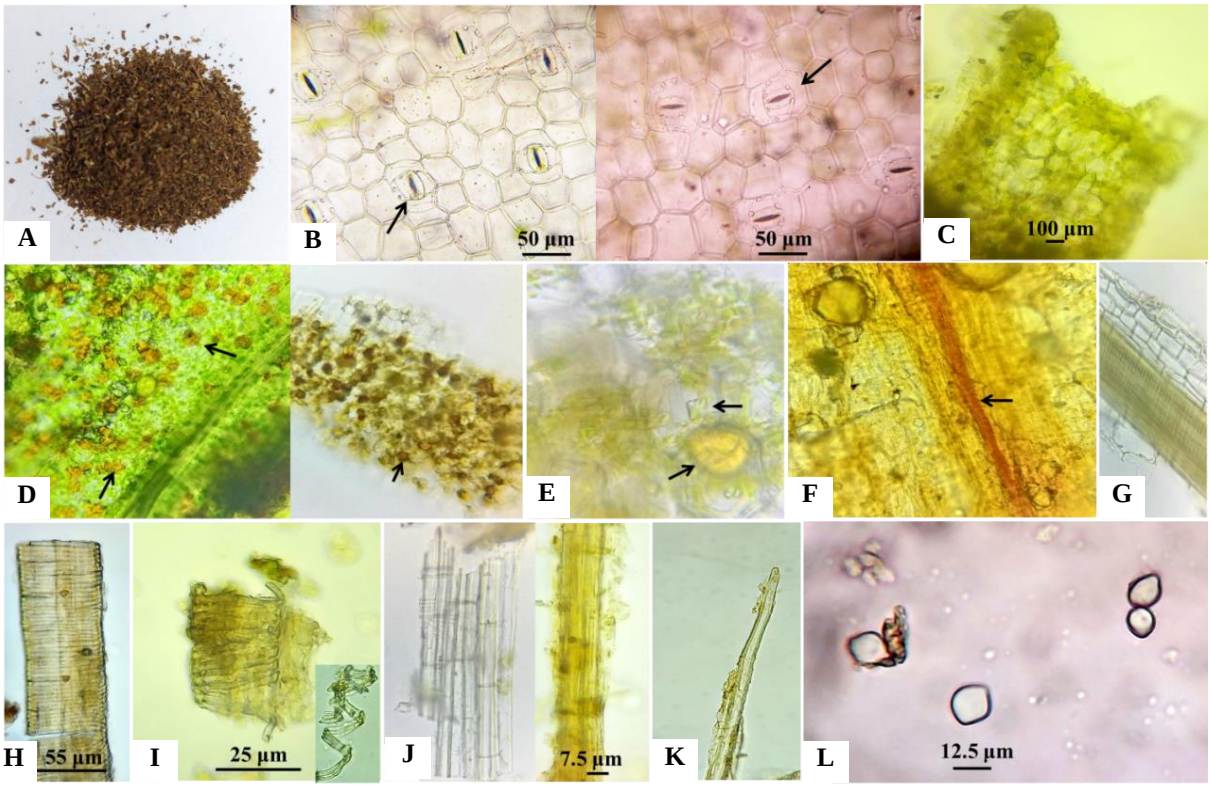

Figure 8. The features of dried leaf powder of *C. sahuynhensis* (with magnifications 40X): (A) leaf powder; (B) surface view epidermis with stomata (arrows); (C) fragment of epidermal cells; (D) parenchymatous cells with red-brown oleoresin (arrows); (E) parenchymatous cells with oil cell and calcium oxalate crystals (rhomboid-shaped) (arrows); (F) parenchymatous cells with thick red-brown fiber; (G, H) fragments of scalariform xylem vessel; (I) spiral xylem vessel; (J) bundle of fibers; (K) sclereid fiber; (L) starch granules

Microscopic inspection of powdered material is essential for the accurate identification of medicinal herbs (WHO 1998). In addition, microscopy has proven to be a simple and fast tool for testing the purity of raw material powders based on plant tissue characteristics (Jackson et al. 1990; Osman et al. 2019). By using microscopy, the main features of Vegetable Turmerics' powdered rhizomes are the characteristic of starch granules, epidermal cells, parenchymatous cells, xylem vessels, oleoresin masses, and oil cells. Nonetheless, calcium oxalate crystals are not found in the *C. sahuynhensis* rhizome, a result similar to that of other *Curcuma* species (Upton et al. 2016; Osman et al. 2019); therefore, the detection of these crystals in turmeric powders indicates adulteration with non *Curcuma* species. In particular, curcumin containing species such as *C. longa* (Khatoon et al. 2014), *C. zedoaria* (*C. malabarica*), and *C. zanthorrhiza* (Osman et al. 2019) were not found in *C. sahuynhensis*. Additionally, the red-brown oleoresin masses in the *C. sahuyhensis* rhizome are also present in *C. caesia* and *C. longa* (Paliwal et al. 2011; Danapur and Venugopal 2019).

Combined with previous research on the morphological rhizome and root tuber, our current study is added and modified. The key to taxonomic identification is presented like this (Uma et al. 2014; Wijayasiriwardene et al. 2016; Yee et al. 2019; Anu et al. 2020):

Phytochemical evaluation

Phytochemical screening

The phytochemical screening of the powder sample extracts of *C. sahuynhensis* was reported in Table 2. The result reveals the presence of several main phytochemical constituents including essential oil, reducing sugars, amino acids, coumarins, triterpenoids, and polyuronides in both. In addition, lipids, alkaloids, and flavonoids (absent in the inflorescence) were found in the rhizome, while tannins were only found in the inflorescence. However, it does not contain carotenoids, saponins, and cardiac glycosides under the analysis conditions.

Phytochemical screening is known as an analytical method to determine the secondary metabolites discovered in plants due to their special ability to react to certain reagents. The presence of chemical constituents in plants extracted with solvents of different polarities suggested that specific components were soluble in that particular solvent used for extraction to achieve high performance.

In general, the main compounds such as essential oil, carbohydrates, amino acids, triterpenoids, lipids, alkaloids, and flavonoids present in the rhizome of *C. sahuynhensis* are also found in other *Curcuma* species (e.g., *C. caesia*, *C. aeruginosa* (Jose et al. 2014; Mahato et al. 2018), *C. longa* (Oghenejobo et al. 2017; Maithilikarpagaselvi et al. 2020)).

In previous studies, these phytochemicals compounds have the potential to make valuable contributions to various medicinal as well as nutritional supplements from these herbs. Currently, only essential oils extracted from *C. sahuynhensis* have been reported to inhibit pathogenic bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans* (Sam et al. 2020). Furthermore, the highlight of this work is the first time that the phytochemical and experimental criteria of *C. sahuynhensis* have been reported in the literature. In extended studies, the majority of compounds (flavonoids, coumarins, tannins, alkaloids, and essential oils) were reported to display potent biological importance, as well as have positive effects on disease treatments such as antibacterial, antioxidant, anti-inflammatory, anti-allergic, anti-diabetic, anti-tumor, anti-cancer and other biological activities (Bouchelaghem et al. 2022; Xiao 2022; Gulcin et al. 2022; Van Chen et al. 2022b). It has been shown that extracts of parts of *C. sahuynhensis* showed promising biological effects.

Key-based on the morphological rhizome, root tuber for the taxonomic identification of some *Curcuma* species

1a. Rhizome branched, epidermis uniseriate, crystal cells absent 2
2a. Single epidermis with layer cutinized, crystal cells and curcumin absent ……. *C. sahuynhensis*
2b. Single epidermis, crystal cells absent, curcumin cells present 3
3a. Trichome absent, cut surface of dark blue or blackish rhizome …….. *C. caesia*
3b. Trichome unicellular …… 4
4a. Cambium layers present (3 layered), 8-9 layered peridermic, endodermis layer discontinuous ..*C. montana*
4b. Cambium layers absent, peridermic layers present, endodermis layer continuous …….. 5
5a. 6-8 layered peridermic, primary vascular bundle < 50 bundles, bundle sheath present ……. *C. zedoaria*
5b. Primary vascular bundle > 50 bundles ……. 6
6a. Orange-yellow to reddish yellow rhizome, stele region < 0.5 cm, mild camphoraceous aroma …..…… ……. ……. *C. longa*
6b. Rhizome not orange-yellow to reddish yellow, stele region > 0.5 cm ……..…….. 7
7a. Rhizome not bitter, raw mango aroma, lack any intercellular spaces in stele region ….. *C. amada*
7b. Rhizome bitter, camphoraceous aroma …… 8
8a. Bundle sheath present, cut surface of cream-colored rhizome, starch granules ellipsoid and phaseoliform in shape (33.07 x 14.51 µm) …… *C. aromatica*
8b. Bundle sheath absent 9
9a. Light-yellow root tubers, light-yellow rhizome, taste warm bitter, larger oil cells (173 ± 11.69 µm) …..….. *C. zanthorrhiza*
9b. Whitish root tubers …….. 10
10a. Bluish-green, inwardly pale pearly rhizome, strong camphoraceous aroma, medium oil cells (106 ± 16.70 µm), curcumin cells (202 ± 17.38 µm) …… ……..……. *C. aeruginosa*
10b. Creamy rhizome, mild camphoraceous aroma, smaller oil cells (77 ± 7.97 µm), curcumin cells (142 ± 12.25 µm) …… *C. haritha*

1b. Rhizome branched, epidermis uniseriate, crystal cells present ……..…. ……. 11
11a. White rhizome and no tubers, prismatic crystals present in parenchyma cells but absent cortical region ……. ……. *C. albiflora*
11b. Pale yellow or orange-yellow rhizome, with unicellular hair and calcium oxalate crystals …… *C. comosa*

1c. Rhizome not branched, epidermis multiseriate, crystal cells present, starch granules circular or ovoid in shape (17.78 x 11.73 µm) …… *C. aurantiaca*

Table 2. Phytochemical screening of *C. sahuynhensis* rhizomes and inflorescences

Chemical constituents	The name of the test	The rhizome extract of *C. sahuynhensis*			The inflorescence extract of *C. sahuynhensis*		
		Ether extract	Ethanol extract	Water extract	Ether extract	Ethanol extract	Water extract
Fats	Stain test	+	-	-	-	-	-
Carbohydrates	Molisch's test, Fehling's test	+	+	+	+	+	+
Essential oil	Scent test	+	+	-	+	+	-
Carotenoids	H_2SO_4 test	-	-	-	-	-	-
Alkaloids	Wagner's test, Dragendoff's test	+	+	-	-	-	-
Amino acids	Na_2CO_3 test	-	+	+	-	+	-
Cardiac glycosides	Raymond's test, Xanthydrol test	-	-	-	-	-	-
Coumarins	Lactone ring test	+	+	-	+	+	-
Flavonoids	Shinoda test	+	+	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-
Tannins	Gelatin test, $FeCl_3$ test	-	-	-	-	-	+
Triterpenoid	Salkowski test	+	+	-	+	+	-
Polyuronides	Ethanol 90% test	-	-	+	-	-	+

Note: "+" indicates the presence and "-" indicates the absence

Physico-chemical parameters

The average of three independent measurements of the rhizome powders' moisture content, total ash value, and acid-insoluble ash value was 11.79 ± 0.20%, 7.32 ± 0.60%, and 0.24 ± 0.08%, respectively. The excess moisture content of the raw drug combined with the relatively high temperature of the environment is responsible for its decomposition due to chemical changes as well as microbial attacks. The total ash values established in this work may be useful in the determination of the purity and quality of *C. sahuyhensis*. The total ash for the rhizome is relatively high (7.32 ± 0.60%), indicating a high amount of inorganic salts. Besides, the acid-insoluble ash value is very low (0.24 ± 0.08%). The acid-insoluble ash value shows a very small amount of the acid-insoluble inorganic component. All results of pharmacognostical are diagnostically important. In short, with established pharmacognostic parameters in this work, the sample achieved purity in terms of moisture content, total ash, and acid-insoluble ash values, as well as was within the allowable limits of the Vietnamese Pharmacopoeia V for medicinal materials uses.

In conclusion, the current study provided information for the first time on the micromorphological, pharmacognostical, and phytochemical characteristics of *C. sahuynhensis* in Quang Ngai Province, Vietnam. The comparative microscopic observations of the *C. sahuynhensis*'s cross-sectioned roots, rhizomes, leaf, and crude powder with other *Curcuma* species (e.g., *C. longa*, *C. aroma*, *C. zedoaria*, *C. albiflora*, etc) revealed that many of these micromorphological characteristics are homologous. Accordingly, a generalized description to account for these similarities was drawn up: the root and rhizome structure consists of the epidermis, the cortex region (outer and inner cortex), and the stele region (endodermis, pericycle, and parenchymatous pith), which are relatively similar. The powder characteristics consist of fragments of the epidermis, fragments of parenchyma, red-brown oleoresin masses, oil cells, starch granules, calcium oxalate crystals, fragments of spiral xylem vessels, and fragments of scalariform xylem vessels, all of which are commonly present in the observation field. Its rhizome and inflorescence contain essential oil, amino acids, coumarins, triterpenoids, polyuronides, and reducing compounds. However, lipids, alkaloids, and flavonoids (present in the rhizome) were not found in the inflorescence of *C. sahuynhensis*, while tannins were only found in the inflorescence. These results are used as a monograph in the identification and standardization of medicinal material *C. sahuynhensis* as well as contribute the information to carrying on further studies on the chemical compositions and their biological activities.

ACKNOWLEDGEMENTS

The authors thank the partial facility support of the Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, Viet Nam for the identification of micromorphological features, pharmacognostical parameters, and phytochemical screening of *Curcuma sahuynhensis*. We also thank Prof. Nguyen Thi Ai Nhung, Đinh Thanh Cuong, and Mrs. Nguyen Thi Xuan Nong for their assistance in providing the research samples from Quang Ngai province, Viet Nam.

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