PLANT SCIENCE TODAY
Vol 12(3): 1-9
https://doi.org/10.14719/pst.7930

eISSN 2348-1900

RESEARCH ARTICLE

# Microscopic anatomy and phytochemical profiling of *Curcuma cotuana*

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Received: 26 February 2025; Accepted: 09 May 2025; Available online: Version 1.0: 20 August 2025; Version 2.0: 16 September 2025

**Cite this article:** Vo VT, Ngoc NT, Van HT, Le-thi TT, Luu HT, Nguyen-Phi N, Truong DH. Microscopic anatomy and phytochemical profiling of *Curcuma cotuana*. Plant Science Today. 2025; 12(3): 1-9.https://doi.org/10.14719/pst.7930

## Abstract

*Curcuma cotuana* is a rare species belonging to the Zingiberaceae family. In present study, the micromorphological characteristics of the leaf sheath, leaf, root, root tuber and petiole of *C. cotuana* are reported for the first time. Additionally, phytochemical analysis confirmed that the leaf and rhizome extracts of the species contained various bioactive compounds, including phenolics, tannins, alkaloids, flavonoids, coumarins, saponins, steroids and terpenoids. Furthermore, the total flavonoids (TFC), total polyphenol content (TPC) and total triterpene content (TTC) of the leaf and rhizome extracts were quantified. The leaf extracts exhibited higher concentrations (TPC: 39.273 mg GAE/g DW, TTC: 41.831 mg OAE/g DW, TFC: 2.072 mg QE/g DW) compared to the rhizome extracts (TPC: 17.108 mg GAE/g DW, TTC: 4.226 mg OAE/g DW, TFC: 4.232 mg QE/g DW).

**Keywords:** anatomical traits; *Curcuma cotuana*; medicinal properties; phytochemical screening; Vietnam

## Introduction

*Curcuma* L. is one of the largest genera within the Zingiberaceae family, with over 120 species widely found in subtropical and tropical areas, specifically in Vietnam, Thailand, Indochina, Malaysia, India and northern Australia (1, 2). Alongside other members of the Zingiberaceae family, many *Curcuma* species have been well-documented for their medicinal properties as well as their application in cosmetics, dyes, spices, perfumes and ornamental plants (3-8). The rhizome is the most frequently used part because of its active constituents, like volatile oil and non-volatile curcuminoids (9). In traditional medicine across several Asian countries, species of the genus *Curcuma* have been used to cure insect bites, infectious wounds or abscesses, diarrhoea, diarrhoea, bronchial complaints, pneumonia, leucorrhea and dysentery (9).

*Curcuma cotuana* Luu, Škorničk. & H.Đ.Trần is a rare plant and recently described species (10). So far, this species has been identified only in Axan Commune, Tây Giang District, Quảng Nam Province, Vietnam (10). Our prior works investigated the chemical constituents and antibacterial activity of the acetone extracts of *C. cotuana*. In total, 31 chemical components were detected in the acetone extract of *C. cotuana* with (E)-labda-8 (17),12-diene-15,16-dial (14.58 %), n-hexadecanoic acid (10.96 %), 3,7,11,15-tetramethylhexadec-2-en-1-yl acetate (8.13 %) and γ-sitosterol (7.97 %) being the major compounds (11). Additionally, the extract demonstrated antibacterial activity against *Bacillus cereus* (11).

To further elucidate the biological characteristics and chemical compositions of this species, we present for the first time the phytochemical screening and micro-morphological characteristics of *C. cotuana*.

## Materials and methods

### Plants

The samples of *C. cotuana* were collected from the Botanical Garden of the Institute of Advanced Technology, Viet Nam Academy of Science and Technology (Fig. 1).

### Micro-morphological traits

The anatomical characteristics of the leaf sheath, leaf, root, root tuber and petiole of *C. cotuana* were examined following the experimental procedures described in a previous study (12).

### Sample preparation

The freshly harvested rhizome and leaves of *C. cotuana* were subjected to a drying process at a controlled temperature of

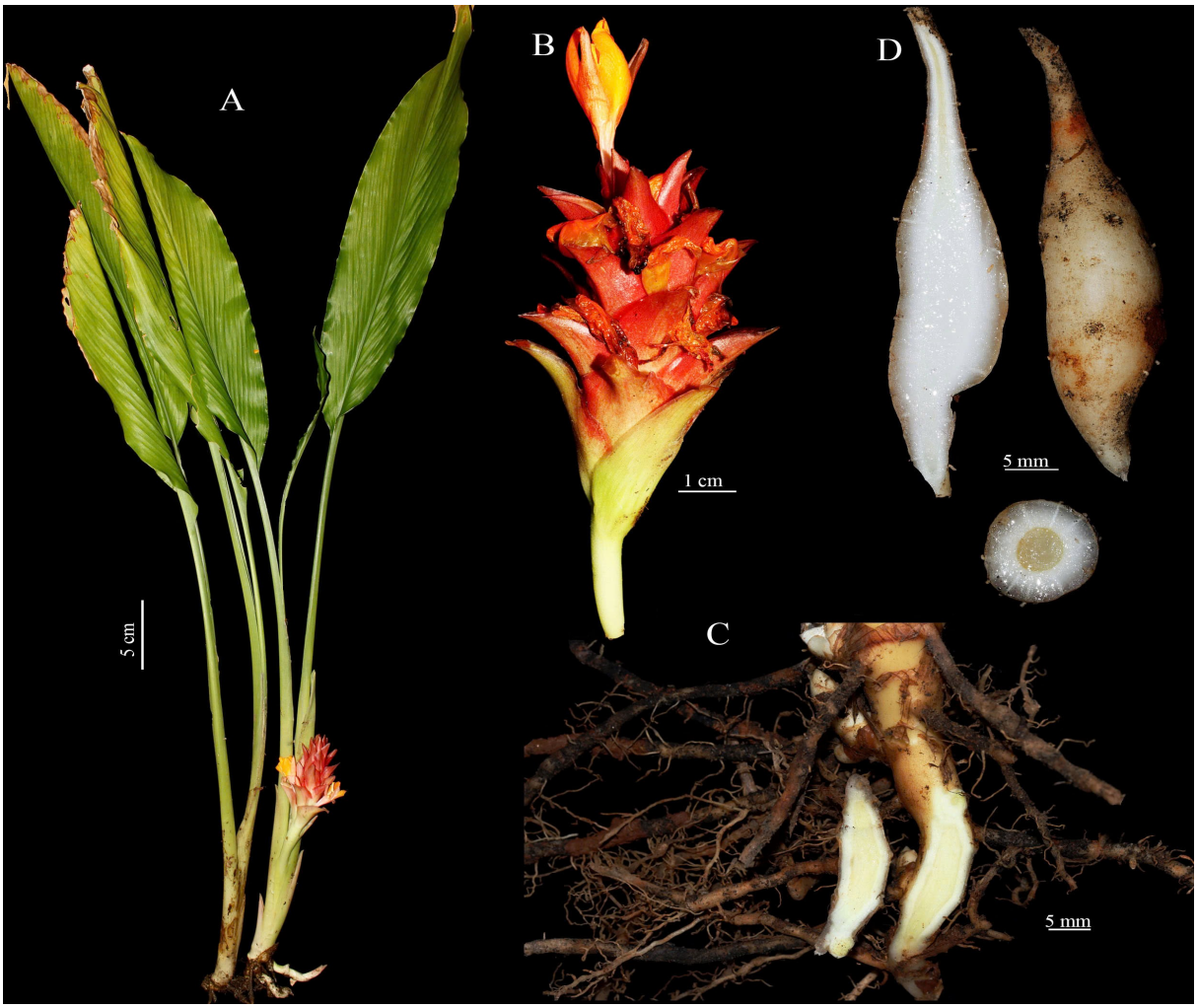


**Fig. 1.** *Curcuma cotuana*. A) Whole plant; B) Flower; C) Root tuber; D) Rhizome and root.

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50 ℃. Subsequently, the dried leaf material was mechanically ground and passed through a sieve with a pore size of 0.45 mm. Following this, a precise mass of 1 g of the prepared samples was then extracted using ethanol at a solvent-to-sample ratio of 1:30 for duration of 8 hr. The resulting supernatant was separated through filtration. This extraction process was repeated twice more under the same conditions (1:30 ratio for 8 hr each) yielding extracts labelled as solutions 2 and 3. Finally, all three solutions were amalgamated to produce the composite sample extract.

## Qualitative phytochemistry of the studied extract

The presence of various biologically active compounds in *C. cotuana* was qualitatively assessed using a series of established biochemical methods. Phenolic and tannin compounds were detected by combining 2 mL of the plant extract with 2 mL of distilled water and 2 - 3 drops of a 5 % $FeCl_3$ solution; a positive result was indicated by the formation of a blue-black or brown-green precipitate (3). For the identification of alkaloids, 2 mL of the extract was treated with 3 - 4 drops of Wagner reagent, with the appearance of a red-brown precipitate confirming their presence (13). Flavonoids were qualitatively determined by mixing 2 mL of the extract with 2 mL of 10 % $Pb(COOH)_2$, which yielded a characteristic yellow precipitate (14). The presence of saponins was indicated by the formation of persistent foaming after boiling 2 mL of the extract in 10 mL of distilled water for 2 min (15). Terpenoids and steroids were detected through a colorimetric reaction; 5 mL of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated $H_2SO_4$, resulting in a reddish-brown coloration (16). Finally, the presence of coumarins was confirmed by the appearance of a yellow colour when 2 mL of the extract was combined with 3 mL of a 10 % NaOH solution (17).

## Quantitative phytochemistry of the studied extract

### Total polyphenol content (TPC)

Add 0.1 mL of the extract and 1.8 mL of the Folin-Ciocalteu reagent to the designated test tube and allow the mixture to rest for 5 min after thorough agitation. Subsequently, 1.2 mL of a 15 % sodium carbonate ($Na_2CO_3$) solution to create an alkaline environment and add distilled water to adjust the total volume to 10 mL. Secure the container, agitate the contents and incubate in a dark environment for a period of 90 min. After incubation, photometric measurements should be taken at a wavelength of $\lambda$ = 734 nm (18). The TPC was identified using Eqn. 1.

$$TPC = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100 - W)} \times K \qquad \text{(Eqn. 1)}$$

Where, TPC: Total polyphenol content (mg GAE/g DW), $C_x$: the total polyphenol concentration in the extract calculated

from the standard curve (ppm), V: sample volume (mL), a: initial sample mass (g), w: moisture content (%), K: dilution factor, $10^3$: conversion factor.

**Total triterpene content (TTC)**

1 mL of the extract was combined with 0.2 mL of 5 % acetic acid and 1.2 mL of $HClO_4$ in a test tube. The mixture was thorough mixed and incubated at 70 °C for 15 min, followed by a cooling period of 2 min. Thereafter, the resultant mixture was adjusted to 5 mL using ethyl acetate. Photometric analysis was conducted at a wavelength of 550 nm (19). TTC was identified using Eqn. 2.

$$TTC = C_x \times \frac{V}{10^3} \frac{100}{a \times (100-w)} \times K \qquad \text{(Eqn. 2)}$$

Where, TTC: Total triterpene content (mg OAE/g DW), $C_x$: the total triterpenoid concentration in the extract calculated from the standard curve (ppm), V: sample volume (mL), a: initial sample mass (g) , w: moisture content (%), K: dilution factor, $10^3$: conversion factor.

**Total flavonoid content (TFC)**

Add 1 mL of the extract and 0.3 mL of 5 % $NaNO_2$ solution to a test tube. Shake the mixture and allow it to stand for 5 min. Then, add 0.3 mL of 10 % $AlCl_3$ solution. Mix well and allow to rest for 5 min. Then, introduce 2 mL of 1 M NaOH, shake thoroughly and add distilled water to make 10 mL. Photometric measurement at wavelength $\lambda = 510$ nm. For the control sample, replace the sample solution with distilled water. Total flavonoid content was expressed as grams of quercetin equivalent QE in Eqn. 3 (20).

$$TFC = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100-w)} \times K \qquad \text{(Eqn. 3)}$$

Where, TFC: Total flavonoid content (mg QE/g DW), $C_x$: the total flavonoid concentration in the extract calculated from the standard curve (ppm), V: sample volume (mL), a: initial sample mass (g), w: moisture content (%), K: dilution factor, $10^3$: conversion factor.

## Results

### Anatomical traits of *C. cotuana*

**Root**

The root cross-section exhibits an approximately circular shape and is composed of two distinct regions: the cortex, which occupies two-thirds of the radial extent and the stele, which accounts for the remaining one-third (Fig. 2).

**Cortex region:** The piliferous layer comprises a single layer of polygonal cells that vary in size and possess cellulose walls, which are occasionally torn on the outer surface. Root hairs are scattered throughout this layer. The exodermis consists of 3-5 layers of cork-impregnated cells with undulating walls, forming various shapes and arranged in a radial pattern. The cortical parenchyma is composed of 10-12 outer layers of polygonal cells arranged irregularly, forming small polygonal shapes or intercellular spaces. This is followed by 6-8 layers of flat, oval-shaped cells that gradually decrease in size and are organized in radial rows and concentric rings. Secretory cells are scattered throughout the cortical parenchyma. The endodermis is characterized by U-shaped wall thickenings.

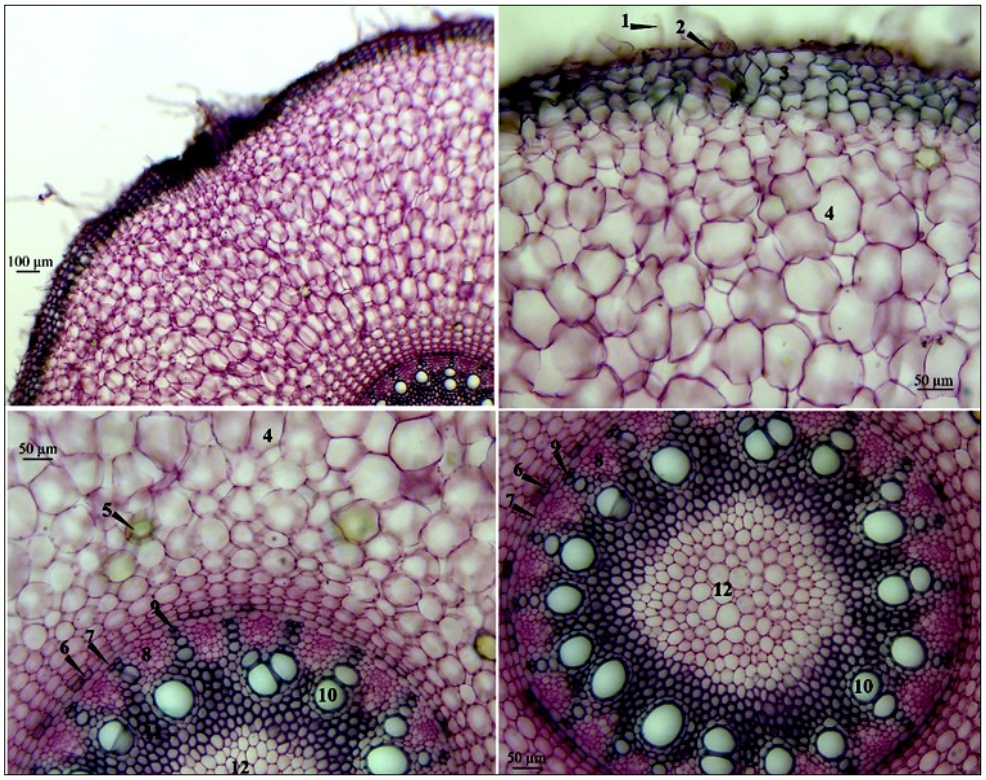



**Fig. 2.** Microsurgery of root. 1) Root hairs; 2) Piliferous layer; 3) Exodermis; 4) Cortical parenchyma; 5) Secretory cell; 6) Endodermis with U-shaped; 7) Pericycle; 8) Phloem; 9) Protoxylem; 10) Metaxylem; 11) Pith parenchyma with lignified cell walls; 12) Pith parenchyma with cellulose cell walls.

**Stele:** The pericycle comprises a single layer of regularly arranged polygonal cells with cellulose walls, positioned alternately with the endodermis. The vascular system includes 18-22 primary phloem bundles alternating with 18-22 protoxylem bundles, forming a ring and separated by medullary rays. The phloem bundles are clustered and composed of irregular polygonal cells. Each protoxylem bundle consists of 2-4 polygonal vessels that exhibit centripetal differentiation. Additionally, 16-22 metaxylem vessels are typically located individually or in pairs beneath the protoxylem and phloem. The medullary rays are formed by 1-2 rows of narrow, polygonal parenchyma cells with cellulose walls, aligned transversely. The medullary parenchyma consists of irregular polygonal cells; the 8-10 outer layers have thick, lignified walls and are tightly packed, while the 6-8 inner layers possess cellulose walls and are more loosely arranged with intercellular spaces.

**Rhizome**

The cross-section of the rhizome exhibits a circular shape (Fig. 3).

**Cortex region:** The cork layer includes 6-10 layers of rectangular, flattened cells with cork-impregnated walls, arranged radially. The cortical parenchyma is usually solid or has small intercellular spaces. Scattered small vascular bundles are located in the parenchyma. The endodermis is characterized by the presence of Casparian strips.

**Stele:** The pericycle consists of 1-2 layers of polygonal cells with cellulose walls. Vascular bundles are irregularly distributed throughout the parenchyma. In the rhizome, these vascular bundles are either not surrounded or only slightly surrounded by sclerenchyma tissue. The parenchyma also contains numerous secretory cells.

**Root tuber**

The cross-section is circular (Fig. 4).

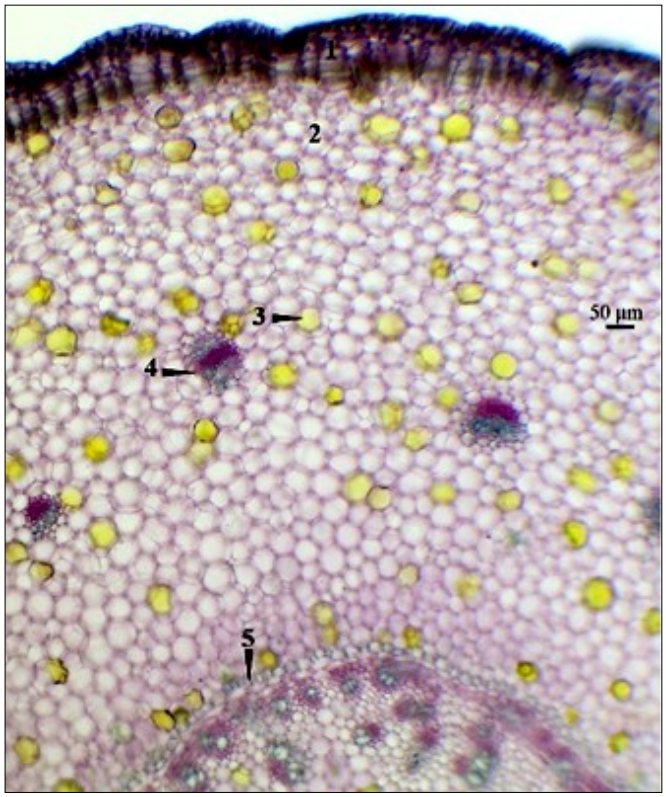
**Cortex Region:** The cork consists of 2-5 layers of rectangular, flattened cells with cork-impregnated walls. The cortical parenchyma is typically compact, exhibiting either solid tissue or small intercellular spaces. The endodermis is characterized by the presence of a casparian strip.

**Stele:** The pericycle comprises 1-2 layers of cells with cellulose walls, interspersed with endodermal cells. Xylem and phloem bundles are arranged alternately in a ring beneath the pericycle. Each xylem bundle contains 2-5 centripetally differentiated vessels. Both the medullary and cortical parenchyma includes specialized secretory cells.

**Leaves**

The midrib is concave on the upper surface and convex on the lower surface (Fig. 5). Both the upper and lower epidermis consists of a single layer of fairly regular polygonal cells. The vascular bundle, with xylem positioned above and phloem below is arranged in rows just above the lower epidermis. Each xylem bundle contains 3-7 small protoxylem vessels and 1-3 large metaxylem vessels. The phloem is composed of polygonal cells of irregular size, arranged irregularly. Sclerenchyma is present at both ends of the vascular bundle, comprising 3-8 cell layers on the phloem side and 2-4 cell layers on the xylem side. The chlorenchyma surrounding the vascular bundles contains numerous chloroplasts and at the center of the bundles, the parenchyma is often degraded, forming air cavities. Additionally, 5-6 small vascular bundles are located within the parenchyma beneath the upper epidermis. Secretory cells may occasionally be found in the parenchyma region.

**Lamina:** The upper and lower epidermis each consists of a single layer of polygonal cells with cellulose walls. The upper epidermal cells are larger than those of the lower epidermis, which contains numerous stomata. Beneath the epidermis on both leaf surfaces are 1-2 layers of large hypodermal cells with



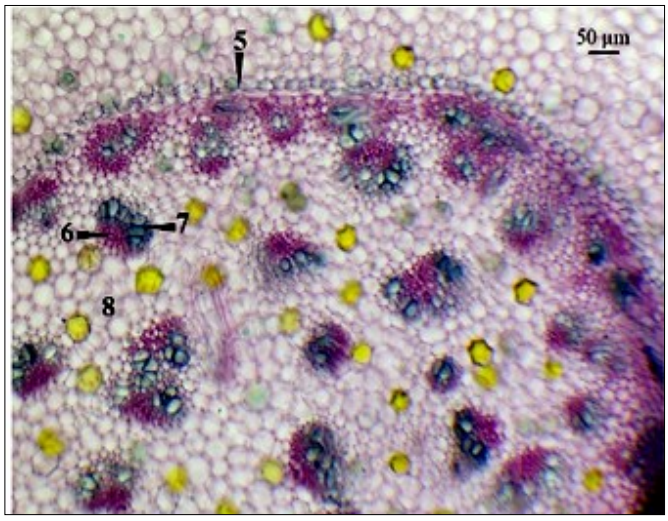




**Fig. 3.** Microsurgery of rhizome. 1) Phellem; 2) Cortical parenchyma; 3) Secretory cell; 4) Vascular bundle; 5) Endodermis with casparian strip; 6) Phloem; 7) Xylem; 8) Medullary parenchyma.

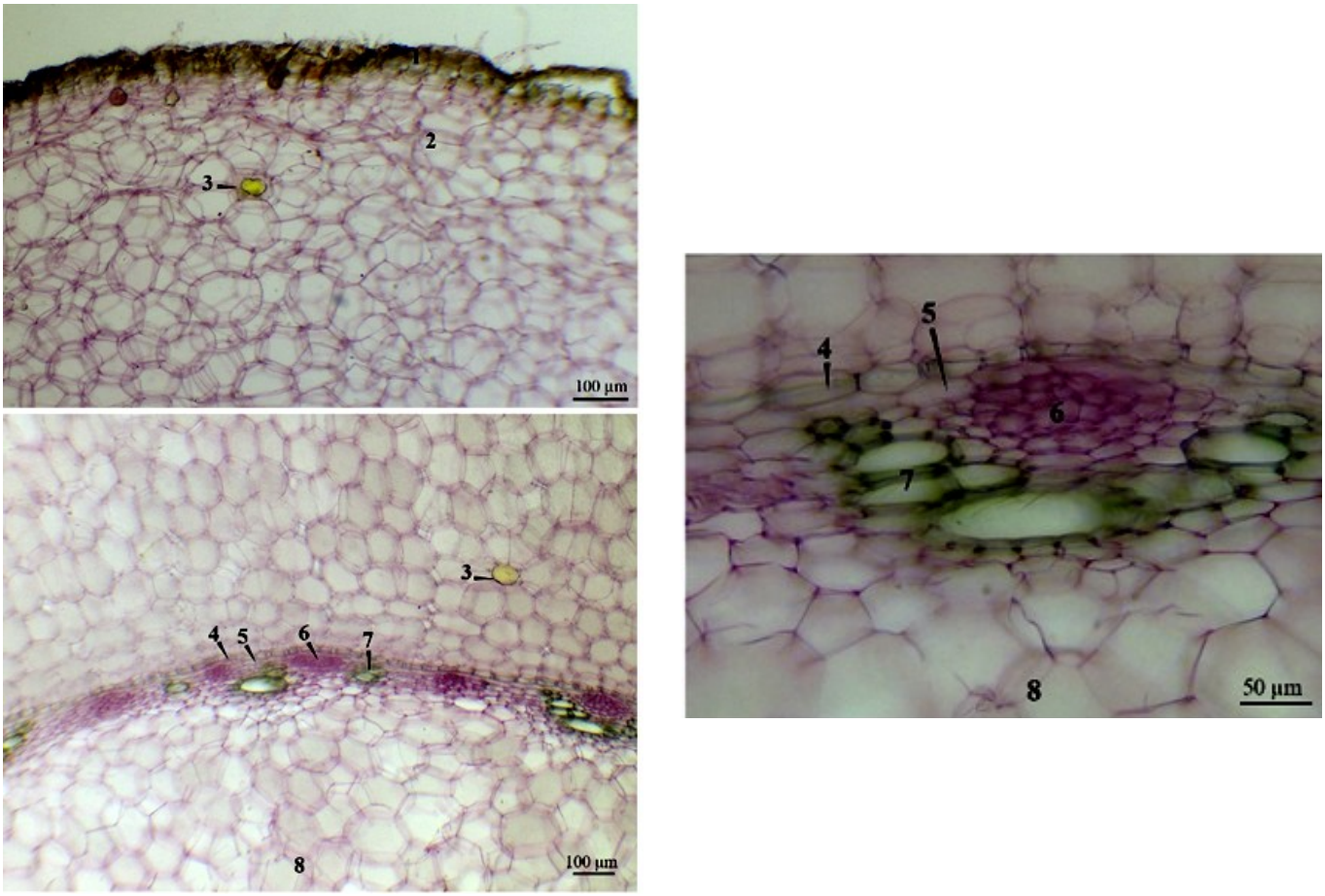



**Fig. 4.** Microsurgery of root tuber. 1) Phellem; 2) Cortical parenchyma; 3) Secretory cell; 4) Endodermis with casparian strip; 5) Pericycle; 6) Phloem; 7) Xylem; 8) Medullary parenchyma.

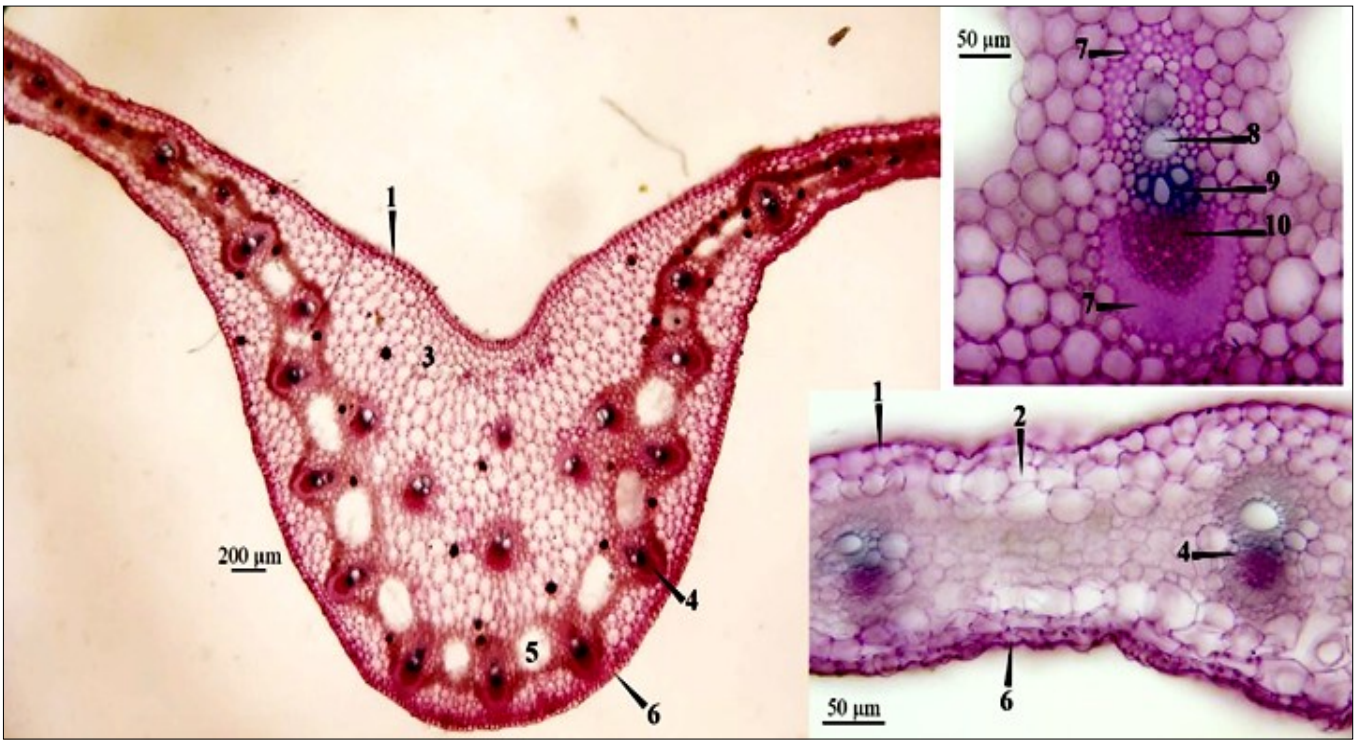



**Fig. 5.** Microsurgery of leaf blade. 1) Upper epidermis; 2) Hypodermis; 3) Parenchyma; 4) Vascular bundle; 5) Air cavity; 6) Lower epidermis; 7) Sclerenchyma; 8) Metaxylem; 9) Protoxylem; 10) Xylem.

cellulose walls. The vascular bundles are arranged in a row and have a structure similar to that of the vascular bundles in the midrib. The parenchyma contains abundant chloroplasts as well as several secretory cells.

**Petiole**

Concave on the upper surface and convex on the lower surface, exhibiting a structure similar to that of the midrib of the lamina (Fig. 6).

**Leaf sheath**

Curled around the pseudostem, the upper and lower epidermis consists of a single layer of rectangular cells with cellulose walls (Fig. 7). The parenchyma is composed of irregular, polygonal cells, which are either closely packed or separated by small spaces. The sclerenchyma occurs in clusters above the vascular bundles of the lower epidermis. These vascular bundles, with xylem positioned above the phloem, are arranged in one to three rows within the parenchyma region. Below the phloem and above the xylem, the bundles are supported by one to three

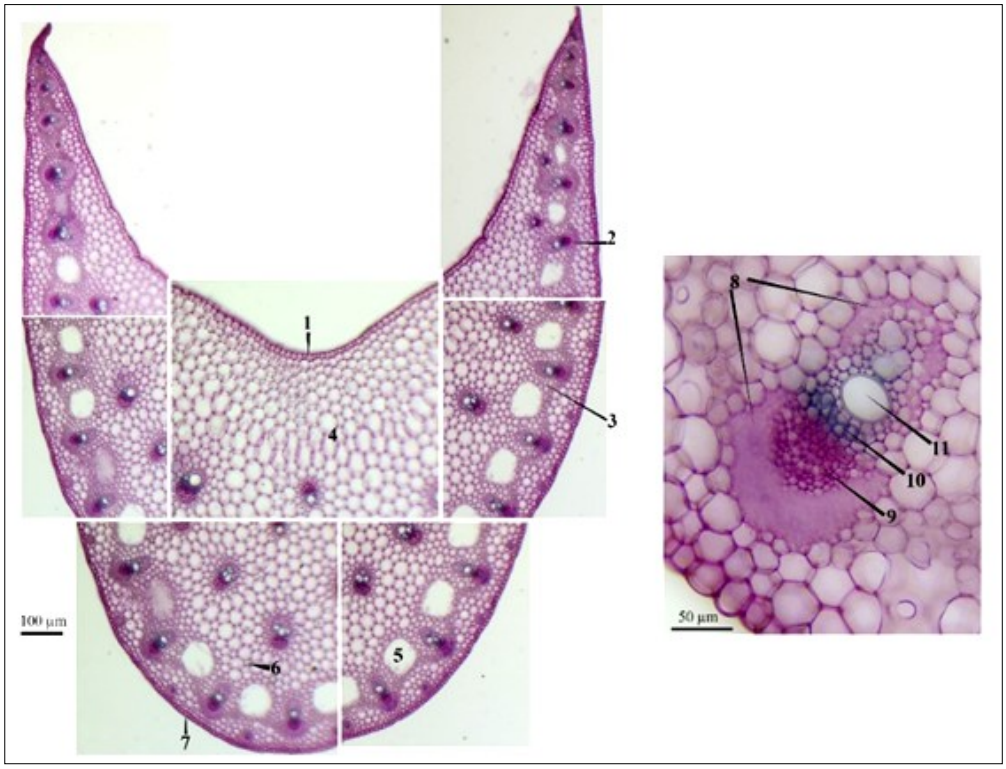



**Fig. 6.** Microsurgery of petiole. 1) Upper epidermis; 2) Vascular bundle; 3) Chlorenchyma; 4) Parenchyma; 5) Air cavity; 6) Secretory cell; 7) Lower epidermis; 8) Sclerenchyma; 9) Xylem; 10) Protoxylem; 11) Metaxylem.

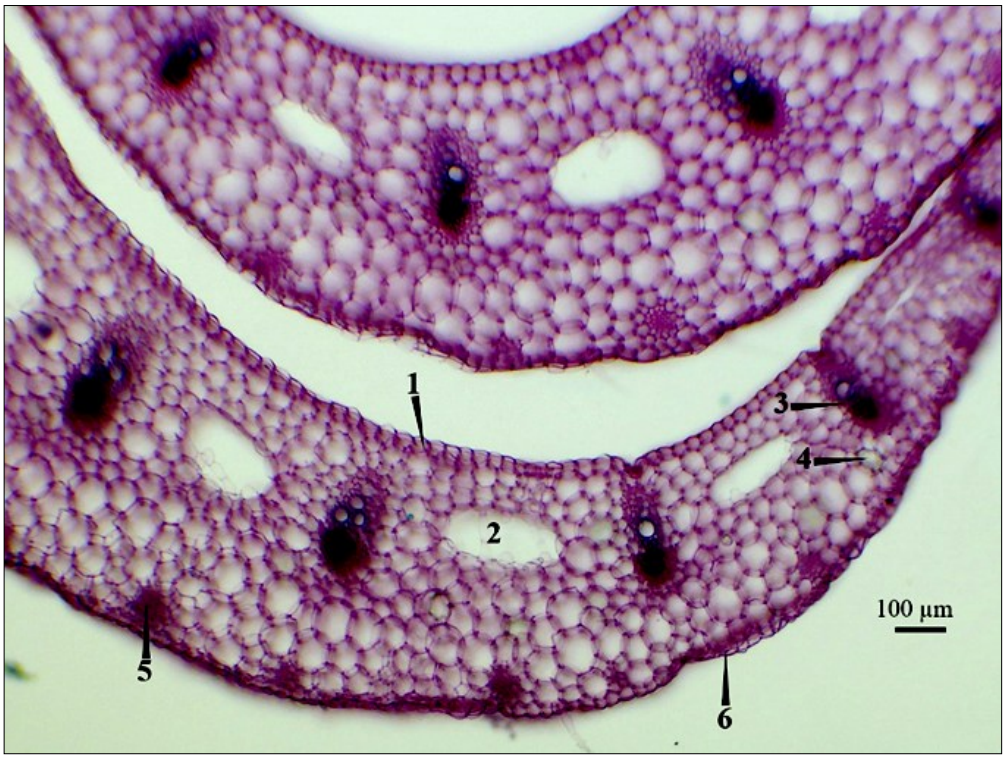



**Fig. 7.** Microsurgery of leaf sheath. 1) Upper epidermis; 2) Air cavity; 3) Vascular bundle; 4) Secretory cell; 5) Sclerenchyma; 6) Lower epidermis.

layers of sclerenchyma cells. In the spaces between the large vascular bundles, parenchyma cells are often degraded, resulting in the formation of intercellular spaces. Additionally, a few cells containing secretions are scattered throughout the parenchyma region.

**Table 1.** Qualitative phytochemistry of the leaf (A) and rhizome (B) extracts

| Phytochemistry | Leaf | Rhizome |
|---|---|---|
| Phenolic | ++ | ++ |
| Tannin | ++ | ++ |
| Alkaloid | + | + |
| Flavonoid | +++ | +++ |
| Saponin | + | + |
| Terpenoid | +++ | +++ |
| Steroid | +++ | +++ |
| Coumarin | + | ++ |

(+) less; (++) medium; (+++) very abundant

### Qualitative phytochemistry of the studied extract

The qualitative analyses conducted for the preliminary phytochemical assessment of the leaf and rhizome of *C. cotuana* (Table 1 & Fig. 2). The evaluated extracts were found to contain a variety of bioactive constituents, including phenolic compounds, tannins, alkaloids, flavonoids, coumarins, saponins, steroids and terpenoids.

### Quantitative phytochemistry of the studied extract

The quantitative assessments related to the preliminary phytochemical analysis of the leaf and rhizome of *C. cotuana* is presented in Table 2. Overall, the cumulative concentrations of triterpenes, polyphenols and flavonoids extracted from the leaf

**Table 2.** Quantitative phytochemistry of the leaf and rhizome extracts

| Constituents | Leaf | Rhizome |
|---|---|---|
| TFC (mg QE/g DW) | 2.07 ± 0.70 | 4.23 ± 0.20 |
| TPC (mg GAE/g DW) | 39.27 ± 0.19 | 17.11 ± 0.11 |
| TTC (mg OAE/g DW) | 41.83 ± 0.20 | 4.23 ± 0.90 |

were higher than those from the rhizome. Consequently, the leaf extract contained total concentrations of flavonoids, polyphenols and triterpenes at concentrations of 2.072 mg QE/g DW, 39.273 mg GAE/g DW and 41.831 mg OAE/g DW, respectively, whereas the rhizome extract demonstrated total concentrations of flavonoids, polyphenols and triterpenes measured at 4.232 mg QE/g DW, 17.108 mg GAE/g DW and 4.226 mg OAE/g DW, respectively.

## Discussions

Previous studies have reported the phytochemical screening of various *Curcuma* species. Ethanol, water and ether extracts isolated from rhizome the *C. sahuynhensis* from Vietnam contained various bioactive components, including fats, essential oil, carbohydrates, carotenoids, amino acids, alkaloids, coumarins, cardiac glycosides, flavonoids, tannins, saponins, polyuronides and triterpenoid (21). Similarly, the different extract such as petroleum ether, acetone, methanol, ethanol, chloroform and water of the *C. caesia* rhizome frown in India was found to include oxalates alkaloids, carbohydrates, cardiac glycosides, phenols, flavonoids, proteins, phlobatannins, tannins, saponins, sterols, quinones, terpenoids and oxalates (22). Methanol and ethanol extracts obtained from the rhizome of *C. aromatica* from India were reported to contain tannin, alkaloid and flavonoid (23).

The total phenol contents of water, methanol, ethyl acetate, chloroform, benzene, petroleum ether and hexane extracts from the fresh rhizome of *C. caesia* collected in India were reported as 34.39, 32.58, 45.48, 57.53, 56.64, 38.42, 50.44 mg GAE/g, respectively. In contrast, the total phenolic content for the same extracts in another study was reported as 48.49, 28.33, 86.29, 109.41, 96.68, 26.43, 53.44 mg GAE/g , respectively (24). The total phenol content of the dichloromethane extracts from the rhizome of the 7 *Curcuma* species, including *C. rakthakanta*, *C. malabarica*, *C. caesia*, *C. brog*, *C. aromatica*, *C. amada*, *C. aeruginosa* were ranging from 23.0 to 69.0 mg GAE/g (25). The total phenolic content from the rhizomes of four *Curcuma* plants such as *C. zedoaria*, *C. amada*, *C. aromatica*, *C. xanthorrhiza* and two taxa of *C. longa* (collected from 2 regions, Ryudai Okinawa and Gold) were ranging from 37.9 to 154.4 mg GAE/g while the total flavonoid content of these species were ranging from 15.3 to 797.5 GAE/g (26).

The hexane extract and essential oils from *C. aromatica* were found to be rich in 1H-3a, 7–methanoazulene, curcumene and xanthorrhizol (27). Moreover, the phytochemical properties of the rhizome of *C. longa* have also been reported. Specifically, 11 bioactive compounds were identified in the ethanol extract such as tannins, anthraquinones, flavonoids, steroids, saponins, carbo-hydrates, glycosides, alkaloids, proteins, terpenoids and phlobotannins. The chloroform extract consisted of 7 out of 11 compounds except proteins, terpenoids, tannins and flavonoids, whereas the hydroalcoholic extract included 10 constituents, with the exception of phlobotannins (28).

Another study showed chemical components of the methanolic extract obtained from the rhizomes of 3 *Curcuma* species. For instance, *C. longa* extract was found to contain ar-tumerone, tumerone and curlone as the major compounds. The extract from *C. angustifolia* was characterized by the predominance of oleic acid, nitrous oxide, n-hexadecanoic acid and acetic acid. In contrast, the extract from *C. decipiens* was rich in trispiro [4.2.4.2.4.2.] heneicosane; 4, 4-dimethyl-2, 4, 5, 6-tetrahydro-1H-inden-2-yl) acetic acid; and cycloheptyl ethyl methylphosphonate (29).

## Conclusion

This study highlights the distinct anatomical and phytochemical characteristics of *C. cotuana*, affirming its value as a medicinal plant. The higher bioactive compound concentrations in leaves suggest organ-specific therapeutic potential. These findings support the importance of its conservation and warrant further pharmacological exploration. Future studies should focus on evaluating its bioactivity and potential clinical applications. This foundational study supports the pharmacognostic relevance of

*C. cotuana* and its prospective integration into natural product-based drug discovery pipelines.

## Acknowledgements

This research is supported by Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Viet Nam. The authors would like to thank Ms. Nguyen Thi Minh Thu for her cooperation.

## Authors' contributions

VTV participated in the design of the study, conducted the experiments, carried out the statistical analysis and drafted the manuscript. NTN, HTV, HTL, NNP and TTL carried out experimental work and participated in research data. VTV and DHT drafted the manuscript and resolved all the query of editors and reviewers. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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