

Anatomical traits and phytochemical screening of *Curcuma newmanii* Škorničk.

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SUMMARY

Introduction: *Curcuma newmanii* Škorničk. has been recently described as a new species to science which the type specimens collected from Vietnam. **Aim:** The present study firstly described the pharmacological properties of this species, including micro-morphological features and phytochemical screening. **Methodology:** the iodine green-carmin double staining method was used to provide the details of anatomical features whereas the qualitative and quantitative assays were used to perform the phytochemical screening of this plant. **Results:** the micro-morphological characteristics of the different organs of *C. newmanii*, including the petiole, root, root tuber, leaf, leaf sheath, and rhizome were firstly provided. Additionally, the ethanolic extracts obtained from leaf, flower, and rhizome of *C. newmanii* consisted of various bioactive components, including coumarin, terpenoid, steroid, flavonoid, saponin, alkaloid, phenolic, and tannin. Furthermore, the rhizome extract possessed the highest contents of the triterpene, flavonoid, and polyphenol with the contents of 18.57 mg OAE/g DW, 44.84 mg QE/g DW, and 8.38 mg GAE/g DW, followed by the leaf extract (3.08 mg OAE/g DW, 28.78 mg QE/g DW, and 5.48 mg GAE/g DW), and the flower extract (0.39 mg OAE/g DW, 16.85 mg QE/g DW, and 3.71 mg GAE/g DW). **Conclusion:** the pharmacological properties of *C. newmanii* obtained from this study hopefully provide the potential application of this plant the pharmacological fields in the future.

Keywords: *Curcuma newmanii*; micro-morphological features; qualitative and quantitative assays.

RESUMEN

Características anatómicas y análisis fitoquímico de *Curcuma newmanii* Škorničk.

Introducción: *Curcuma newmanii* Škorničk. ha sido descrita recientemente como una nueva especie para la ciencia, cuyos especímenes tipo se recolectaron en Vietnam. **Objetivo:** El presente estudio describió inicialmente las propiedades farmacológicas de esta especie, incluyendo las características micromorfológicas y el análisis fitoquímico. **Metodología:** Se utilizó el método de doble tinción con verde de yodo-carmín para obtener los detalles de las características anatómicas, mientras que los ensayos cualitativos y cuantitativos se emplearon para realizar el análisis fitoquímico de esta planta. **Resultados:** En primer lugar, se proporcionaron las características micromorfológicas de los diferentes órganos de *C. newmanii*, incluyendo el pecíolo, la raíz, el tubérculo radicular, la hoja, la vaina foliar y el rizoma. Además, los extractos etanólicos obtenidos de hoja, flor y rizoma de *C. newmanii* consistieron en varios componentes bioactivos, incluyendo cumarina, terpenoide, esteroide, flavonoide, saponina, alcaloide, fenólico y tanino. Además, el extracto de rizoma poseía los contenidos más altos del triterpeno, flavonoide y polifenol con los contenidos de 18,57 mg OAE/g PS, 44,84 mg QE/g PS y 8,38 mg GAE/g PS, seguido por el extracto de hoja (3,08 mg OAE/g PS, 28,78 mg QE/g PS y 5,48 mg GAE/g PS), y el extracto de flor (0,39 mg OAE/g PS, 16,85 mg QE/g PS y 3,71 mg GAE/g PS). **Conclusión:** Se espera que las propiedades farmacológicas de *C. newmanii* obtenidas en este estudio permitan su posible aplicación en el campo farmacológico en el futuro.

Palabras clave: *Curcuma newmanii*; características micromorfológicas; ensayos cualitativos y cuantitativos.

RESUMO

Características anatômicas e triagem fitoquímica de *Curcuma newmanii* Škorničk.

Introdução: *Curcuma newmanii* Škorničk. foi recentemente descrita como uma nova espécie para a ciência, a partir de espécimes-tipo coletados no Vietnã. **Objetivo:** O presente estudo descreveu inicialmente as propriedades farmacológicas desta espécie, incluindo características micromorfológicas e triagem fitoquímica. **Metodologia:** O método de dupla coloração com verde-iodo-carmim foi utilizado para fornecer os detalhes das características anatômicas, enquanto ensaios qualitativos e quantitativos foram utilizados para realizar a triagem fitoquímica desta planta. **Resultados:** As características micromorfológicas dos diferentes órgãos de *C. newmanii*, incluindo pecíolo, raiz, tubérculo radicular, folha, bainha foliar e rizoma, foram inicialmente fornecidas. Além disso, os extratos etanólicos obtidos de folhas, flores e rizomas de *C. newmanii* consistiram em vários componentes bioativos, incluindo cumarina, terpenoide, esteroide, flavonoide, saponina, alcaloide, fenólico e tanino. Além disso, o extrato do rizoma possuía os maiores teores de triterpeno, flavonoide e polifenol com os teores de 18,57 mg OAE/g DW, 44,84 mg QE/g DW e 8,38 mg GAE/g DW, seguido pelo extrato da folha (3,08 mg OAE/g DW, 28,78 mg QE/g DW e 5,48 mg GAE/g DW) e o extrato da flor (0,39 mg OAE/g DW, 16,85 mg QE/g DW e 3,71 mg GAE/g DW). **Conclusão:** as propriedades farmacológicas de *C. newmanii* obtidas neste estudo proporcionam potencial aplicação desta planta em áreas farmacológicas no futuro.

Palavras-chave: *Curcuma newmanii*; características micromorfológicas; ensaios qualitativos e quantitativos.

1. INTRODUCTION

Curcuma L., the third largest genus belonging to the family Zingiberaceae, comprises about 130 species widely found in islands of the South Pacific, South East Asia, and northern Australia [1]. In herbal remedies, species of this genus have been used as the medicinal plants to treat many diseases such as bronchial complaints, diarrhea, insect bites, leucorrhea, pneumonia, abscesses, and infectious wounds [2]. In addition, a large number of the *Curcuma* species have been reported to consist of bioactive compounds as well as pharmaceutical properties [2].

Currently, accurate classification of plant species, especially medicinal plants, is very important in their research and application. Studies provided that *Curcuma* is one of the genera of the family Zingiberaceae with high diversity due to morphological and genetic variation as well as the wide hybridization and polyploidization [1]. The annual flowering cycle of species in this genus is short. Moreover, the vegetative organs are usually have similar morphological characteristics among species, leading to difficulty in classifying species [3].

To solve the difficulties in taxonomy using the comparative morphological method, many supporting methods have been applied, of which the micro-morphological characteristics are considered to play an important role in classification and standardization of medicinal plants [4]. It is necessary to study on the micro-morphological features to provide taxonomic information as well as bioactive compounds of newly discovered species in the *Curcuma* genus. In 2013, Leong-Škorničková and Tran were firstly discovered and described *Curcuma newmanii* Škorničk. as a new species to science which the type samples were collected from Ban Don village, Dak Lak province, Vietnam [5]. The present study, therefore, firstly investigated the micro-morphological features and phytochemical screening of *C. newmanii*.

2. MATERIALS AND METHODS

2.1. Plants

The specimens of the *C. newmanii* were collected from Krong Na commune, Ban Don District, Daklak province, Vietnam, at the altitude of 190-195 m, coordinates of 12°54'43.4"N/107°43'58.2"E. The voucher sample (NPN-DK-025) was deposited at the Herbarium of University of Science, Vietnam National University-HCMC (PHH).

2.1.1. Anatomical characteristics

The petiole, root, root tuber, leaf, leaf sheath, and rhizome of *C. newmanii* were cut into the thin slices of using razor blade. The Javel solution was used to soak these thin slices to remove the undesirable constituents in the tissue. The microscopic specimens were stained using the iodine green-carmin double staining method. These specimens were washed by the distilled water and then, the 10% glycerol was used to preserve them [6]. The studied samples were observed and taken the picture with the Olympus BX53 Digital Upright Microscope.

2.1.2. Extraction procedures

The fresh leaf, flower, and rhizome of *C. newmanii* were washed and dried at 50 °C using drying cabinet. The samples were then grinded into the powder. Ethanol solution was used to soak five grams of studied powder according to the ratio of 1 sample: 30 ethanol (w/w) for 8 hours and then it was filtered using whatman paper. This extraction was repeated twice more

with the residue. The supernatant was and all filtrate fractions were combined to collect the final extract.

2.1.3. Qualitative phytochemistry of *C. newmanii*

The qualitative phytochemistry of *C. newmanii* were determined using the methods as follows, (1) the phenolic and tannin: 2 mL of studied extract were taken into the tube, 2 mL of H₂O and 2-3 drops of FeCl₃ (5%) were added; if the blue-black or brown-green precipitate appeared after completion of the reactions, it was considered as positive [7]; (2) Alkaloids: 2 mL of studied extract and 3-4 drops of Wagner reagent were taken; if the red brown precipitate appeared after completion of the reactions, it was positive [8]; (3) Flavonoids: 2 mL studied extract and 2 mL 10% Pb(COOH)₂ were taken; if the yellow precipitate appeared after completion of the reactions, it was positive [9]; (4) Saponin: 2 mL studied extract were taken into the tube, 10 mL of distilled water were added and boiled for 2 minutes; if the mixture formed foam, it was positive [10]; (5) Terpenoids and steroids: 5 mL of studied extract were taken, 2 mL chloroform and 3 mL concentrated H₂SO₄ were added; if the reddish brown colour appeared, it was positive [11]; (6) Coumarin: 2 mL of studied extract were taken and 3 mL NaOH (10%) were added; if the yellow colour appeared, it was positive [12].

2.2. Quantitative phytochemistry of *C. newmanii*

2.2.1. Total triterpene content (TTC)

1.0 mL of the studied extract was taken into a tube, then 5% acetic acid (0.2 mL) and perchloric acid (1.2 mL) were added, this mixture was shaken slightly, incubated at 70 °C for 15 minutes, and cooled quickly within 2 minutes. 2.6 mL of ethyl acetate was added the mixture to make 5.0 mL and then it was photometrically measured at 550 nm wavelength. Acetic acid (5%) was used as a control agent. The total triterpene concentration (milligrams of oleanoic acid equivalent-OAE) in the studied extract was identified based on the photometric results and standard curve graph [13]. The TTC was calculated according to the formula:

$$\text{TTC (mg OAE/g DW)} = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100 - W)} \times K$$

Where, C_x: the total triterpenoid content in the studied extract obtained from the standard curve (ppm); a: initial sample mass (g); V: sample volume (mL); K: dilution factor; W: humidity (%); 10³: conversion factor.

2.2.2. Total flavonoid content (TFC)

1.0 mL of the studied extract and 0.3 mL of NaNO₂ 5% were added into a tube, this mixture was shaken slightly. The tube was kept at room temperature for 5 minutes and then, 0.3 mL of AlCl₃ solution 10% were added, then the tube was well shaken and leaved at room temperature for 5 minutes. 2.0 mL of 1 M NaOH solution was added and shaken, then, 6.4 mL of distilled water were also added to make 10.0 mL. The mixture was measured photometrically at wavelength λ= 510 nm. Distilled water was used as a control agent. The total flavonoid content (milligrams of quercetin acid equivalent-QE) in the studied extract was identified based on the photometric results and standard curve graph [14]. The TFC was calculated according to the formula:

$$\text{TFC (mg QE/g DW)} = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100 - W)} \times K$$

Where, C_x : the total flavonoid content in the studied extract obtained from the standard curve (ppm); a : initial sample mass (g); V : sample volume (mL); K : dilution factor; W : humidity (%); 10^3 : conversion factor.

2.2.3. Total polyphenol content (TPC)

A volume of 0.1 mL of the studied extract and 1.8 mL of Folin-Ciocalteu solution were added into a tube, this mixture was shaken slightly and the tube was kept at room temperature for 5 minutes. Then, 1.2 mL of 15% Na_2CO_3 solution were added, and finally 8.7 mL of distilled water were added to make 10 mL. The tube was covered, shaken, and incubated at room temperature and dark conditions for 90 minutes. The mixture was measured photometrically at wavelength $\lambda = 734$ nm. Distilled water was used as a control agent. The total polyphenol content (milligrams of gallic acid equivalent-GAE) in the studied extract was identified based on the photometric results and standard curve graph [15]. The TPC was calculated according to the formula:

$$\text{TPC (mg GAE/g DW)} = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100 - W)} \times K$$

Where, C_x : the total polyphenol content in the studied extract obtained from the standard curve (ppm); a : initial sample mass (g); V : sample volume (mL); K : dilution factor; W : humidity (%); 10^3 : conversion factor.

3. RESULTS

Photos of all parts of studied *Curcuma newmanii* Škorničk. samples are shown in Fig. 1.

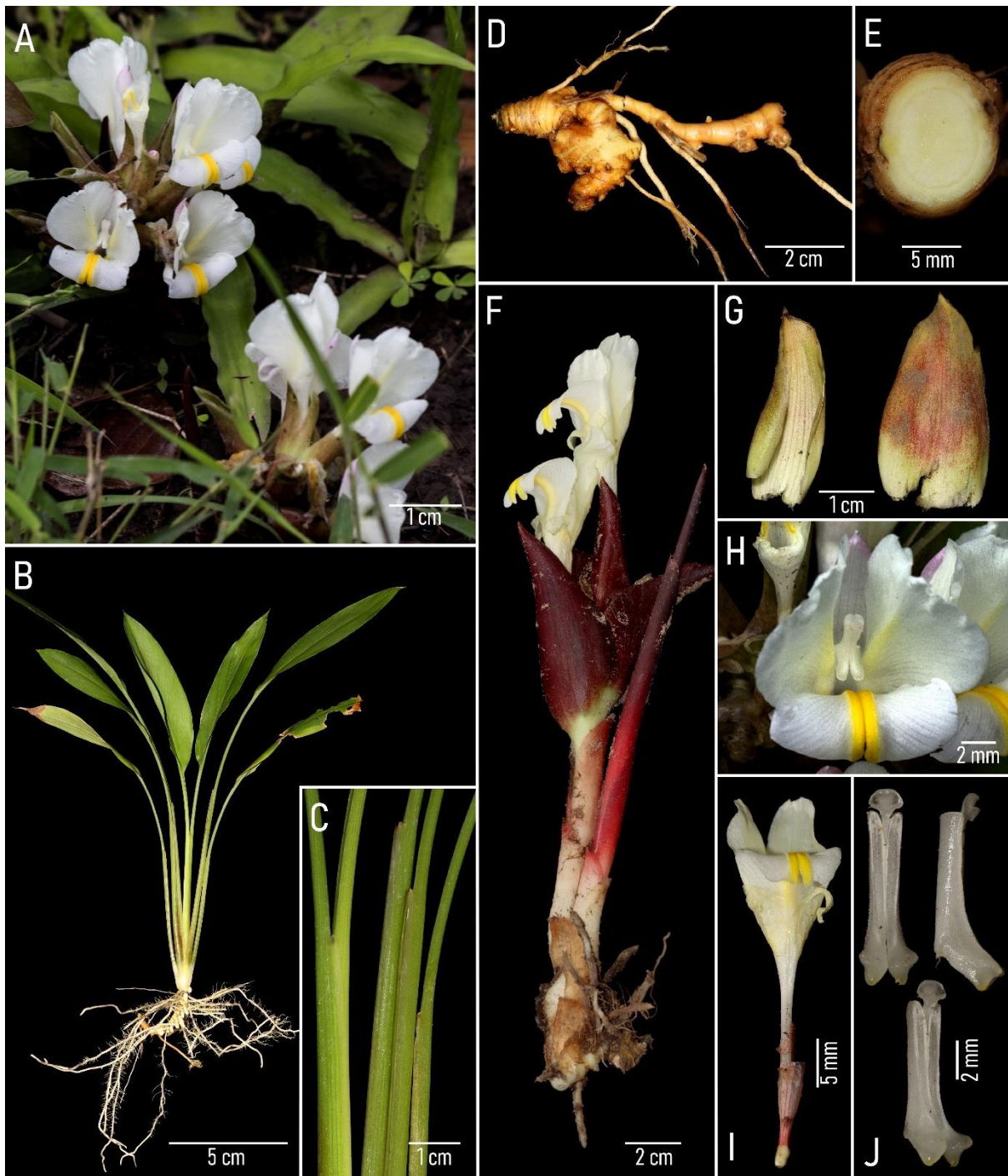


Figure 1. *Curcuma newmanii*. A. Habit (flowering stage). B. Whole plant (after flowering). C. Petioles and ligules. D. Rhizome and roots. E. Cross section of rhizome. F. Inflorescence with flower. G. Bracts. H. Flower (front view). I. A flower in detail. J. Anthers in different views. Photos: Diep Quang Dinh: A-I, Nga Nguyen-Phi: J.

3.1. Anatomical characteristics of *C. newmanii*

3.1.1. Root

The cross-section of root is nearly circular, divided into 2 regions, the cortical area includes $\frac{3}{4}$ of the radius of cross-section while $\frac{1}{4}$ of the remaining region is stele. The piliferous layer consists of a layer of polygonal or rectangular cells, the walls are impregnated with phellem, approximately equal size, closely arranged, and many root hairs. The exodermis consists of 2-

3 layers of rectangular or square cells, walls impregnated with phellem, radially arranged. The cortical parenchyma is divided into 2 regions, outer parenchyma consists of 8-10 layers of nearly polygonal round cells, cellulose walls, irregular size, haphazardly arranged, leaving quite large intercellular spaces (spongy parenchyma); inner parenchyma includes 5-6 layers of rectangular or square cells with cellulose walls, gradually smaller in size towards the center, arranged in concentric rings and radial rows to create small intercellular spaces. There are many cells containing yellow-brown secretions in the cortical parenchyma. The endodermis with Casparian *strip* comprises 1 layer of rectangular cells, quite regular. The pericycle consists of a single layer of polygonal cells with cellulose walls, alternately arranged with endodermis. The vascular system consists of 18 - 22 primary phloem bundles, alternately arranged with 18 - 22 primary xylem bundles on a ring close to the pericycle. The primary phloem has 3-5 layers of polygonal cells, cellulose walls, irregular, radially differentiated. The protoxylem bundle consists of 2-4 vessels, polygonal shape, lignin-impregnated walls, radially differentiated. There are 12-14 metaxylem veins located in a ring under primary phloem and protoxylem bundle. The metaxylem veins are polygonal in shape, slightly rounded, with cellulose or lignin-impregnated walls, large size. The medullary parenchyma is divided into 2 regions, the outer medullary parenchyma consists of 6-7 layers of polygonal cells, lignin-impregnated walls, tightly packed together; Inner medullary parenchyma includes of 2-4 layers of polygonal cells, cellulose walls, irregular, with small intercellular spaces.

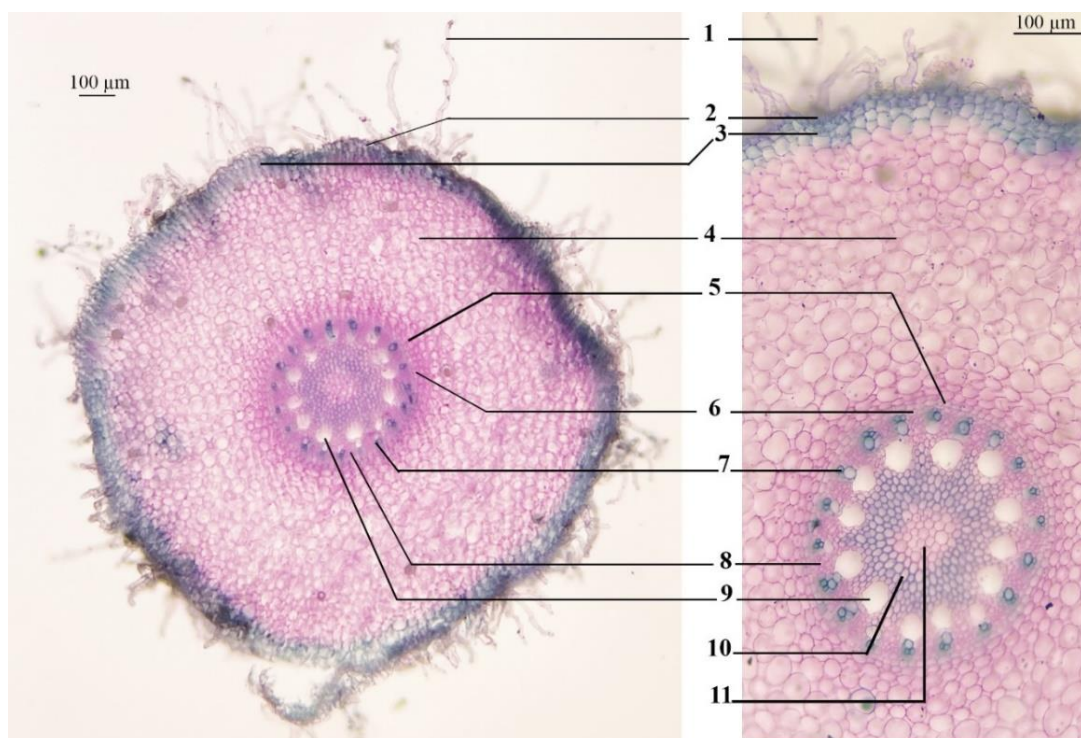


Figure 2. The cross-section of root. 1: root hair; 2: piliferous layer; 3: exodermis; 4: cortical parenchyma; 5: endodermis with Casparian strip; 6: pericycle; 7: primary xylem; 8: primary phloem; 9: metaxylem; 10: sclerenchymatous conjunctive tissues; 11: parenchymatous pith.

3.1.2. Root tuber

The cross-section is often distorted, rarely round, divided into 2 regions, the cortex is very thick and grows according to the growth of tuber, the stele is thin. The piliferous layer includes a layer of rectangular, distorted, very flat, small cells, and scattered with hairs. The phellem

has 3-4 layers of rectangular, flat cells, walls impregnated with cork, radially arranged. The phelloderm consists of 4-6 layers of polygonal cells with curved cellulose walls, large size, radially arranged. The cortical parenchyma is a type of spongy parenchyma with polygonal or nearly round cells, cellulose walls; there are many cells containing yellow secretions in the cortical parenchyma. The endodermis with Casparian *strip occurs*. The pericycle has 1 layer of polygonal, regular cells with cellulose walls. There are 14-18 protoxylem bundles alternately arranged with 14-18 primary phloem bundles per a ring. The protoxylem bundle has 2-4 polygonal xylem vessels, walls impregnated with lignin, and radially differentiated. The primary phloem bundle consists of 3-5 layers of polygonal cells, cellulose walls, haphazardly arranged. There are 14-16 polygonal metaxylem vessels with walls impregnated with lignin, usually located close to the protoxylem bundle. The medullary parenchyma is a dense tissue with polygonal cells, cellulose walls, closely arranged.

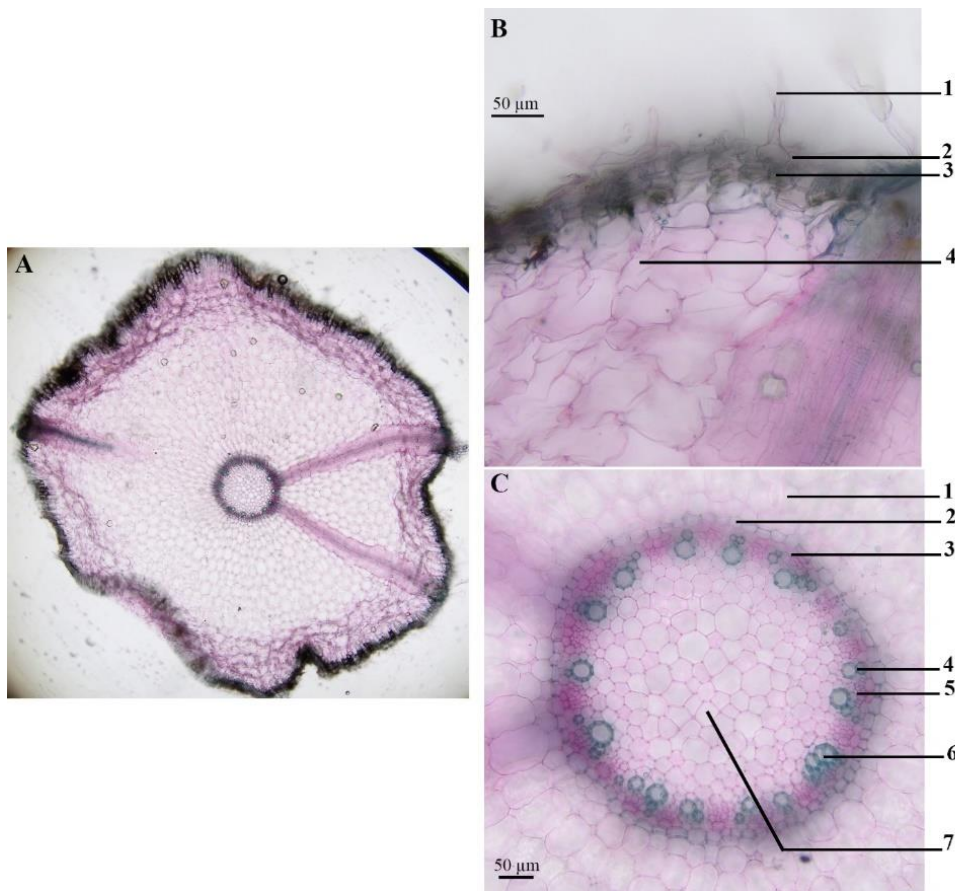


Figure 3. The cross-section of root tuber. A. the whole view of cross-section. B. Cortex (1: root hair, 2: piliferous layer, 3: phellem, 4: phelloderm). C. Stele (1: cortical parenchyma, 2: endodermis with Casparian strip, 3: pericycle, 4: primary xylem, 5: primary phloem, 6: metaxylem, 7: medullary parenchyma)

3.1.3. Rhizome

The cross-section is often distorted, rarely round. The epidermis includes 1 layer of rectangular cells, very flat, and small. The phellem has 3-8 layers of distorted rectangular cells, 1-2 layers of phellem under the epidermis are large size, the lower cell layers are very flat rectangular, the walls are impregnated with phellem, radially arranged. The phelloderm consists of 2-3 layers of rectangular cells, with cellulose walls. The cortical parenchyma belongs to the medullary parenchyma type, consisting of round or nearly round polygonal cells, scattered with

primary vascular bundle with xylem inside and phloem outside. The endodermis with Casparian *strip occurs*. The pericycle consists of a single layer of cellulose wall cells, many locations of which are unclear. Many vascular bundles are arranged in an orderly manner from the endodermis into the medullary parenchyma; the differentiation of the xylem bundles is unclear. The medullary parenchyma includes nearly round or polygonal cells and cellulose walls. There are many cells containing yellow secretions scattered throughout the cortical and medullary parenchyma.

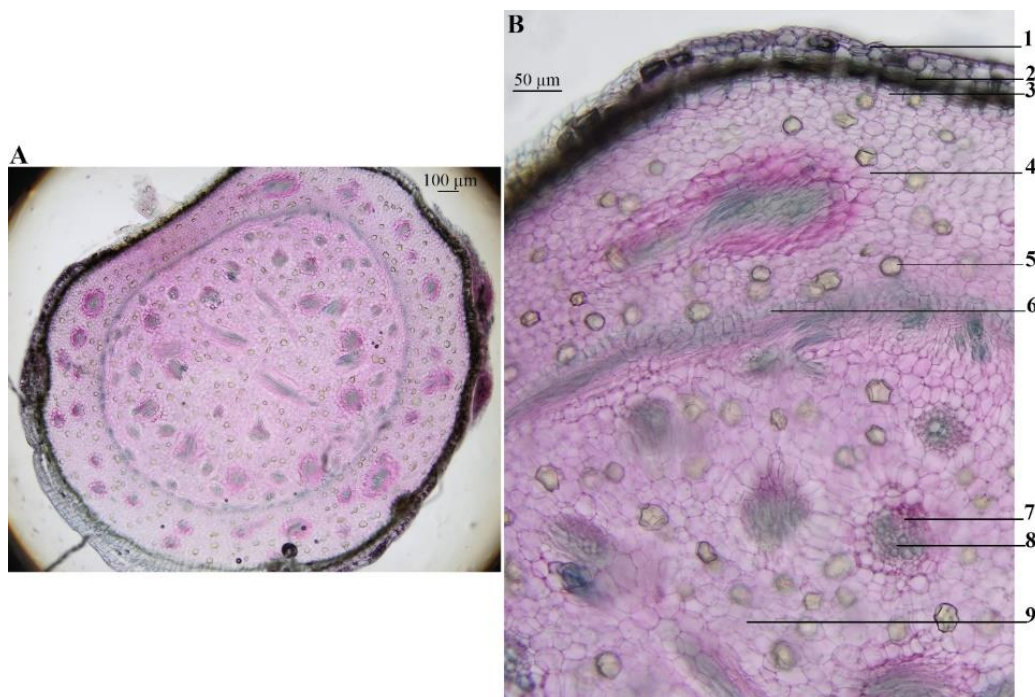


Figure 4. The cross-section of rhizome. A. The whole view of cross section. B. The details of cross section (1: epidermis, 2: phellem, 3: phelloderm, 4: cortical parenchyma, 5: secretory cell, 6: endodermis with Casparian strip, 7: primary phloem, 8: primary xylem, 9: medullary parenchyma).

3.1.4. Leaves (Figure 5)

3.1.4.1. Midrib: The upper surface is concave, the lower surface is convex. The epidermis consists of closely rectangular or polygonal cells, sometimes with unicellular protective hairs. The angular collenchyma consists of 1-2 layers of cells located below the concave part of the upper epidermis. The parenchyma has polygonal cells, sometimes distorted and irregular size. There are vascular bundles arranged in rows above the lower epidermis with xylem located above the phloem, there are large air cavities among these vascular bundles. The xylem includes: 1-3 metaxylem vessels, 2-6 small protoxylem vessels located below the metaxylem; under the phloem, horseshoe-shaped sclerenchyma cluster includes polygonal cells and very thick walls impregnated with lignin. In the middle of the parenchyma, there are 4-5 vascular bundles, arranged in rows; below xylem and above phloem, there are 2 layers of sclerenchyma cells with thick walls by cellulose or impregnated with lignin. **Lamina:** The upper and lower epidermis consist of a layer of rectangular cells, cellulose walls, and stomata scattered in both epidermis. The hypodermis is 1 layer of polygonal cells, cellulose wall, located below the epidermis on both sides. The vascular bundles are the same structure as those in the midrib. The

spongy parenchyma comprise round or oval cells, near the upper epidermis, there are 2-3 layers of slightly elongated cells arranged in rows like the chlorenchyma, containing many chloroplasts.

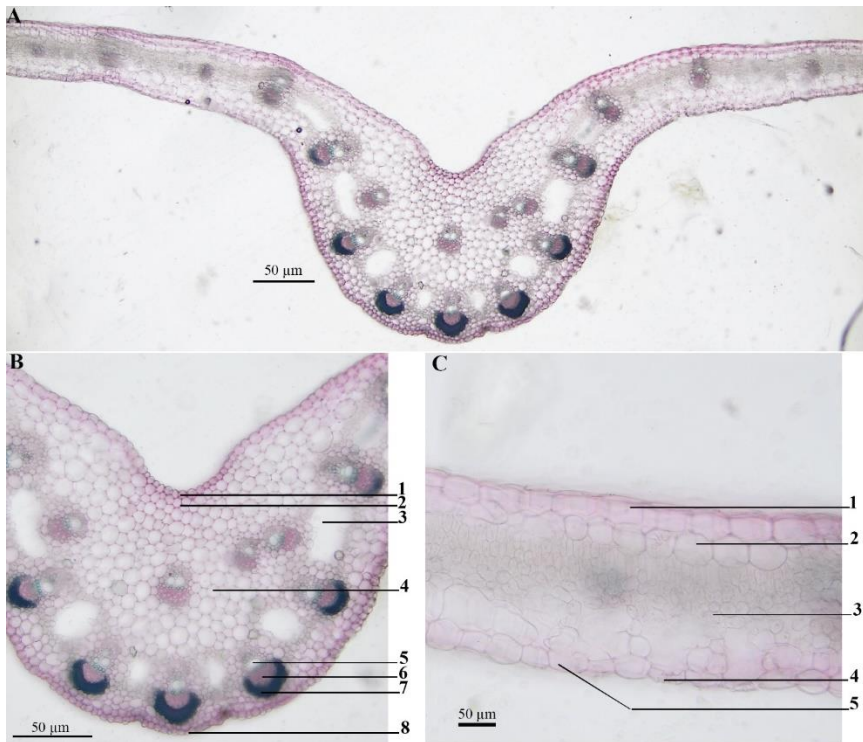


Figure 5. The cross-section of leaf. A. The whole view of cross-section. B. Midrib (1: upper epidermis, 2: angular collenchyma, 3: air cavity, 4: parenchyma, 5: xylem, 6: phloem, 7: sclerenchyma, 8: lower epidermis). C. Lamina (1: upper epidermis, 2: hypodermis, 3: spongy parenchyma, 4: stomata, 5: lower epidermis).

3.1.5. Petiole (Figure 6)

The outline is open 'U' shaped. The cross section has a concave at upper surface and a convex at lower surface, with the same structure as those in the lamina. The epidermis on the petiole has many unicellular protective hairs.

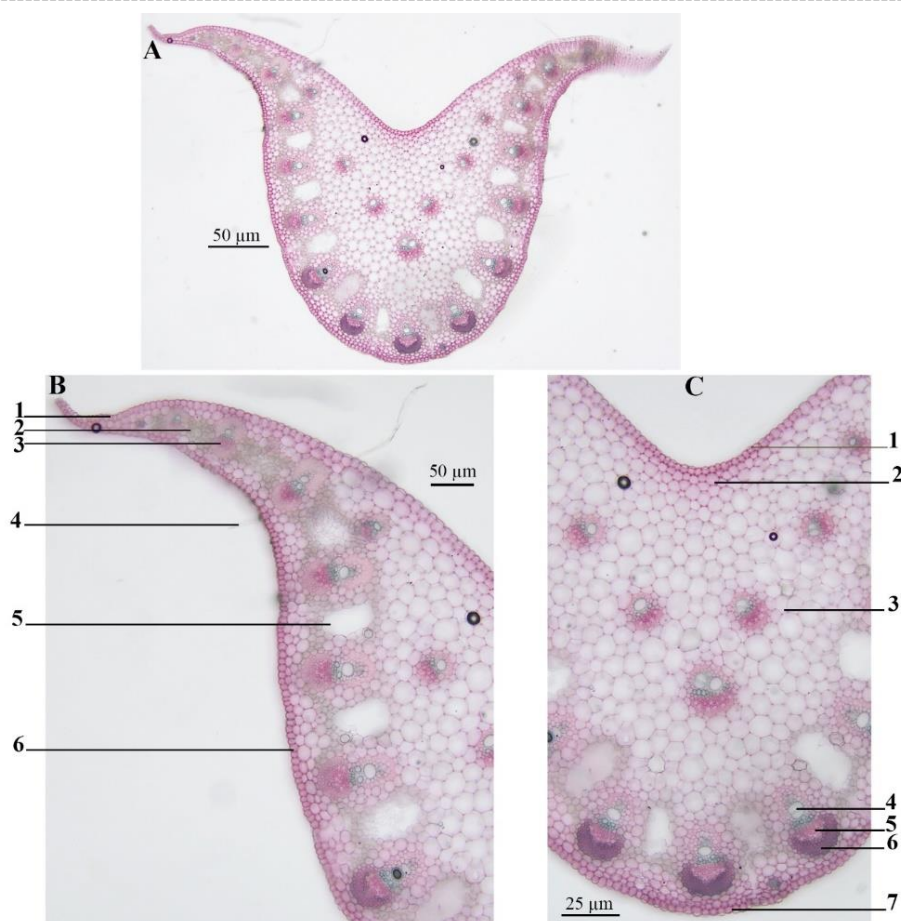


Figure 6. The cross-section of petiole. A. The whole view of cross section. B. Side view of cross section (1: upper epidermis, 2: spongy parenchyma, 3: vascular bundle, 4: unicellular protective hair, 5: air cavity, 6: lower epidermis). C. The middle of the cross section (1: upper epidermis, 2: angular collenchyma, 3: parenchyma, 4: xylem, 5: phloem, 6: sclerenchyma, 7: lower epidermis).

3.1.6. Leaf sheath (Figure 7)

The cross section is curved with a deeply concave at upper surface, thick in the middle and gradually thinner on both sides. The upper and lower epidermis are the same, consisting of 1 layer of rectangular cells, the upper epidermal cells are larger than the lower epidermis. The parenchyma includes polygonal cells. On the lower epidermis, there are large and small vascular bundles arranged in alternating rows; in the middle, there are the large vascular bundles with large air cavities. The vascular bundle includes xylem above, phloem below. Xylem comprises 1-2 metaxylem vessels, 3-5 protoxylem vessels; sclerenchyma clusters located above xylem and below phloem, 2-3 layers of sclerenchyma cells above the xylem have thin cellulose walls; 5-6 layers of sclerenchyma cells under the phloem have thick walls, lignin-impregnated walls. There are small vascular bundles also arranged in rows in the middle of the parenchyma area with a similar structure to the large vascular bundles. The vascular bundles gradually become smaller in size and are only arranged in a row towards the thin parts on both sides of the leaf sheath.

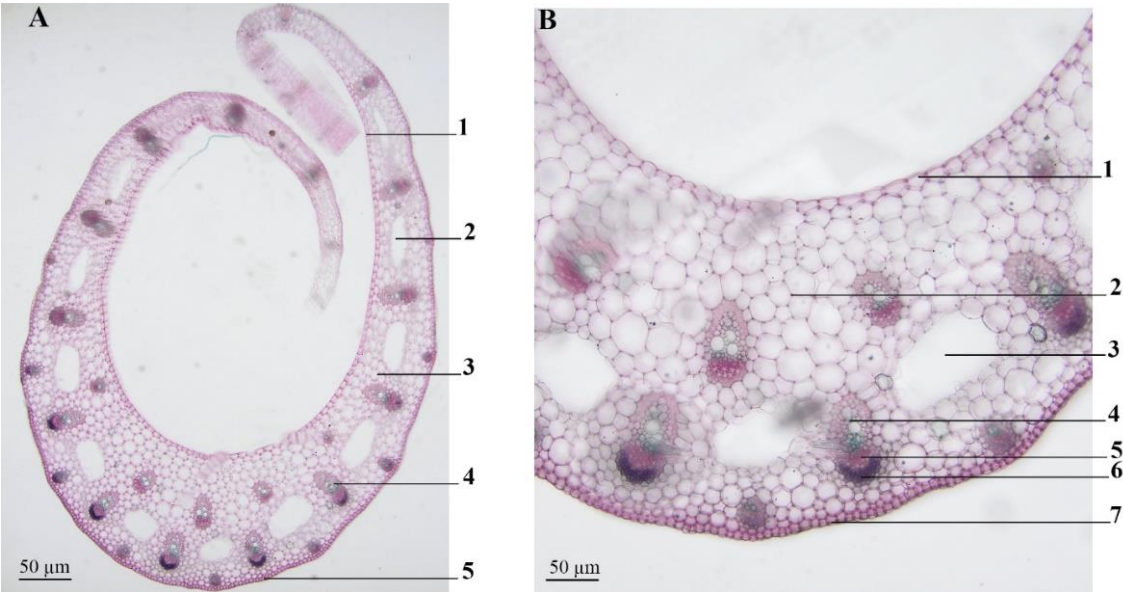


Figure 7. The cross-section of leaf sheath. A. The whole view of cross section (1: upper epidermis, 2: air cavity, 3: parenchyma, 4: vascular bundle, 5: lower epidermis). B. the middle of the cross section (1: upper epidermis, 2: parenchyma, 3: air cavity, 4: xylem, 5: phloem, 6: sclerenchyma, 7: lower epidermis).

3.2. Qualitative phytochemistry of *C. newmanii*

The results in Tab. 1 demonstrated that the ethanolic extracts obtained from leaf, flower, and rhizome of *C. newmanii* consisted of various bioactive components, including coumarin, terpenoid, steroid, flavonoid, saponin, alkaloid, phenolic, and tannin.

Table 1. Preliminary phytochemistry of *C. newmanii*

Compounds	Leaf	Flower	Rhizome
Phenolic	+++	++	+++
Tannin	+++	++	+++
Alkaloid	+	++	++
Flavonoid	++	+	+++
Saponin	+	+	+
Terpenoid	+++	+++	+++
Steroid	+++	+++	+++
Coumarin	++	++	++

Note: (+) Less, (++) Medium, (+++) Very abundant.

3.3. Quantitative phytochemical content of *C. newmanii*

The data in Tab. 2 showed the total of triterpene, flavonoid, and polyphenol contents of the ethanolic extracts from *C. newmanii*. Accordingly, the rhizome extract possessed the highest contents of the triterpene, flavonoid, and polyphenol with the contents of 18.57 mg OAE/g DW, 44.84 mg QE/g DW, and 8.38 mg GAE/g DW, followed by the leaf extract (3.08 mg OAE/g DW, 28.78 mg QE/g DW, and 5.48 mg GAE/g DW), and the flower extract (0.39 mg OAE/g DW, 16.85 mg QE/g DW, and 3.71 mg GAE/g DW).

Table 2. The triterpene, flavonoid, and polyphenol contents of *C. newmanii*

Phytochemical contents	Leaf	Flower	Rhizome
TTC (mg OAE/g DW)	3.08 ± 0.30	0.39 ± 0.30	18.57 ± 0.34
TFC (mg QE/g DW)	28.78 ± 0.23	16.85 ± 0.26	44.84 ± 0.60
TPC (mg GAE/g DW)	5.48 ± 0.70	3.71 ± 0.10	8.38 ± 0.40

Note: TTC: total triterpene content, TFC: total flavonoid content, TPC: total polyphenol content.

4. DISCUSSION

The anatomical traits of some *Curcuma* species have been also provided by prior studies. Overall, micro-morphological features of *C. newmanii* are relative to other *Curcuma* species in having: open 'U' shaped petiole; vascular bundles distributed close collateral alternating with air cavities in the leaf, petiole, and leaf sheath; thick layer of cortical parenchyma in root etc. However, the studied species can be distinguished from *C. singularis* in having: (1) the cortical parenchyma of *C. newmanii* root mostly consists of polygonal or nearly round cells while the cortical parenchyma of *C. singularis* root usually contained elongated oval cells in a radial direction, the metaxylem vessel of *C. singularis* root has thick walls that are almost always impregnated with lignin while the metaxylem vessel of *C. newmanii* root has walls that are usually made of cellulose; (2) in the *C. newmanii* rhizome, the vascular bundles in the cortical region are often larger than that in the stele, the direction of differentiation of phloem and xylem is unclear whereas the vascular bundles in the cortical region and stele of *C. singularis* rhizome are quite uniform in size with the phloem overlapping the xylem, the xylem is centrifugally differentiated, this can be explained by the fact that the *C. singularis* rhizome has less branching, so the structure and differentiation of the conductive bundles are clear; (3) the *C. singularis* leaf blade has many protective hairs on the lower epidermis while the *C. newmanii* leaf blade has very few or almost no protective hairs [16].

Anu and Dan (2020) [17] provided the anatomical traits of the petiole of 12 different *Curcuma* species. Accordingly, the outline shape of these plants were divided into 5 groups, including horseshoeshaped (*C. longa*), V shaped (*C. oligantha* and *C. aurantiaca*), open V shaped (*C. aromatica*, *C. zedoaria* and *C. vamana*), U shaped (*C. haritha* and *C. caesia*), and open U shaped (*C. pseudomontana*, *C. aeruginosa*, *C. zanthorrhiza*, and *C. amada*). According to Figure 6A, the outline shape of the micro-morphological features of the *C. newmanii* petiole has open U shaped like that of *C. pseudomontana*, *C. aeruginosa*, *C. zanthorrhiza*, and *C. amada* [17]. In addition, the shape of epidermal cells of the *C. newmanii* petiole are rectangular which are similar to that of *C. aeruginosa*, *C. amada*, *C. aurantiaca*, *C. haritha*, *C. longa*, and *C. vamana* [17].

The phytochemical screening of the *Curcuma* species have been reported by prior studies. For instance, Akter *et al.* provided the total phenolic and flavonoid contents as well as antioxidant effects of the methanol extracts isolated from the rhizomes of six *Curcuma* samples from Japan, including *C. longa* (collected from 2 regions, Ryudai Gold and Okinawa), *C. xanthorrhiza*, *C. aromatica*, *C. amada*, and *C. zedoaria*. Accordingly, the total phenolic content from these samples were 154.4, 59.2, 38.5, 37.9, 48.7, and 43.7 mg GAE/g, respectively while 797.5, 310.7, 89.3, and 15.3 GAE/g, respectively were the total flavonoid content towards the same species [18]. Rajamma *et al.* showed that the total phenol content isolated from the rhizome dichloromethane extracts of 7 *Curcuma* species such as *C. aeruginosa*, *C. amada*, *C. aromatica*, *C. broga*, *C. caesia*, *C. malabarica*, and *C. rakthakanta* collected from Kerala, India, were 34.0, 23.0, 69.0, 40.0, 63.0, 46.0, and 46.0 mg GAE/g [19]. Similarly, the total phenol contents of the various solvent

extracts obtained from the fresh and dried rhizome of *C. caesia* grown in India were also investigated. Accordingly, the hexane, petroleum ether, benzene, chloroform, ethyl acetate, methanol, and water extracts isolated from fresh and dried rhizome were found to contain the total phenol contents of 50.44 and 53.44, 38.42 and 26.43, 56.64 and 96.68, 57.53 and 109.41, 45.48 and 86.29, 32.58 and 28.33, 34.39 and 48.49 mg GAE/g [20].

The qualitative phytochemicals of the distilled water and methanol extract from the rhizome of *C. aromatica* and *C. xanthorrhiza* grown in Kerala, India showed that the distilled water extracts of these plants contained flavonoid, tannin, saponin, carbohydrate, terpenoids, sterols, protein, and phenols. The methanol extract from *C. aromatica* consisted of 5 later compounds while the same components and flavonoid were presented in the *C. xanthorrhiza* methanol extract. Also, the quantitative identification of curcumin provided that *C. aromatica* and *C. xanthorrhiza* contained curcumin with the contents of 0.0175 g/100 g and 1.0863 g/100 g [21]. The ethanol extract of *C. zedoaria* rhizomes grown in Savar, Bangladesh, contained some phytochemical components, including steroids, carbohydrates, terpinoids, alkaloids, saponins, flavonoids, and tannins [22]. Joshi *et al.* provided the qualitative phytochemicals of the ethanol and methanol extracts from *C. longa* and *C. aromatica* rhizome collected from India. The results showed that both extracts from these plants contained flavonoid and alkaloid while tannin was found in the *C. longa* ethanol and methanol extracts [23].

The phytochemical screening of the various extracts, including water, ethanol, methanol, acetone, ethyl acetate, chloroform, and petroleum ether of the *C. caesia* rhizome collected from Raipur, India have been also investigated. Experiments were carried out in a total of 13 compounds, including alkaloids, cardiac glycosides, carbohydrates, flavonoids, phenols, phlobatannins, proteins, saponins, sterols, tannins, terpenoids, quinones, and oxalates. The results showed that the methanol is the best solvent which contained 11 out of 13 compounds (except phlobatannins and oxalates) [24]. In addition, the ethanol extracts isolated from *C. xanthorrhiza* and *C. domestica* grown in Indonesia have been also reported of which saponin, steroid, alkaloid, and flavonoid were found in the extract of the first plant while the later species contained steroid and flavonoid [25]. The different extracts of *C. sahuynhensis* rhizomes and inflorescences collected from Quang Ngai province, Vietnam, were also investigated using 13 tests for the phytochemical screening, including fats, carbohydrates, essential oil, carotenoids, alkaloids, amino acids, cardiac glycosides, coumarins, flavonoids, saponins, tannins, triterpenoid, and polyuronides. Accordingly, the ether and ethanol extracts of the rhizome comprised 7 out of 13 compounds, the water extract of this organ consisted of 3 out of 13 constituents. Meanwhile, the water, ether, and ethanol extracts of inflorescences contained 3, 4, 5, respectively out of 13 components [26].

5. CONCLUSION

The present study firstly provided the details of micro-morphological features of the different organs of *C. newmanii*, including the petiole, root, root tuber, leaf, leaf sheath, and rhizome. In addition, the ethanolic extracts obtained from leaf, flower, and rhizome of *C. newmanii* consisted of various bioactive components, including coumarin, terpenoid, steroid, flavonoid, saponin, alkaloid, phenolic, and tannin. Also, the studied plant consisted of the significant amounts of triterpene, flavonoid, and polyphenol contents. Like many valuable species of the genus *Curcuma*, the results from this study are expected to be a scientific basis for the application of this species in the pharmaceutical field in the future.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors have reported no conflicts of interest

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