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RESEARCH ARTICLE

Anatomical characteristics and phytochemical screening of *Homalomena perplexa*: An endemic species from Con Dao National Park, Vietnam

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Abstract

Homalomena perplexa has been recently described as a new species in the flora of Vietnam. In this study, the micro-morphological features of the different organs of *H. perplexa*, including the root, leaf and rhizome were provided for the first time using the iodine green-carmine double staining method. Moreover, the phytochemical screening of *H. perplexa* was also conducted for the first time. Accordingly, the leaf, petiole and rhizome of the studied plant were found to contain various compounds, including coumarin, steroid, terpenoid, saponin, flavonoid, alkaloid, tannin, tannin and phenolic. In addition, the total triterpene and polyphenol contents in the rhizome extract were the highest (44.82 mg OAE/g DW and 15.51 mg GAE/g DW), followed by the petiole extract (14.02 mg OAE/g DW and 11.31 mg GAE/g DW) and the leaf extract (1.44 mg OAE/g DW and 10.97 mg GAE/g DW). Furthermore, the leaf extract possessed the highest quantitative flavonoid content (77.04 mg QE/g DW), followed by the petiole and rhizome extracts with the contents of 39.68 and 35.76 mg QE/g DW, respectively.

Keywords

anatomy; Con Dao National Park; *Homalomena*; phytochemical screening

Introduction

Homalomena schott, one of the large genera belonging to family Araceae, comprises approximately 250 species distributed in Malesia, Solomon Islands, India, Southern China and Southeast Asia (1-4). Many species within this genus are traditionally used in medicine. For instance, the extracts from *H. aromatica* rhizome and leaves were widely used to cure kidney problems, stomach pain and diarrhoea, joint-pains, asthma, skin infections, common cold in infants and jaundice stomach (5, 6). The extract from *H. aromatica* rhizome has been used to treat stomach and rheumatoid arthritis (7). In addition, *Homalomena* plants

have been considered a rich source of essential oils which found to be rich in the different chemical groups such as monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes and oxygenated sesquiterpenes (8). A large number of *Homalomena* plants have been reported possessed various biological activities, including antibacterial, antioxidant, nematicidal and insecticidal properties (8, 9).

In the book "Cay Co Viet Nam" published in 2000, Professor Pham Hoang Ho recorded six *Homalomena* species for the flora of Vietnam, including *H. occulta, H. tonkinensis, H. cochinchinensis, H. gigantea, H. pierreana* and *H. tonkinensis* (10). Recently, two new species belonging to this genus, *H. vietnamensis* (11) and *H. perplexa* (12), were described for the flora of Vietnam bringing the total number of *Homalomena* species recorded in Vietnam to eight. Also, studies demonstrated that *Homalomena* is a genus in the Araceae family with high variety of morphological characteristics. The annual flowering cycle of the plants within this genus is short while their vegetative organs usually have morphologically similar traits, leading to difficulty in distinguishing species (12–15). Notably, the micro-morphological features are considered an effective method to classify and standardize the medicinal plants (14). Therefore, it is necessary to study on the anatomical characteristics to provide taxonomic information as well as the chemical components of newly discovered species in the genus *Homalomena*.

In 2023, *Homalomena perplexa* K.Z.Hein, Vuong, Bao & V.S.Dang was first described for the flora of Vietnam. The type specimens of this species were collected from Con Dao National Park, Ba Ria-Vung Tau province, Vietnam (12). To date, *H. perplexa* is a rare species and it has been only discovered in the type location so far (12). The present study, thus, investigated the anatomical characteristics and phytochemical screening of *H. perplexa* for the first time.

Materials and Methods

Plant materials

The samples of *H. perplexa* were collected from Con Dao National Park, Ba Ria-Vung Tau province, Vietnam in May 2024. The voucher specimen (Van H.T 86B) was deposited in the herbarium of the Institute of Tropical Biology (VMN), Vietnam Academy of Science and Technology, Vietnam. The species grows under tropical semi-evergreen forest on the humus soils. The flowering time of this species is from May to September.

Morphological characteristics

The studied specimen was collected and identified according to the guidelines of the Royal Botanic Gardens, Kew (16). The vegetative and reproductive traits of *H. perplexa* were compared with those of prior publications on *Homalomena* genus (10-13, 15).

Anatomical characteristics

The root, rhizomes and leaf of *H. perplexa* were sliced transversely into thin sections. The samples were bleached with sodium hypochlorite solution (Javel water) and then stained using the iodine green-carmine double staining technique. After multiple washing with water, the slices were preserved in a 10% glycerol solution (17). The samples were examined using an Olympus BX53 Digital Upright Microscope.

Extraction procedures

The *H. perplexa* leaf, petiole and rhizome were dried at 50°C and then ground into a powder. Five grams of each specimen was soaked in 99% ethanol absolute (1:30, w/w) for 8 hr. The Whatman paper was used to filter the supernatant and then the residue was soaked and filtered more two times to collect all supernatant fractions.

Qualitative phytochemistry of the extract

The detection of biologically active compounds was carried out using the following methods: Coumarin: 2 mL of extract was mixed with 3 mL of 10% NaOH, resulting in a yellow color indication (+) (18); terpenoids and steroids: 5 mL of extract was combined with 2 mL of chloroform and 3 mL of concentrated H_2SO_4, which produced a reddish brown color (+) (19); saponin: 2 mL of extract was added to 10 mL of distilled water and boiled for 2 min, forming foam (+) (20); flavonoids: 2 mL of extract was reacted with 2 mL of 10% $Pb(COOH)_2$, resulting in a yellow precipitate (+) (21); alkaloids: 2 mL of extract was treated with 3-4 drops of Wagner's reagent, leading to a red-brown precipitate (+) (22); phenolic and tannin: 2 mL of extract was mixed with 2 mL of water and 2-3 drops of 5% $FeCl_3$, indicated by a blue-black or brown-green precipitate (+) (23).

Quantitative phytochemistry of the studied extract

Total polyphenol content (TPC): 0.1 mL of the extract was added to a tube along with 1.8 mL of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 5 min. Following this, 1.2 mL of a 15% Na_2CO_3 solution was added to achieve a pH of 8 and the volume was topped up with distilled water to a total of 10 mL. The tube was covered, shaken and incubated in the dark for 90 min. After incubation, the absorbance was measured at a wavelength of λ=734 nm (24). The total phenolic content (TPC) was determined using the following formula:

$$\text{TPC (mg GAE/g DW)} = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100 - W)} \times K \quad \text{(Eqn. 1)}$$

Where,

Cx: total concentration of polyphenols in the extract, determined using the standard curve (ppm); V: volume of the sample (mL); a: initial mass of the sample (g); W: percentage of humidity (%); K: dilution factor; and 10^3: conversion factor.

Total triterpene content (TTC): In a test tube, 1 mL of the extract, 0.2 mL of 5% acetic acid and 1.2 mL of $HClO_4$ were mixed thoroughly and incubated the mixture at 70°C for 15 min. Immediately after, it was cooled for 2 min. Then, the solution was diluted to a final volume of 5 mL with ethyl acetate. The absorbance was measured at a wavelength of 550 nm (25). The TTC value was determined using the following formula:

$$\text{TTC (mg OAE/g DW)} = C_x \times \frac{V}{10^3} \times \frac{100}{a \times (100 - W)} \times K \quad \text{(Eqn. 2)}$$

Where,

Cx: total concentration of triterpenoids in the extract, determined using the standard curve (ppm); V: volume of the sample (mL); a: initial mass of the sample (g); W: humidity (%); K: dilution factor; and 10^3: conversion factor.

Total flavonoid content (TFC): 1 mL of the extract was placed into a test tube and 0.3 mL of a 5% $NaNO_2$ solution was added. The mixture was thoroughly mixed and allowed to stand for 5 min. Afterward, 0.3 mL of a 10% $AlCl_3$ solution was added to the test tube. The mixture was again mixed thoroughly and left to stand for another 5 min. Subsequently, 2 mL of 1M NaOH was added to the reaction mixture, which was then mixed well. The solution was diluted with distilled water to achieve a final volume of 10 mL. The absorbance of the resulting solution was measured at a wavelength of 510 nm using distilled water as a control in place of the extract solution (26). The total flavonoid content was reported as grams of quercetin equivalent (QE).

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

Where,

Cx: total flavonoid concentration in the extract, determined using the standard curve (ppm); V: volume of the sample (mL); a: initial mass of the sample (g); W: humidity (%); K: dilution factor; and 10^3: conversion factor.

All experiments were repeated three times to ensure the reliability of the data. The standard deviation (± SD) of the data has also been calculated and specified in the analysis results.

Results

Homalomena perplexa K.Z.Hein, Vuong, Bao & V.S.Dang

Evergreen herb, rhizomes 15-20 cm in length, 3-6 cm in width, bearing many roots (Fig. 1). Leaves 10-15. Petiole 30-60 cm long, about 1-2 cm wide at base, reddish-brown, 0.4-0.6 cm at apex, grey-green. Leaf blade 30-40 cm long, 25-30 cm wide, light green on the under side, darker on the upper side, cordato-sagittate or triangular-sagittate, midrib prominent abaxially and impressed adaxially, lateral veins diverging from the midrib and toward the margin. Inflorescence many, peduncle much shorter than petiole, 9-12 cm long, ca. 3 mm wide, pale green. Spathe longer than spadix, 4.5-5.5 cm long, 0.4-0.8 cm wide, green, no distinction between blade and tube, elliptical, crescent-shaped or boat-shaped. The spadix short than spathe, 4-5 cm long, almost sessile; the female part ca. 1.5 cm long, 0.5-0.8 cm in diameter, cylindrical, loosely arranged. Ovary short bottle-shaped, pale yellow to pale green, ca. 1.5 mm high, ca. 1 mm in diameter, 3-locular, ovules numerous; stigma discoid, convex, ca. 1 mm in diameter, pale yellow; style almost absent; staminode slender, subcylindric to slightly clavate, white, interspersed between the ovaries. Male part 2.5-3.5 cm long, ca. 4 mm in diameter, cylindrical, rounded apex, opaque white, densely arranged, anthers opening by lateral slits near apex. Flowering: May-July.

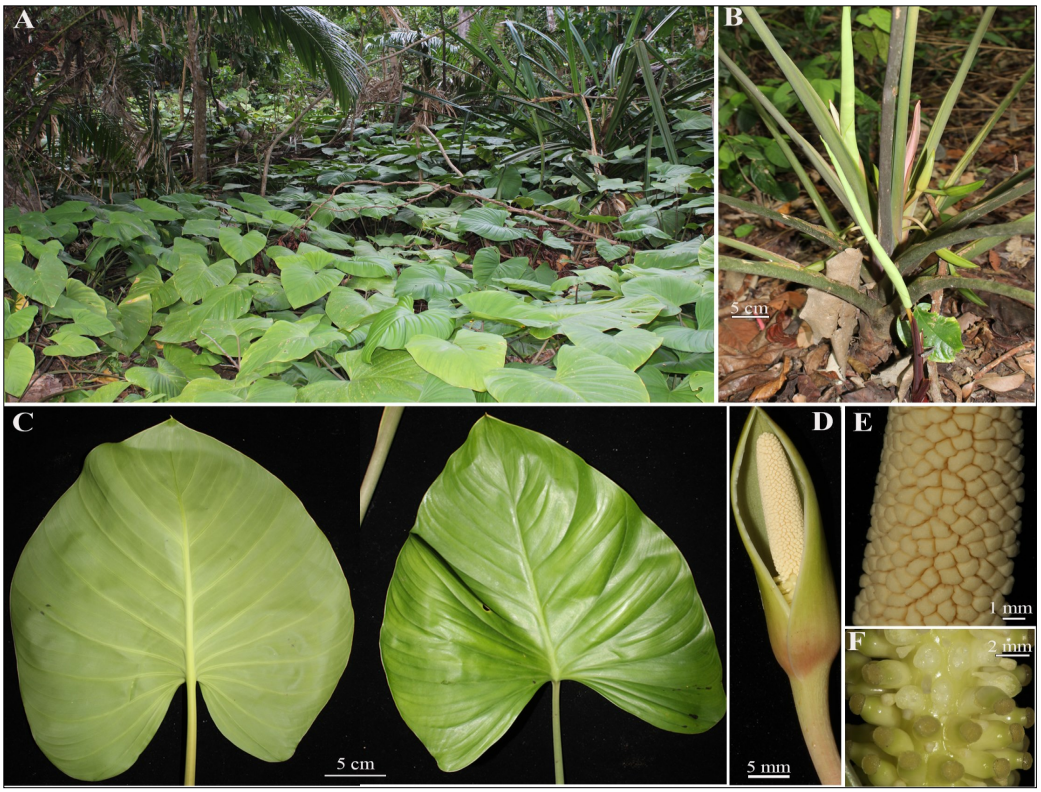

Fig. 1. Morphological features of *H. perplexa*. A. Species in its habitat, B. Inflorescence, C. Leaf blade, D. Spathe and spadix, E. Stamens, F. Ovary and stigma.

Type: BV 1691 (isotype: VNM 00043082!; holotype: VNM 00043081!), Con Dao National Park, Ba Ria-Vung Tau province, Vietnam; 10 May 2022.

Studied specimens: Van H.T 86B (VMN), Con Dao National Park, Ba Ria-Vung Tau province, Vietnam, 04 May 2024; Van H.T 86A (SGN), Con Dao National Park, Ba Ria-Vung Tau province, Vietnam, 17 May 2015.

Distribution: *H. perplexa* has only been found in Con Dao National Park, Ba Ria-Vung Tau province, Vietnam so far.

Habitat: The species grows on litter, under the canopy of moist evergreen forest, about 150-200 m above sea level.

Anatomical characteristics

***Roots*:** The cross-section of root is nearly circular and divided into 2 distinct areas, ¾ of the radius of cross-section is the cortical area whereas a pith area accounts for ¼ of the radius (Fig. 2). *Cortex* piliferous layer consists of a layer of polygonal, irregular cells, distorted walls impregnated with phellem, scattered with root hairs. The exodermis includes 3-5 layers of polygonal cells, walls impregnated with thin phellem or cellulose, haphazardly arranged and closely together. The cortical parenchyma has cellulose walls, divided into 2 regions: the outer cortical parenchyma has many layers of round or polygonal cells, haphazardly arranged, leaving small intercellular spaces; the inner cortical parenchyma has 5-6 layers of nearly round polygonal cells, arranged in radial rows and concentric rings. The schizogenous cavities are abundant in the cortical parenchyma with a border consisting of a ring of 6-8 irregular polygonal cells. The endodermis with casparian strip includes an almost rectangular layer of cells, usually divided into segments of 2-4 cells, the segment at the top of the xylem bundle are cells with cellulose walls, the segment at the top of the phloem bundle are cells impregnated with xylem. *Stele*: the pericycle consists of a layer of flattened polygonal cells, interspersed with endodermic cells. The vascular bundles are concentrated right under the pericycle layer, including 23-24 phloem bundles and 23-24 protoxylem bundles arranged alternately on a ring. Phloem bundles are small oval clusters, including polygonal cells, cellulose walls, radial differentiation. The protoxylem bundle consists of 3-6 vessels, polygonal shape, walls impregnated with xylem, radially differentiated. The Metaxylem consists of 28-30 vessels, larger than the protoxylem vessels, irregular size. The medullary ray includes 1-2 rows of flattened polygonal cells in a radial direction between the phloem and xylem bundles, cellulose wall. The sclerenchymatous conjunctive tissues are polygonal cells, arranged closely together.

Rhizomes: The cross-section of rhizome is nearly circular and divided into 2 regions, the thin cortical area and the thick stele area (Fig. 3). Cortical region: the phellem consists of many layers of flattened rectangular cells, arranged in radial rows. Phelloderm includes 1-2 layers of rectangular cells, cellulose wall. The parenchyma has many layers of nearly round cells, cellulose walls, randomly arranged with the polygonal or triangular intercellular space. Stele region: the medullary parenchyma is similar to the cortical parenchyma, but the cells often contain many starch granules. There are many vascular bundles and are scattered throughout the medullary parenchyma; they can be individual bundles with phloem above and xylem below, but are usually arranged in clusters of 2 or many bundles; each cluster has a phloem in the middle, the xylem vessels can be arranged in an arc, a ring, on opposite sides or in three clusters. The calcium oxalate crystals divided into 2 forms, the first form includes spherical crystals scattered throughout the parenchyma area while the second one is the needle-shaped crystals stuck in bundles in elongated cells. The schizogenous cavities with large diameters scattere are scattered in the parenchyma; the edge of the cavities has 3-4 layers of very flat cells stacked on top of each other in a radial direction; the cells in the inner layer often have no clear shape and surrounding by the schizogenous cavities with 3-4 layers of polygonal parenchyma cells alternately arranged and closely together.

Leaves: The midrib is slightly concave on the upper surface, convex on the lower surface (Fig. 4). The epidermal cells have a thin layer of cuticle; stomata are scattered throughout both epidermal layers. Thick tissue under the upper epidermis arranged in clusters, 1-2 layers of polygonal cells, cellulose wall. There are 2 forms of parenchyma; the first region is close to collenchyma which contains 3-5 layers of nearly round cells, small size, loosely arranged, with few chloroplasts while the remaining area is spongy parenchyma, usually a series of cells connected together to create many irregular large and small intercellular space; these intercellular spaces gradually

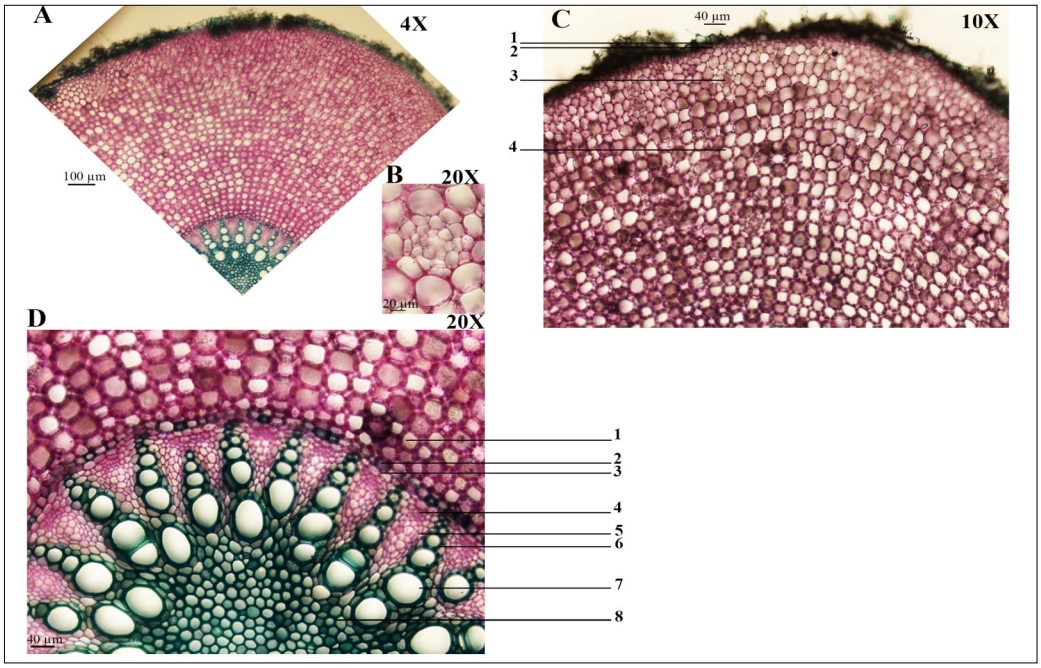

Fig. 2. Cross-section of the root: A. Whole cross-section, B. Schizogenous cavity, C. Cortex region (1: piliferous layer, 2: exodermis layer, 3: schizogenous cavity, 4: cortical parenchyma), D. Stele region (1: medullary parenchyma, 2: endodermis with casparian strip, 3: pericycle, 4: phloem, 5: medullary ray, 6: protoxylem, 7: metaxylem, 8: sclerenchymatous conjunctive tissues).

become smaller and disappear when approaching the lower epidermis. The vascular bundle has irregular size and is scattered in the parenchyma. Each bundle includes xylem above and phloem below. Above the phloem and below the xylem are sometimes clusters of hard tissue cells. These clusters consist of 2-4 layers of polygonal cells. The xylem has thin, impregnated walls, which are close together. The xylem includes 3-7 irregular vessels. The phloem has many large-sized sieve tubes. The lower collenchyma contains 6-7 layers of cells with clearly thickened walls at the corners, forming a continuous arc, scattered with small schizogenous cavity. There are 2 forms of the calcium oxalate crystals such as (1) spiny spheres scattered throughout the parenchyma and (2) needle shaped bundles located in large, oval cells. The schizogenous cavities are usually found in the concavity of the lower collenchyma arch, schizogenous cavity with a border of an irregular ring of 6-8 cells.

Qualitative phytochemistry of H. perplexa

The Table 1 shows the preliminary phytochemistry of the ethanolic extracts of *H. perplexa*. Accordingly, the leaf, petiole and rhizome of the studied plant contain different compounds, including coumarin, steroid, terpenoid, saponin, flavonoid, alkaloid, tannin and phenolic compounds.

Table 1. Preliminary phytochemical screening of *H. perplexa* extracts

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

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

Quantitative phytochemistry of H. perplexa extract

The total contents of triterpene, polyphenol and flavonoid compounds in the ethanolic extracts from *H. perplexa* were presented in the Table 2. As a result, the total triterpene and polyphenol contents in the rhizome extract had the highest contents *(*44.82 mg OAE/g DW and 15.51 mg GAE/g DW), followed by the petiole extract (14.02 OAE/g DW and mg 11.31 mg GAE/g DW) and the leaf extract (1.44 mg OAE/g DW and 10.97 mg GAE/g DW). Furthermore, the leaf extract possessed the most quantitative flavonoid content (77.04 mg QE/g DW), followed by the petiole and rhizome extracts with the contents of 39.68 and 35.76 mg QE/g DW, respectively.

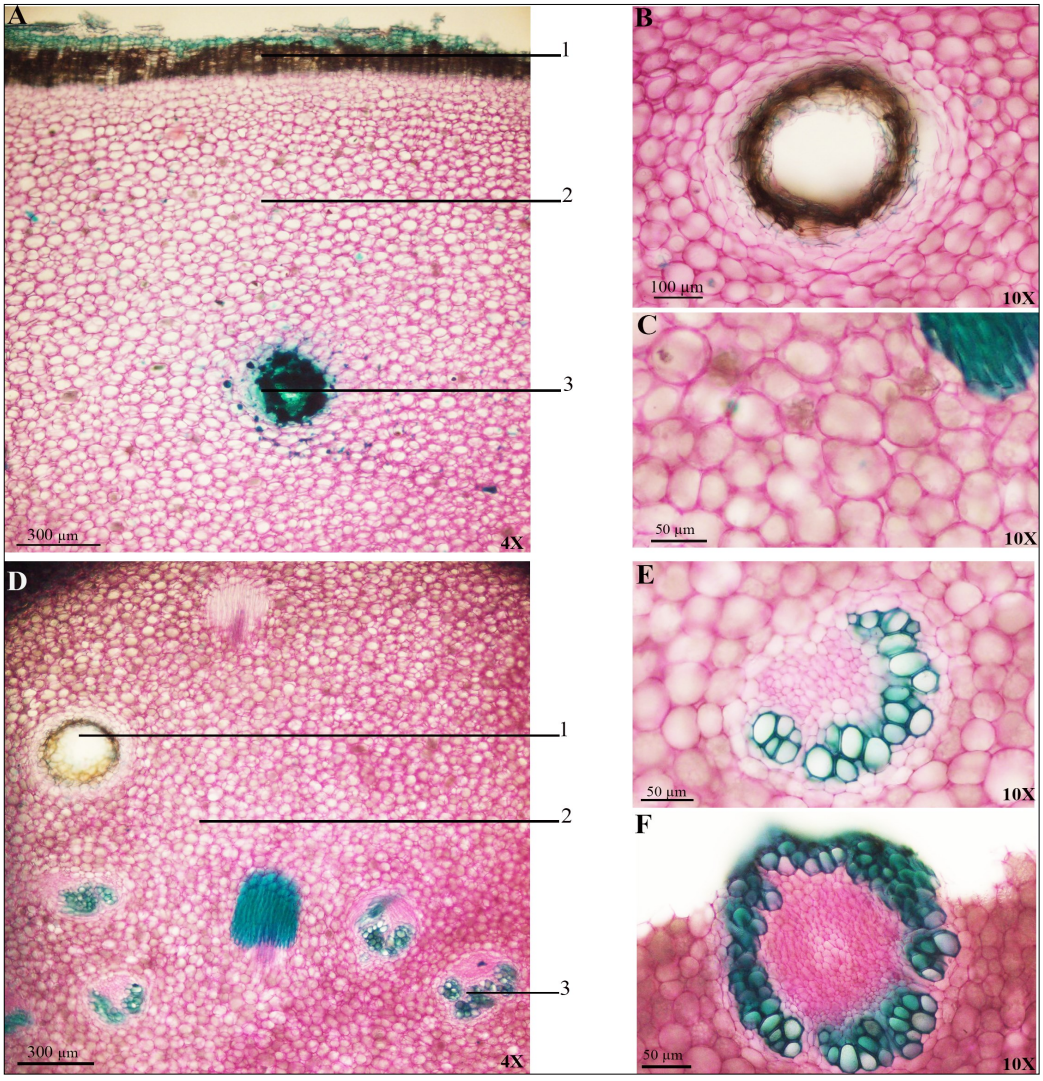

Fig. 3. Cross-section of the rhizome: A. Cortical region (1: phellem, 2: parenchyma, 3: schizogenous cavity), B. Schizogenous cavity, C. Spherical calcium oxalate crystals, D. Stele region (1: schizogenous cavity, 2: parenchyma, 3: vascular bundle), E & F. Vascular bundle.

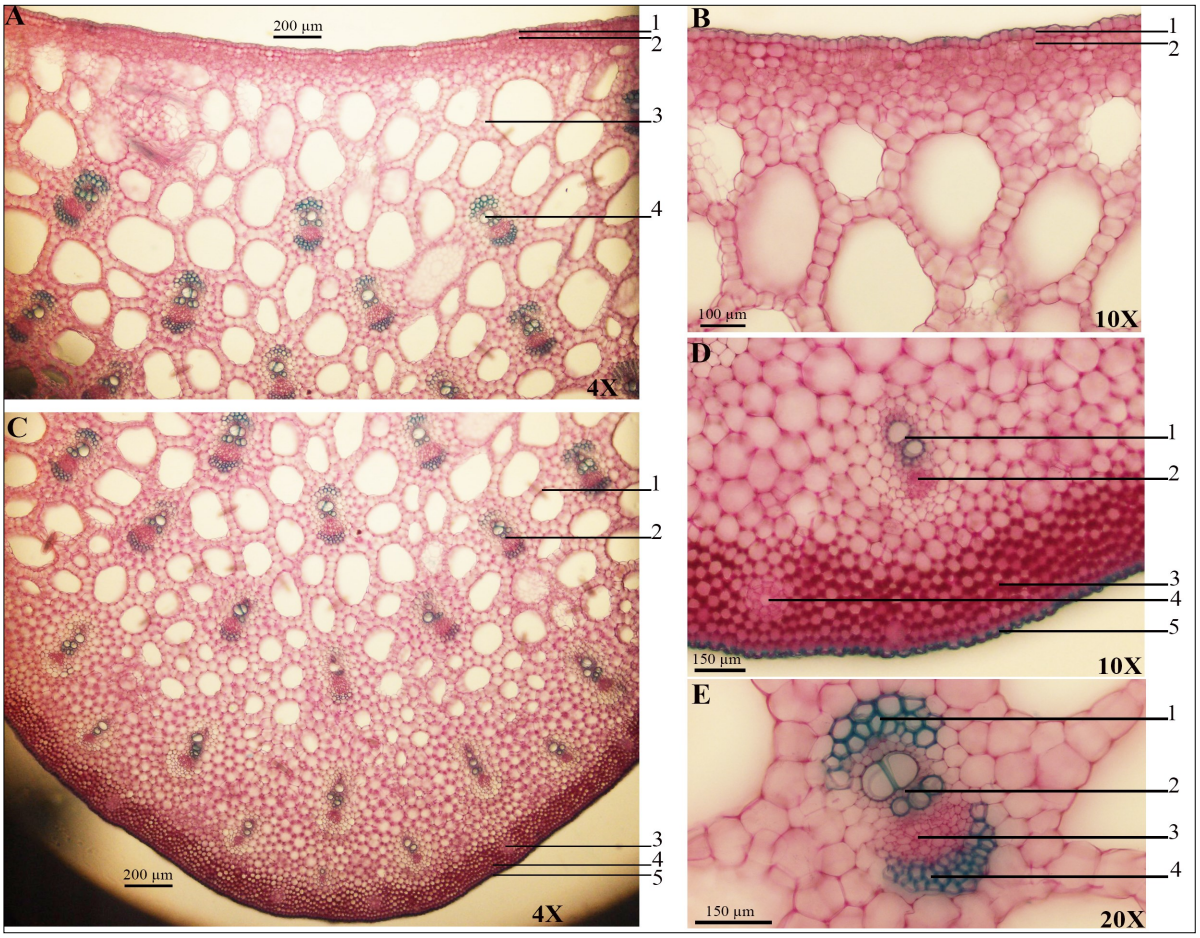

Fig. 4. Cross-section of the leaves: A. Upper surface (1: upper epidermis, 2: collenchyma, 3: parenchyma, 4: vascular bundle), B. Enlarged image of the upper surface (1: upper epidermis, 2: collenchyma), C. Lower surface (1: needle-shaped calcium oxalate crystals, 2: vascular bundle, 3: schizogenous cavity, 4: collenchyma, 5: lower epidermis), D. Enlarged image of the lower surface (1: xylem, 2: phloem, 3: collenchyma, 4: schizogenous cavity, 5: lower epidermis), E. Vascular bundle (1: sclerenchyma, 2: xylem, 3: phloem, 4: sclerenchyma)

Table 2. Total triterpene, polyphenol and flavonoid contents from *H. perplexa* extracts

	TTC (mg OAE/g DW)	TFC (mg QE/g DW)	TPC (mg GAE/g DW)
Leaf	1.44 ± 0.02	77.04 ± 0.07	10.97 ± 0.03
Petiole	14.02 ± 0.05	39.68 ± 0.03	11.31 ± 0.04
Rhizome	44.82 ± 0.01	35.76 ± 0.01	15.51 ± 0.04

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

Discussion

In Vietnam, there have been many taxonomic confusions on the taxonomic status of the genus *Homalomena* although few species have been recorded. For instance, *H. occulta* was the first species to be described for the flora of Vietnam in 1832 (27). In 1912, four new species belonging to the genus were described for the flora of Vietnam such as *H. tonkinensis* Engl., *H. philippinensis* Engl., *H. cochinchinensis* Engl. and *H. pierreana* Engl. as well as *H. occulta* was classified as a synonym of *H. aromatica* (Spreng.) Schott (28). In 1942, after removing *H. philippinensis* from the list of *Homalomena* species in Vietnam and adding *H. gigantea* Engl., Gagnepain recorded five species for Vietnam, including *H. tonkinensis* Engl., *H. pierreana* Engl., *H. cochinchinensis* Engl., *H. aromatica* (Spreng.) Schott and *H. gigantea* Engl. (15). In the book "Cay Co Viet Nam" published in 2000, Professor Pham Hoang Ho recorded 6 species of *Homalomena* for the flora of Vietnam, including *H. occulta, H. tonkinensis, H. cochinchinensis, H. gigantea, H. pierreana* and *H. tonkinensis* (10). In 2017, Nguyen synonymized *H. gigantea* as *H. pendula*; *H. aromatica* and *H. cochinchinensis* as *H. occulta* as well as added one new species, *H. vietnamensis* (11), to revise five species for Vietnam such as *H. occulta, H. tonkinensis, H. pendula, H. pierreana* and *H. vietnamensis* (13). In our view, the number of *Homalomena* species in Vietnam according to statistics of Pham (10) is a fuller information and thus, combined with the two recently described species, *H. vietnamemsis* (11) and *H. perplexa* (12), the total numbers of *Homalomena* species in Vietnam during this period is eight.

The researchers provided the micro-morphological features of *Homalomena pendula* (9). Overall, the anatomical characteristics of *H. perplexa* are similar to those of *H. pendula* in having, the transverse section of leaf and root have many secretory ducts in the parenchyma; there are two type of calcium oxalate crystals, spiny spheres and long needles, which are agglutinated into bundles within large oval-shaped cells. Additionally, both species have numerous air cavities of varying sizes in the transverse section of their leaves. However, the anatomical characteristics of *H. pendula* can be distinguished from those of *H. perplexa* by the presence of the unicellular protective hairs and secretory hairs in the epidermal cells of the transverse section of leaves (absent in *H. perplexa*) (9).

Phytochemical screening has been conducted on other *Homalomena* species in prior studies. For instance, the

water and methanol extracts obtained from rhizome and petiole/leaf of *H. aromatica* collected from Assam, India were analyzed using various phytochemiscal tests. Accordingly, the rhizome methanol extract was found to contain in alkaloid, tannin, saponin, cardiac glycoside, phenolic, coumarin, phlobatannin and terpenoid while the water rhizome extract contained alkaloid, tannin, saponin, flavonoid, phenolic, quinone, fixed oil, coumarin, gum and mucilage and terpenoid. Furthermore, the methanol leaf/petiole extract contained alkaloid, tannin, saponin, cardiac glycoside, phenolic, fixed oil, coumarin whereas the water leaf/petiole extract was characterized by the presence of alkaloid, tannin, saponin, flavonoid, phenolic, fixed oil, coumarin, gum and mucilage and terpenoid (29).

Similarly, the methanol extract obtained from the *H. aromatica* rhizome grown in Kamrup, Assam India also contained carbohydrate, alkaloid, saponin, glycoside, flavonoid and fixed oil and fat (30). In addition, few researchers the total phenolic of the water extract from the *H. occulta* grown in China with the content of 1.14 mg GAE/g (31). The ethanol extract obtained from the aerial parts of *H. occulta* were collected in Thua Thien Hue province, Vietnam contained of the total phenolic, flavonoid and triterpenoid compounds with the contents of 36.87 mg GAE/g, 26.83 mg QUE/g and 52.09 mg oleanolic acid/g, respectively (9).

Conclusion

This study is the first to investigate the micro-morphological features and the phytochemical screening of *H. perplexa*. The identified micro-morphological traits are valuable for classifying and standardizing this species as a potential medicinal plant. In addition, the phytochemical screening suggests promise for further application of this species in pharmaceutical field. The outcome of this study hold promise for using as a monographic literature in the classification and standardization of the medicinal plants as well as further application of this species in pharmaceutical field.

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Authors' contributions

DHT and HTV drafted the manuscript, participated in the design of the study and performed the experiments and statistical analysis. NNT, NTN, VSD, HTL, TTTT and NNP performed experiments and handled the research data. HTV drafted the manuscript and resolved all the queries 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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