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RESEARCH ARTICLE

Anatomical traits and phytochemical screening of *Amorphophallus synandrifer*, an endemic species from Vietnam

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Abstract

Amorphophallus synandrifer is a rare and native species to Vietnam. The present study is to provide the micro-morphological characteristics and the phytochemical screening of this species. As a result, the micro-morphological features from the different organs of *A. synandrifer* are firstly provided in which they are like those of *Amorphophallus scutatus* and *A. curvistylis*. The preliminary phytochemistry of the ethanol extracts isolated from the leaf blade, petiole, spathe/spadix and penduncle of *A. synandrifer* were found to be rich in phenolic, tannin, alkaloid, flavonoid, saponin, terpenoid, steroid and coumarin. Furthermore, the leaf blade extract showed the highest quantitative concentration with the total triterpene, flavonoid and polyphenol contents of 8.08 mg OAE/g DW, 33.39 mg QE/g DW and 71.48 mgGAE/g DW, respectively, followed by the spathe/spadix, peduncle and petiole extracts.

Keywords: *Amorphophallus synandrifer*, Araceae, micro-morphological traits, preliminary phytochemistry

Introduction

Amorphophallus Blume ex Decne. is one of the largest genera of the family Araceae which over 250 species widely distributed in over the world, especially in tropical and subtropical regions. About 30 species belonging to this genus have been recorded for the flora of Vietnam so far (1). Some *Amorphophallus* species have been known as the economically valuable plants and have been used in the production of food products as well as for medicinal applications (2). Some edible plants belonging to the genus *Amorphophallus*, including *A. campanulatus, A. krausei, A. nanus, A. yuloensis, A. paeoniifolius, A. yunnanensi, A. konjac, A. mulleri, A. onchophyllus* and *A. riveiri* are classified as perennial crop in several Asian countries (3). The tubers are a rich source of starch and protein and starch applications (4). Notably, gluconmannan, a water-soluble polysaccharide, isolated from the tuber of *A. konjac* has been reported to possess many important health benefits, including improving blood sugar levels (5), wound dressing (6), reducing cholesterol (7), promoting intestinal activity (8), normalizing triglyceride concentration in blood (9) and immune function (10).

A. synandrifer Hett. & V D Nguyen was firstly described as a new species for the flora of Vietnam by Hetterscheid and Van Der Ham in 2001 of which the type specimen was collected from Vinh Hao Community, Ca Na District, Ninh Thuan Province, Vietnam (11). To date, it is a rare species and the micro-morphology and chemical profiles have been unknown. Therefore, this study firstly provided the phytochemical screening and anatomical traits of *A. synandrifer* .

Materials and Methods

Plant materials

The specimens of *A. synandrifer* were collected from Binh Chau-Phuoc Buu Nature Reserve, Xuyen Moc District, Ho Chi Minh City, Vietnam (Fig. 1).

Anatomical characteristics

The thin slices of the leaf blade, petiole and petiolule of *A. synandrifer* were cut using the razor blade. These samples were soaked into the javel solution to remove the unwanted constituents in plant tissue. They were stained by the iodine green-carmine double staining method. The distilled water was used to wash these samples and the glycerol solution (10 %) was used to preserve them (12).

Extraction procedures

Five grams of the dried powder of the leaf blade, petiole, spathe/

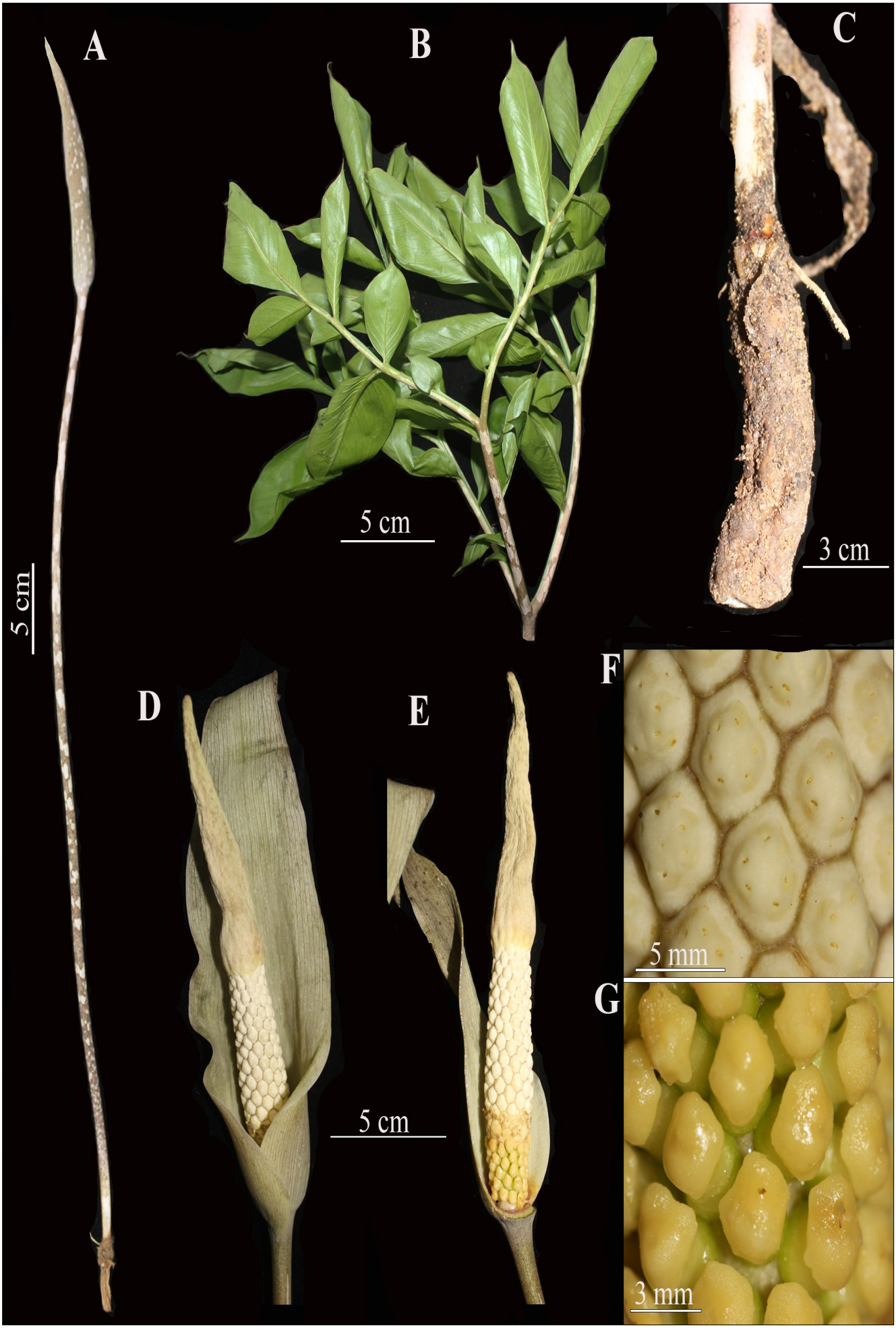

Fig. 1. *A. synandrifer* Hett. & V D Nguyen. A: inflorescence; B: leaves; C: tuber; D: spathe; E: spadix; F: male zone; G: femle zone.

spadix and penducle of *A. synandrifer* were soaked by 99 % ethanol (1:30, w/w) within 8 hrs. The Whatman paper was used to filter the first supernatant and this procedure was repeated more two times with the residue. All supernatant fractions were combined into only one extract.

Qualitative phytochemistry of *A. synandrifer*

The qualitative phytochemistry of *A. synandrifer* were identified using the methods shown in the Table 1.

Quantitative phytochemistry of *A. synandrifer*

Total polyphenol content

Add 0.1 mL of the sample into a test tube, followed by 1.8 mL of Folin-Ciocalteu solution. Mix thoroughly and leave for 5 min for Folin-Ciocalteu to react completely with polyphenols. Next, add 1.2 mL of 15 % Na_2CO_3 solution to create alkaline pH and finally add distilled water to make 10 mL. Cover the tube, shake well and incubate in the dark for 90 min. Afterward, measure the photoluminescence at a wavelength of λ =734 nm, using distilled water as the control. The total polyphenol content of the samples was calculated using a gallic acid standard curve and expressed in milligrams of gallic acid equivalent (GAE). The total concentration of polyphenols in the samples was determined based on the photometric results and the standard curve (19). The calculation for total polyphenol content is as follows:

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

Where:

C_x: total polyphenol concentration in the extract calculates 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

To determine the total flavonoid content, add 1 mL of the sample to a test tube, then introduce 0.3 mL of 5% $NaNO_2$ solution. Mix thoroughly and leave for 5 min. Next, add 0.3 mL of 10 % $AlCl_3$ solution, shake well and wait another 5 min. Following this, add 2 mL of 1M NaOH solution, mix again and then add distilled water to the total volume to 10 mL. Measure the absorbance photometrically at a wavelength of λ = 510 nm, using distilled water as the control. The total flavonoid content is expressed in milligrams of quercetin equivalent (QE). The total flavonoid concentration in the samples was calculated based on the photometric data and the standard curve (20). The formula used for calculating the total flavonoid content is:

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

Where:

C_x: the total flavonoid 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

To assess the total triterpene content, transfer 1 mL of the extracted sample into a test tube. Add 0.2 mL of 5 % acetic acid and 1.2 mL of perchloric acid ($HClO_4$), then mix and incubate at 70 °C for 15 min. Afterward, cool the mixture rapidly for 2 min. Next, add 2.6 mL of ethyl acetate to achieve a total volume of 5 mL and measure the absorbance photometrically at a wavelength of 550 nm, using 5 % acetic acid as the control. The total triterpenoid content is expressed in milligrams of oleanoic acid equivalent (OAE). The total triterpene concentration in the samples was calculated based on the photometric data and the standard curve (21). The total triterpene content was determined using the following formula:

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

Where:

C_x: total triterpenoid concentration in the extract derived 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

Micro-morphological traits of *A. synandrifer*

Leaf blade (Fig. 2)

Midrib: The midrib is concave on the upper surface and convex on the lower surface. The upper and lower epidermis consist of a layer of polygonal cells; the outer surface is cutinized. The angular collenchyma lies in clusters on the lower epidermis, the cells are polygonal, cellulose wall, irregular size. The parenchyma contains small polygonal or intercellular spaces, polygonal cells, cellulose wall. The vascular bundle consists of xylem above, phloem bellow, arranged in rows in the parenchyma, the lower bundle is larger than the upper bundle. Each xylem bundle has 2-3 xylem vessels, polygonal shape.

Lamina

The upper and lower epidermis consist of a layer of polygonal cells with cellulose walls. The leaf flesh has an asymmetrical heterogeneous structure; the chlorenchyma consists of a layer of long, rectangular cells containing many chloroplasts, arranged closely together and perpendicular to the upper epidermis; the spongy parenchyma includes 5-7 layers of polygonal, irregular cells creating air cavities. The vascular bundles are small and arranged in a row in the parenchyma area. In the parenchyma area, many cells contain calcium oxalate crystals, usually needle -shaped, sometimes spherical.

Petiole (Fig. 3)

There is no distinction between the cortex and stele. The epidermis includes 1 layer of polygonal cells, cellulose wall. The angular collenchyma arranged in clusters below the epidermis. The parenchyma with walled cells that are often distorted in various shapes forming small polygonal or intercellular spaces. The vascular bundles with the phloem overlap the xylem, arranged in many circles, the size of the vascular bundles gradually increases towards the inside (the outer bundles are smaller than the inner bundles). Each xylem bundle usually has 1-3 protoxylem veins, centrifugally differentiated and 1-2 large metaxylem veins located below the protoxylem. In the parenchyma area, there are many cells containing needle-

Table 1. The methods used to identify the qualitative phytochemistry of *A. synandrifer*

Phytochemical	Reagent	Positive reaction	References
Phenolic and tannin	2 mL extract + 2 Ml H_2O + 2-3 drops of 5 % $FeCl_3$	Color changes to brownish to blackish green	(13)
Alkaloids	2 mL extract + 3-4 drops of Wagner reagent	Formation of a reddish-brown precipitate	(14)
Flavonoids	2 mL extract + 2 mL of 10 % Pb $(COOH)_2$	Formation of a yellow precipitate	(15)
Saponins	2 mL extract + 10 mL H_2O + 2 min of boiling	Foam formation	(16)
Terpenoids and steroids	5 mL extract + 2 mL CCl_3 + 3 mL concentrated sulfuric acid	Color changes to brownish-red	(17)
Coumarins	2 mL extract + 3 mL of 10 % NaOH	Color changes to yellow or dark yellow	(18)

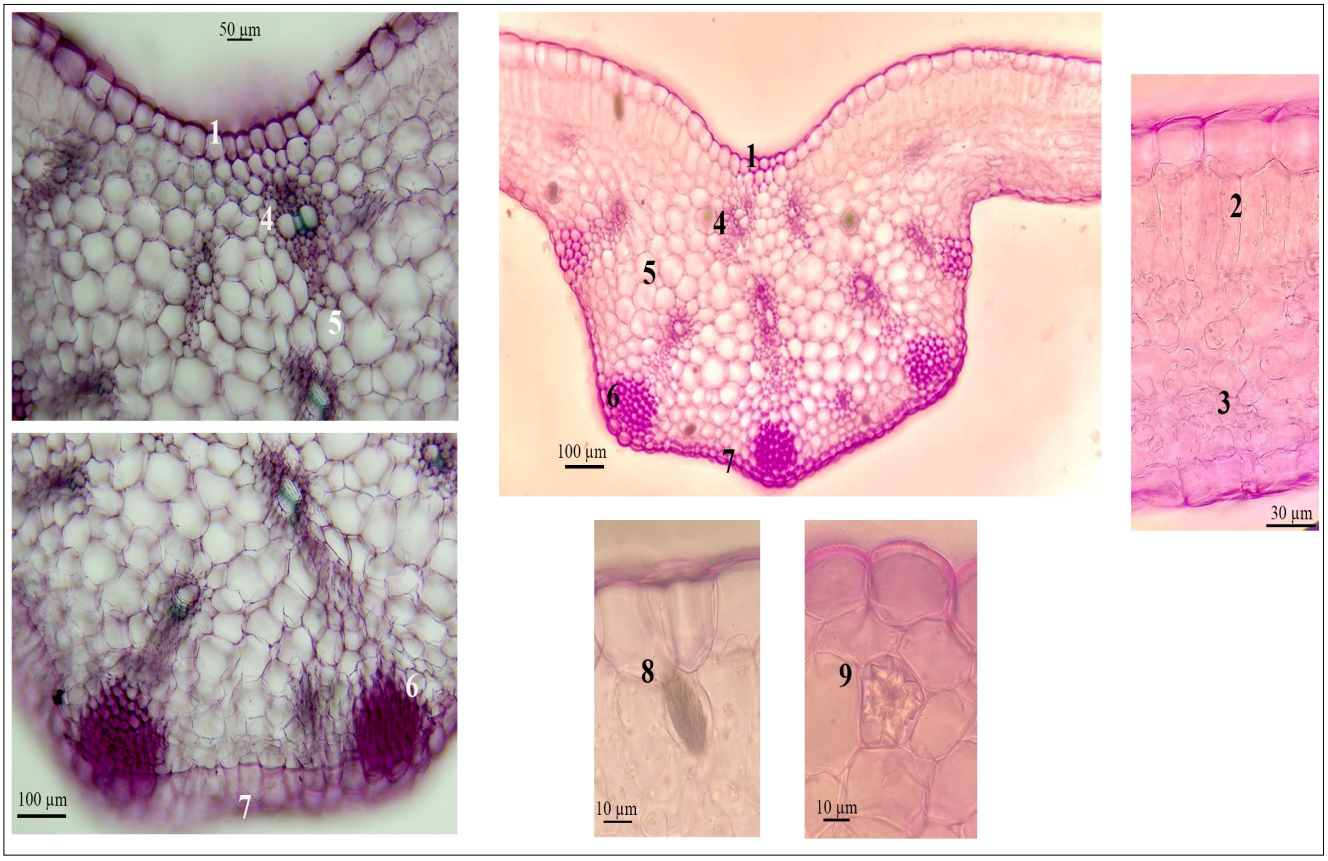

Fig. 2. The cross section of leaf blade. 1: upper epidermis, 2: chlorenchyma cell, 3: spongy parenchyma, 4: vascular bundle, 5: parenchyma, 6: angular collenchyma, 7: lower epidermis, 8: needle-shaped calcium oxalate crystal, 9: spiny-shaped calcium oxalate crystal.

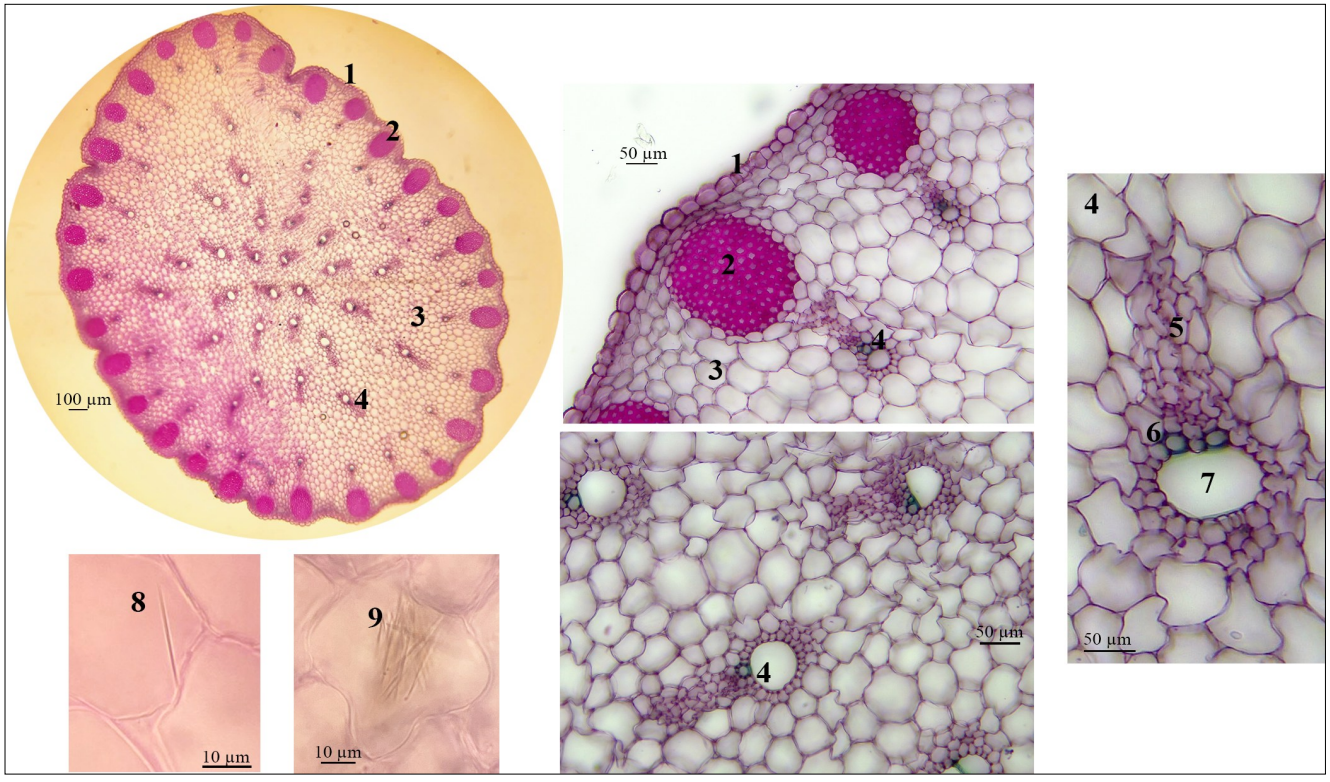

Fig. 3. The cross section of petiole. 1: epidermis, 2: angular collenchyma, 3: parenchyma, 4: vascular bundle, 5: phloem, 6: protoxylem, 7: metaxylem, 8 & 9: needle-shaped calcium oxalate crystals.

shaped calcium oxalate crystals.

Petiolule (Fig. 4)

The cross section is circular with a deeply concave upper surface. Epidermis consists of a single layer of polygonal cells with cellulose walls. The angular collenchyma lies in clusters on the lower epidermis. The parenchyma includes walled cells that are often distorted into different shapes, forming small polygonal or intercellular spaces. The vascular bundles arranged in many circles, gradually increasing in size towards the center. In the parenchyma area, many cells contain calcium oxalate crystals, usually needle-shaped, sometimes spherical.

Preliminary phytochemicals of the ethanol extracts of *A. synandrifer*

The preliminary phytochemistry of ethanol extracts isolated from the leaf blade, petiole, spathe/spadix and penducle of *A. synandrifer* were found to be rich in phenolic, tannin, alkaloid, flavonoid, saponin, terpenoid, steroid and coumarin (Table 2).

Quantitative phytochemical content of *A. synandrifer*

Total triterpene, polyphenol and flavonoid contents of ethanol extracts from different organs of *A. synandrifer* were presented in the Table 3. Accordingly, the leaf blade extract showed the highest quantitative concentration with the TTC, TFC and TPC contents of 8.08 mg OAE/g DW, 33.39 mg QE/g DW and 71.48 mg GAE/g DW, respectively; followed by the spathe/spadix (4.28 mg OAE/g DW, 29.25 mg QE/g DW and 68.09 mg GAE/g DW); peduncle extract (0.53 mg OAE/g DW, 17.52 mg QE/g DW, 59.90 mg GAE/g DW; and petiole extract (3.97 mg OAE/g DW, 19.41 mg QE/g DW, 42.40 mg GAE/g DW).

Discussion

The cross section of midrib of *A. synandrifer* also had five lobed shape and eight vascular bundles (Fig. 1). These structures were like those of several *Amorphophallus* species. For instances the micro-morphological traits of 23 *Amorphophallus* species collected from Thailand. Accordingly, the cross section of midrib of all studied samples were divided into 6 groups, including curved shape occurred in eight species; five lobed shape; six-lobed shape; seven-lobed shape; eight-lobed shape; twelve lobed shape of which the midrib with five lobed shape were

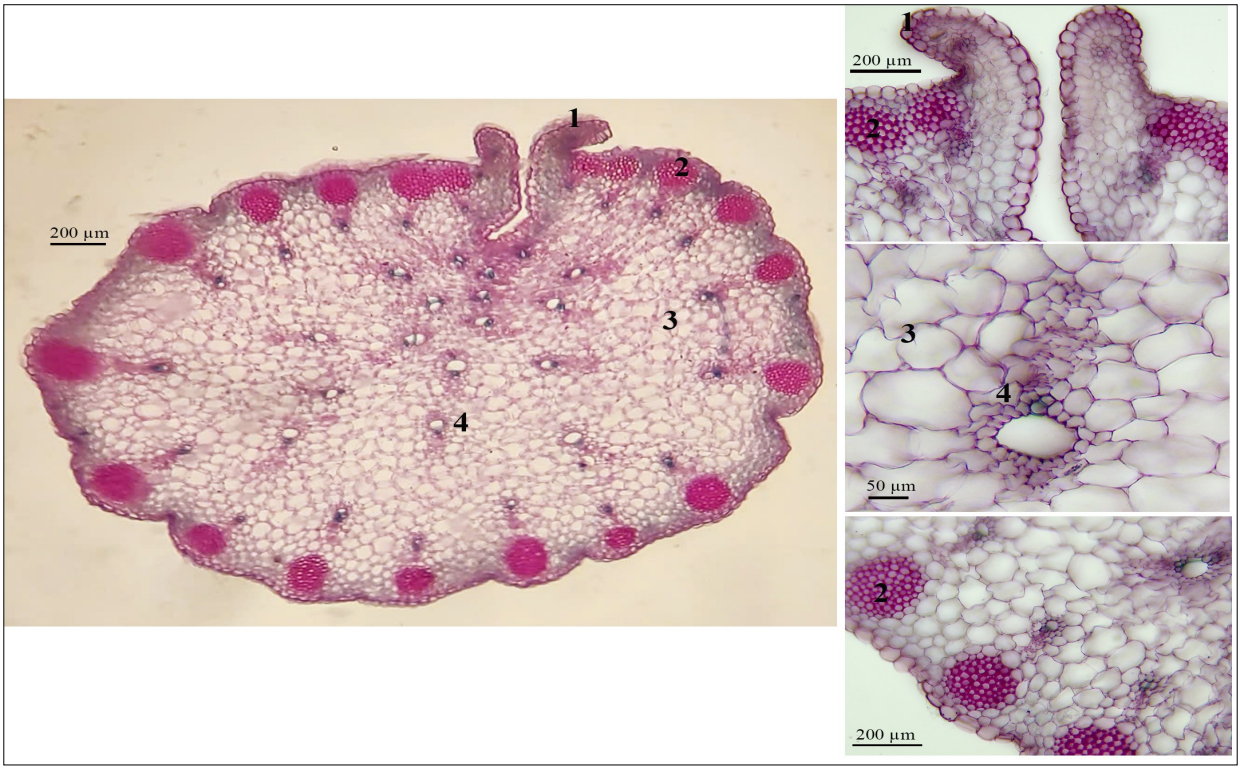

Fig. 4. The cross section of petiolule. 1: epidermis, 2: angular collenchyma, 3: parenchyma, 4: vascular bundle.

Table 2. Preliminary phytochemistry of ethanol extract of A. *synandrifer*

Compounds	Leaf blade	Petiole	Spathe/spadix	Penducle
Phenolic	+++	+++	+++	++
Tannin	+++	+++	+++	++
Alkaloid	+	+	+	+
Flavonoid	+++	+++	+++	+++
Saponin	++	+	++	+
Terpenoid	+++	+++	+++	++
Steroid	+++	+++	+++	++
Coumarin	+	++	++	++

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

Table 3. Total triterpene, polyphenol and flavonoid contents of ethanol extract of *A. synandrifer*

	Leaf blade	Petiole	Spathe/spadix	Peduncle
TTC (mg OAE/g DW)	8.08 ± 0.50	3.97 ± 0.30	4.28 ± 0.10	0.53 ± 0.10
TFC (mg QE/g DW)	33.39 ± 0.49	19.41 ± 0.21	29.25 ± 0.30	17.52 ± 0.13
TPC (mg GAE/g DW)	71.48 ± 0.64	42.40 ± 0.38	68.09 ± 0.27	59.90 ± 0.10

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

presented in *Amorphophallus* sp. 3, *Amorphophallus* sp.4, *A. curvistylis* and *A. scutatus* (22). Furthermore, the study also highlighted variation in the number of vascular bundles among species that *Amorphophallus* sp. 1, *Amorphophallus* sp. 4, *A. scutatus*, *A. curvistylis* and *A. cruddasianus* possessed eight vascular bundles in their midrib structures (22). Based on the anatomical traits of other *Amorphophallus* plants, the anatomical structure of *A. synandrifer* is like *Amorphophallus scutatus* and *A. curvistylis* (22).

Studies also provided the phytochemical screening of other *Amorphophallus* species. For example, the total phenolic and flavonoid contents of the ethanol and the aqueous extracts of *A. campanulatus* tuber collected from West Bengal, India have been reported. Accordingly, the total phenolic contents of the ethanol and aqueous extracts were 194.4 and 104.6 mg w/w while 6.75 and 1.50 mg w/w were the total flavonoid contents towards the same extracts (23). Furthermore, the preliminary phytochemicals of the methanol, aqueous extracts and crude powder of *A. campanulatus* form India were also provided. Accordingly, the methanol and aqueous extracts consisted of some compounds such as alkaloid, flavonoid, tannin, phenol and glycoside whereas alkaloid, saponins, flavonoids and carbohydrate were found in the crude powder (24).

The different extracts of the tuber of *A. paeoniifolius* from West Bengal, India were also tested with various bioactive compounds. As a result, the petroleum extract was found to be rich in alkaloid, sterol and terpenoid, fats and fixed oils. The aqueous extract consisted of tannin, flavonoid, carbohydrate, protein and amino acid. Methanol extract were identified as a mixture of alkaloid, flavonoid, sterol and terpenoid while the chloroform extract was only contained alkaloid (25). In another report, Salunke and Satpute also demonstrated the preliminary phytochemicals of the methanol, aqueous extracts and crude powder of *A. paeoniifolius* form India. As a result, the methanol and aqueous extracts consisted of some compounds such as alkaloid, flavonoid, tannin, phenol and glycoside while the same compounds and carbohydrate were presented in the the crude powder (24). Additionally, the methanol extract of the *A. paeoniifolius* tuber collected from the South-West region of Bangladesh was found to be rich in various bioactive compounds, including alkaloid, albuminoid, anthracene, betulinic acid, flavonoid, free anthraquinone, glucomannan, gums, lupeol, quercetin, reducing compound, rutin, β-sitosterol, steroid, sterol, stigmasterol and terpenoid (26).

The methanol, hexane and water extract of *A. smithsonianus* collected from Kerala, India were also investigated. Accordingly, the water extract contained carbohydrate, reducing sugar, flavonoid, glycoside and saponin. The hexane extract consisted of carbohydrate, reducing sugar, flavonoid, glycoside, phenolic, fat and oil while reducing sugar, tannin, flavonoid, phytotannin, terpenoid, saponin, phenolic, fat and oil were found in the methanol extract (27). The preliminary phytochemicals of the petroleum ether, methanol and water extracts of *A. commutatus* var. *wynadensis* Kerala, India. Accordingly, the petroleum extract consisted of terpenoid, glycoside, coumarin, fixed oils and fat. The methanol extract was found to be rich in alkaloid, carbohydrate, phenol, terpenoid, glycoside, fixed oil and fat, flavanones, quinones, tannins, steroids while the same compounds, except steroids, alkaloid, fixed oil and fat were found in water extract (28).

Conclusion

This studies firstly provided the anatomical traits from different organs of *A. synandrifer*. So many bioactive components also included in different organs of the studied species. The significant amounts of triterpene, flavonoid and polyphenol contents were also detected from the studied samples. These findings contribute valuable baseline data for future pharmacological and taxonomic studies on *A. synandrifer*.

Authors' contributions

TNN drafted the manuscript, participated in the design of the study and performed the experiments, statistical analysis, and resolved all the queries of editors and reviewers. VHT, QNDP, NNT and LTT performed experiments and handled the research data. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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