Microscopic characteristics, chemical compositions and bioactivities of *Alpinia vietnamica*

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Abstract

Background: The genus *Alpinia* is one of the diverse genera in Thua Thien Hue province, in which many species have been used as medicine. But until now, studies on *A. vietnamica* have rarely been reported. **Objectives:** The present study was aimed at the determination of microscopic characteristics and chemical compositions as well as evaluating the antioxidant and acetylcholinesterase inhibitory activities of *A. vietnamica*. **Materials and methods**: *A. vietnamica* was collected in Phong Dien district, Thua Thien Hue province. Anatomic structures and powder properties were determined by the microscopic method. Phytochemical screening was conducted by specific chemical reactions. The Folin-Ciocalteau method and the aluminum chloride-flavonoid assay, respectively, were used to quantify the total polyphenol (TPC) and total flavonoid contents (TFC). Antioxidant activity was assessed using the DPPH assay, while acetylcholinesterase (AChE) inhibitory activity was evaluated using the Ellman method. **Results:** The microscopic characteristics of this species have been described. Phytochemical analysis results revealed the presence of flavonoids, coumarins, and tannins in *A. vietnamica*. The ethanol extract from the aerial part of *A. vietnamica* had higher polyphenol and flavonoid contents than the underground part extract. Moreover, this extract also displayed a stronger DPPH radical scavenging and exhibited AChE inhibitory activities. **Conclusion**: This is the first report on the microscopic characteristics, chemical compositions, and biological activities of *A. vietnamica*.

Keywords: *Alpinia vietnamica, microscopic characteristics, chemical constituents, antioxidant activity, acetylcholinesterase inhibitory*

1. BACKGROUND

Alpinia is a large genus of the Ginger family (Zingiberaceae) with over 250 species that are widely distributed in Asia. In many countries around the world, species in this genus have been used for traditional medicine, food, and spices. Fruits, seeds, leaves, and rhizomes of these medicinal plants are frequently used to treat digestive system diseases such as indigestion, stomach pain, and vomiting, or as anti-inflammatory drugs. Terpenoids, diarylheptanoids, lignans, flavonoids, alkaloids, essential oils, and other compounds are found in *Alpinia* genus. Extracts and compounds isolated from this genus have a variety of beneficial biological properties, such as the ability to inhibit the growth of cancer cells, antioxidant, antimicrobial, cardioprotective activities, and reduce blood sugar levels, etc [1].

There are approximately 31 species of *Alpinia* grown or living under the canopy of forests, streams, and wet places in Vietnam. Many species have been used as medicine, spices, and considered raw materials for essential oil extraction. Studies on the genus *Alpinia* in Vietnam mainly focus on analyzing the essential oil composition of some species, such as *A. oblongifolia*, *A. malaccaensis*, *A. menghaiensis*, *A. pinnanensis*, *A. polyantha*, *A. strobiliformis* and *A. tonkinensis* [2]. *Alpinia* is well known as a biodiversity genus in Thua Thien Hue province, with many valuable medicinal species, including *A. vietnamica* [3]. It was discovered in Central Vietnam and is a newly identified species in 2019 [4]. As far as we know, there has not been any study about the microscopic characteristics, chemical compositions, and bioactivities of *A. vietnamica*. Therefore, the objective of this study is to determine the microscopic characteristics, preliminary phytochemical screening, and evaluate the total polyphenol and total flavonoid contents as well as the antioxidant and anti-acetylcholinesterase activities of *A. vietnamica*.

2. MATERIALS AND METHODS

2.1. Materials

The whole of *Alpinia vietnamica* H.D. Tran, Luu & Škorničk. (Zingiberaceae) was collected in

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Phong Dien district, Thua Thien Hue province in June 2022. The plant material was identified by Dr. Thao Xuan Hoang (Faculty of Biology, University of Education, Hue University). Voucher specimen (AV01) has been deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy, Hue University, Vietnam.

2.2. Methods

2.2.1. Identification of microscopic characteristics

Microsurgery: Fresh leaves and roots were cut into thin sections with a razor blade. The sections were soaked in 5% sodium hypochlorite for approximately 30 min and washed with water. Immersed sections in 1% acetic acid for 3-5 min, and rinsed with water. The sections were then dyed with methylene blue and carmine red solution at a suitable time and washed several times with water. After that, they were placed on a glass slide, mounted with a few drops of 10% glycerol, and a cover glass was applied. The final sections were observed using a microscope (Eclipse E100, Nikon, Japan), and photographed with an attached camera (Nikon, D5100) [5].

Powder characteristics: The aerial and underground parts of the plant were powdered and passed through hand sieve with a mesh size of 0.125 mm to obtain a fine powder. The powder was placed on slides with several drops of 10% glycerol and covered with a coverslip. Observations were made with an optical microscope (Eclipse E100, Nikon, Japan), and pictures were taken with a camera (Nikon, D5100) [5].

2.2.2. Preliminary phytochemical screening

The extracts of the aerial and underground parts of *A. vietnamica* were tested for the presence of alkaloids, anthranoids, coumarins, cardiac glycosides, flavonoids, saponins, tannins, organic acids, and steroids by using specific chemical reactions [6].

2.2.3. Preparation of the extract

The powder of dried aerial and underground parts of *A. vietnamica* (10.0 g, each sample) was macerated with ethanol (EtOH) (100 mL x 3 times) at room temperature for three days, shaken, and stirred occasionally. The EtOH extract was filtered through cotton and filter paper, then recovered the solvent until it was completely ethanol-free.

2.2.4. Determination of total polyphenol and flavonoid contents

2.2.4.1. Determination of total polyphenol content

The total polyphenol content was evaluated by the Folin-Ciocalteu method with slight modifications. Tested samples (0.2 mL) were mixed with 0.8 mL distilled water and 1.0 mL of 10% Folin-Ciocalteu reagent and then shaken for 5 min. Then, a volume of 2.5 mL of 7.5% Na_2CO_3 was added. After incubation at room temperature in a dark condition for 30 min, the absorbance was measured at 760 nm. Gallic acid (GAE) was used as a standard for the calibration curve [7]. All samples were analyzed in 3 replicates and the total polyphenol content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) according to the formula (1):

$$\mathbf{TPC} = \frac{C1 \times V \times k}{m} \quad (1)$$

where TPC was the total polyphenol content in mg/g, in GAE (Gallic acid equivalent), C1 was the concentration of Gallic acid established from the curve in mg/mL, V was the initial volume of the extract in mL, k was the dilution factor, and m was the weight of the plant extract in g.

2.2.4.2. Determination of total flavonoid content

The total flavonoid content (TFC) was measured by the aluminum chloride colorimetric method. Tested samples (2.0 mL) were mixed with 2.0 mL of 2% $AlCl_3$. After incubation at room temperature for 10 min, the absorbance was recorded at 430 nm. Rutin (RE) was used as a standard for the calibration curve [8]. TFC was expressed as mg of rutin equivalents per gram of extract (mg RE/g) according to the formula (2):

$$\mathbf{TFC} = \frac{C1 \times V \times k}{m} \quad (2)$$

where TFC was total flavonoid content in mg/g, in RE (Rutin equivalent), C1 was the concentration of Rutin established from the curve in mg/mL, V was the initial volume of the extract in mL, k was the dilution factor, and m is the weight of the plant extract in g.

2.2.5. Evaluation of antioxidant and anti-acetylcholine esterase activities

2.2.5.1. Evaluation of antioxidant activity

The antioxidant activity was evaluated using the DPPH method with minor modifications, and the absorbance was measured at 517 nm [9]. Quercetin was used as a positive control. The ability of the sample to scavenge DPPH radicals (%) was calculated using the following formula (3):

$$\mathbf{DPPH\ scavenging\ effect} = [(A_{control} - A_{sample}) / A_{control}] \times 100\% \quad (3)$$

where $A_{control}$ was the absorbance of DPPH control solution and A_{sample} was the absorbance of DPPH

solution in the presence of tested samples. The tests were carried out three times. The IC_{50} value is the concentration of 50% free radical neutralizer DPPH calculated in Microsoft Excel.

2.2.5.2. Evaluation of acetylcholinesterase (AChE) inhibitory activity

AChE inhibitory activity was measured based on the method of Ellman *et al.* with some minor modifications [10, 11]. Briefly, for each well of a 96-well microtiter plate, 140 µL of phosphate buffer (pH = 8.0), 20 µL tested samples, and 20 µL of 0.25 IU/mL AChE were mixed and incubated for 15 min at room temperature. Post-incubation, 10 µL of 2.4 mM acetylthiocholineiodide (ATCI) and 10 µL of 2.4 mM 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) were added. The reaction mixture was mixed and incubated for 24 min at room temperature. The absorbance was determined at 405 nm (Elisa EMR-500, Labomed Inc., USA). A control sample contained all the aforementioned constituents without the test extract. A blank sample was performed in the absence of AChE. Berberine was used as the positive control. The AChE inhibitory activity was determined using the formula (4):

$$\%I = [1 - (A_s - A_{b/s}) / (A_c - A_{b/c})] \times 100\% \quad (4)$$

where %I was the percent of AChE activity inhibited; A_s and $A_{b/s}$ were the absorbance of the test sample and the blank of the test sample, respectively. A_c and $A_{b/c}$ were the absorbance of the control sample and the blank of the control sample, respectively.

2.2.6. Statistical analysis

All the data in this research was analyzed using the Microsoft Excel program in triplicate. Values were expressed as mean ± standard deviation (SD) for three replicates for each sample.

3. RESULTS

3.1. Microscopic characteristics

3.1.1. Anatomy structure

Leaf midrib [Fig. 1A, 1B, 1D]: The midrib was concave on the upper side and convex on the lower side in cross section. The upper and lower epidermis (B1, B6) consisted of a layer of rectangular cells arranged adjacently. The parenchyma (B2, B5) was comprised of many layers of polygonal cells that were different-sized, thin-walled, and arranged randomly. In the parenchyma region (D1), there were some cells with a spherical oil droplet inside (D2). The phloem-xylem vasculars were divided into two types, including ovoid-shaped and spoon-shaped bundles. The ovoid-shaped bundles (B3) were located in the center of the midrib, and the spoon-shaped bundles (B4) were adjacent to the lower epidermis.

Leaf blade [Fig. 1C, 1E]: The upper and lower epidermis (C1, C7) had a similar structure to the epidermis in the leaf midrib. The lower epidermis had trichomes (C6). The parenchyma (C2) was under the upper epidermis and comprised a layer of oval-shaped cells, thin-walled and large-sized. The palisade and spongy layers (C3, C5) were clearly separated. The bundles of phloem-xylem (C4) were irregular in size. In the spongy parenchyma (E2), there were some cells containing yellow oil droplets (E1).

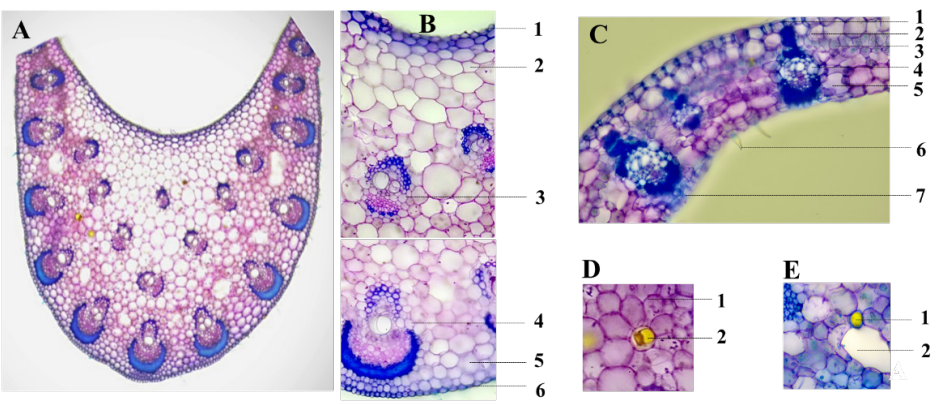

Figure 1. Microscopic characteristics of leaf cross-section of *Alpinia vietnamica*
A, B: *Leaf midrib (1. Upper epidermis, 2, 5. Parenchyma, 3. Ovoid-shaped bundle of phloem-xylem, 4. Spoon-shaped bundle of phloem-xylem, 6. Lower epidermis),* ***C:*** *Leaf blade (1. Upper epidermis, 2. Parenchyma, 3. Palisade parenchyma, 4. Bundle of phloem-xylem, 5. Spongy parenchyma, 6. Trichome, 7. Lower epidermis),* ***D:*** *Parenchyma in leaf midrib (1. Parenchyma cell, 2. Essential oil cell),* ***E:*** *Spongy parenchyma in leaf blade (1. Essential oil cell, 2. Spongy parenchyma cell)*

Root [Fig. 2]: The root of *A. vietnamica* had a circular cross-section; the cortical area occupied more than a half of the microsurgery radius, and the layers from outer to inner included: The epidermis (B1) consisted of a layer of rectangular cells arranged adjacently. The suberoid layer (B2) was made up of a layer of closely spaced polygonal cells. There were many sclereids (B3) close to the outer layer of the cortex. The cortical parenchyma (B4) comprised several layers of parenchyma that were polygonal, unequal-sized and thin-walled. The endoderm (B5) was a thick U-shaped layer of cells forming a caspary belt. The tissues in the innermost part of the endodermis that formed the stele region called the pericycle (B6) comprised a single layer of thin-walled cells. The vascular tissues consisted of many patches of phloem (B7) and xylem (B8) arranged radially. The pith region (B9) was present at the centre of the internal structure and comprised parenchymatous cells along with intercellular spaces composed of many polygonal cells and randomly arranged.

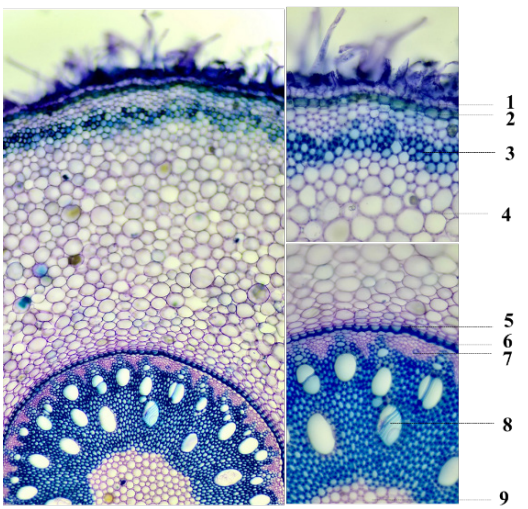

Figure 2. Microscopic characteristics of root cross-section of *Alpinia vietnamica*
1. Epidermis, 2. Suberoid, 3. Sclereid, 4. Cortical parenchyma, 5. Endoderm, 6. Pericycle, 7. Phloem, 8. Xylem, 9. Pith parenchyma

3.1.2. Powder features

The aerial part [Fig. 3]: A green powder had the characteristic of pleasant and aromatic odour. Powder features from the aerial part were observed under a light microscope at 10X and 40X magnifications. The powder had several microscopic characteristics: fragment of epidermis contained trichomes and essential oil (1), fragment of epidermis contained color fragment (2), color fragment (3), fragment of epidermis (4), fragment of epidermis and palisade parenchyma (5), fragment of epidermis contained stomata (6), stomata (7), bundle of fiber (8, 9), fragment of vessel (10), starch (11), fragment of parenchyma (12), fragment of parenchyma contained starch (13), sclereid (14), and trichome (15).

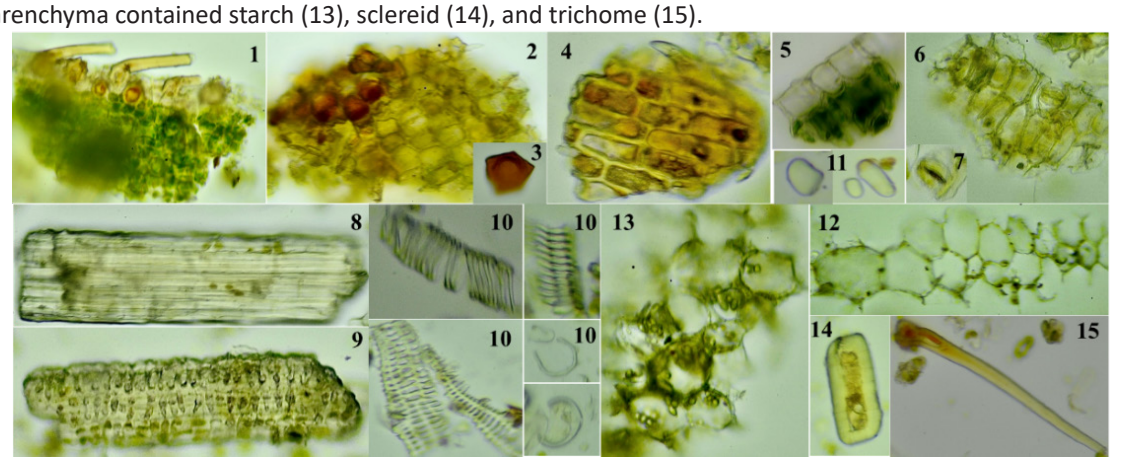

Figure 3. Microscopic features of the aerial part of *Alpinia vietnamica*

The underground part [Fig. 4]: A brown-yellow powder had the characteristic of pleasant and aromatic odour. Some microscopic features of the underground part powder were observed under a light microscope at 10X and 40X magnifications, including: fragment of phellem (1), fragment of epidemis (2), fragment of epidemis contained essential oil cell (3), sclereid (4), fragment of parenchyma (5), starch (6), fragment of parenchyma contained starch (7), bundle of fiber (8, 9), and fragment of vessel (10).

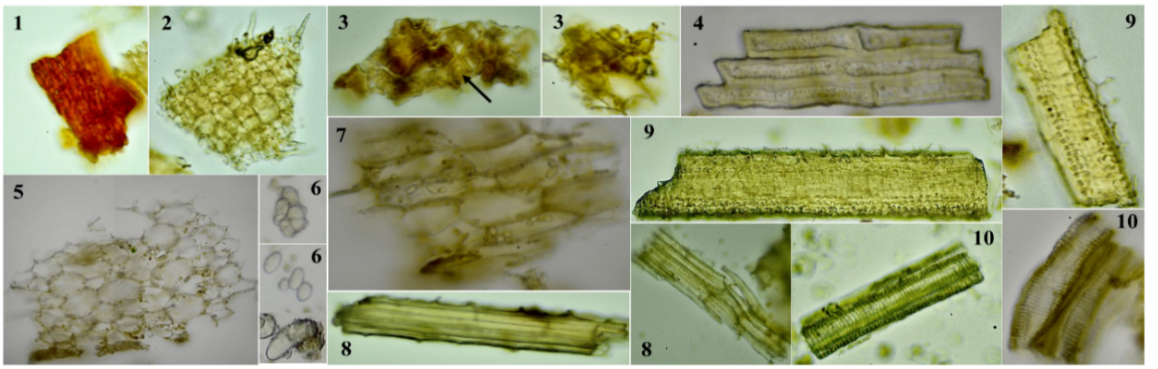

Figure 4. Microscopic features of the underground part of *Alpinia vietnamica*

3.2. Chemical compositions

3.2.1. Phytochemical screening

The phytochemical screening results of *A. vietnamica* revealed that coumarins, flavonoids, and tannins were present in both the aerial and underground parts, while steroids were only found in the aerial part, as shown in Table 1.

Table 1. Phytochemical screening results of *Alpinia vietnamica*

No.	Class of phytochemicals	Test/Reagent	Results	
			The underground part	The aerial part
1	**Alkaloids**	Mayer	–	–
		Dragendorff	–	–
		Bouchardat	–	–
2	**Anthranoids**	Bontraeger	–	–
3	**Coumarins**	Lactone ring-opening	+	+
		Diazo	+	++
4	**Cardiac glycosides**	Liebermann	–	+
		Legal	–	–
		Keller-Killiani	–	–
5	**Flavonoids**	Cyanidin	+	+++
		10% NaOH	+++	+++
		5% $FeCl_3$	++	+++
6	**Saponins**	Foam	–	–
7	**Tannins**	10% $FeCl_3$	+	++
		10% $(CH_3COO)_2Pb$	+	++
		1% Gelatin	+	+
8	**Organic acids**	Na_2CO_3	–	–
9	**Steroids**	Liebermann	–	+

Notes: (+): mildly positive, (++): moderately positive, (+++): highly positive, (-): negative.

3.2.2. Determination of total polyphenol (TPC) and total flavonoid contents (TFC)

The ethanol extracts of *A. vietnamica* possessed significantly high phenolic and flavonoid contents and were displayed in Table 2. The results indicated that TPC and TFC were higher in the aerial part extract (TPC = 284.05 ± 0.28 mg GAE/g, TFC = 51.53 ± 0.06 mg RE/g) compared to the underground part extract (TPC = 172.72 ± 0.40 mg GAE/g, and TFC = 23.33 ± 0.15 mg RE/g).

Table 2. Total polyphenol and total flavonoid contents of *Alpinia vietnamica*

No.	Sample	TPC ± SD (mg GAE/g extract)	TFC ± SD (mg RE/g extract)
1	**Aerial part**	284.05 ± 0.28	51.53 ± 0.06
2	**Underground part**	172.72 ± 0.40	23.33 ± 0.15

3.2.3. Evaluation of antioxidant and AChE inhibitory activities

The antioxidant and AChE inhibitory activities of the ethanol extract from the aerial and underground parts of *A. vietnamica* were summarized in Table 3.

Table 3. Antioxidant and AChE inhibitory activities of *Alpinia vietnamica*

No.	Sample	Antioxidant activity	AChE inhibitory activity
		IC_{50} ± SD (μg/mL)	IC_{50} ± SD (μg/mL)
1	**Aerial part**	10.32 ± 0.11	76.4 ± 3.4
2	**Underground part**	36.65 ± 0.21	> 100
	Quercetin	2.12 ± 0.01	–
	Berberine	–	0.77 ± 0.14

As can be observed, the ethanol extracts of *A. vietnamica* demonstrated remarkable *in vitro* DPPH radical scavenging activity. The aerial part extract exhibited stronger antioxidant activity than the underground part, with IC_{50} values of 10.32 ± 0.11 μg/mL and 36.65 ± 0.21 μg/mL, respectively. Moreover, only the ethanol extract from the aerial part of *A. vietnamica* revealed AChE inhibitory activity with an IC_{50} value of 76.4 ± 3.4 μg/mL. In this study, results showed that DPPH radical scavenging abilities and inhibition of AChE of the extracts of plant parts were less than those of quercetin (IC_{50} = 2.12 ± 0.01 μg/mL) and berberine (IC_{50} = 0.77 ± 0.14 μg/mL) used as positive control, respectively.

4. DISCUSSION

To the best of our knowledge, there was a few studies on the microscopic characteristics of *Alpinia* species. Most publications only focused on the descriptions of morphology. The anatomical structure of the leaf and root of *A. vietnamica* had typical characteristics of monocot plants and Ginger family. In the midrib micromorphological characteristics, there were two different types of phloem-xylem vasculars, including ovoid-shaped and spoon-shaped bundles. In the root anatomy, the endoderm was a thick layer of cells that formed a caspary belt. The pericycle adjacent to endoderm was made up of a single layer of thin-walled cells. In comparison to *A. menghaiensis* and *A. blepharocalyx* [12], [13], the anatomical structure and powder properties of *A. vietnamica* were found to be quite similar to these species and had no specific characteristics to contribute to the difference. As a result, the presence of the key character of identification was not determined in microscopic examination.

Phytochemical screening helped reveal the plant extracts' constituents, which predominated over the others. The preliminary phytochemical examination of the extracts of *A. vietnamica* was rich in tannins, flavonoids, and coumarins. These phytochemical compounds identified may be responsible for the biological activities of *A. vietnamica*.

The ethanol extract of the aerial part of *A. vietnamica* had notably high levels of phenolic and flavonoid contents, with TPC value of 284.05 ± 0.28 Gallic acid equivalent mg/g and TFC value of 51.53 ± 0.06 Rutin equivalent mg/g. Previous studies had shown TPC and TFC in leaf and rhizome extracts of *A. galanga* and *A. calcarata*. Following, TPC and TFC in *A. galanga* were found higher in comparison with *A. calcarata* extracts. The leaf extract of *A. galanga* had TPC = 77.25 ± 1.56 mg GAE/g and TFC = 64.69 ± 1.12 mg Quercetin/g, while the rhizome extract of *A. galanga* had TPC = 32.44 ± 1.35 mg GAE/g and TFC = 39.46 ± 1.05 mg Quercetin/g [14]. Thus, it is obvious that the TPC in the aerial part (284.05 ±

0.28 mg GAE/g) and underground part (172.72 ± 0.40 mg GAE/g) of *A. vietnamica* were much higher than other surveyed species of *Alpinia* genus. Many studies have shown that polyphenol components have antioxidant, anti-inflammatory, and anti-cancer properties [15]. Due to its high polyphenol content, *A. vietnamica* has demonstrated the potential to be a source of raw materials for research and development of healthcare products made from this herb.

The antioxidant and AChE inhibitory activities of the ethanol extract from the aerial part of *A. vietnamica* were stronger than the underground part, which could be attributed to the higher TPC and TFC. *A. vietnamica* had a higher antioxidant capacity than other species in *Alpinia* (the aerial part: IC_{50} = 10.32 ± 0.11 µg/mL, the underground part: IC_{50} = 36.65 ± 0.21 µg/mL). This was demonstrated by a study on alcohol extracts of *A. officinarum* and *A. zerumbet* that had the ability to neutralize DPPH with IC_{50} values of 95.41 ± 1.10 µg/mL and 44.5 ± 4.8 µg/mL, respectively [16], [17]. The AChE inhibitory activity has also been reported in *A. purpurata* [18]. The whole plant methanol extract of *A. purpurata* inhibited AChE with an IC_{50} value of 19.08 ± 0.02 µg/mL, which was stronger than the aerial part ethanol extract of *A. vietnamica* with IC_{50} value of 76.4 ± 3.4 µg/mL. It is possible that the high level of total phenolic in *A. vietnamica* contributed to its potent antioxidant and anti-AChE effects.

5. CONCLUSION

This study provided the first detailed information on the microscopic characteristics, chemical compositions, and biological activities of *A. vietnamica*. The results of this study distinctly show the presence of significant amounts of phenolic and flavonoid components as well as bioactivities of *A. vietnamica*, which could be considered good sources for pharmaceutical and food applications. Further studies are necessary to determine the components of phenolics and flavonoids that may have contributed to the major biological activities.

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