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RESEARCH ON MORPHOLOGICAL CHARACTERISTICS, MICROSCOPIC CHARACTERISTICS AND CHEMICAL COMPOSITION OF "BUOI BUNG" (*ACRONYCHIA PEDUNCULATA* (L.) MIQ.) COLLECTED IN QUANG NINH PROVINCE

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Summary

"Buoi bung" (*Acronychia pedunculata* (L.) Miq.) harvested in Thuong Yen Cong Commune, Uong Bi City, Quang Ninh Province, Vietnam was studied for the first time for its morphological and microscopic characteristics. The results of plant analysis and microdissection of stems, leaves, fruits, and medicinal powders were presented in detail. Qualitative research on some groups of substances in the stems, leaves, and fruits of *A. pedunculata* showed the presence of flavonoids, coumarins, saponins, reducing sugars, fats, sterols, and essential oils. The content of essential oil in fruits of *A. pedunculata* was also determined to be 0.167%, with the main components comprising α-pinene (21.2%), caryophyllene (30.4%), and (-)-spathulenol (12.6%). This essential oil was evaluated for its effects on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, but no effect was observed at the tested concentrations. The research results will contribute to the identification and standardization of *A. pedunculata* in the future.

Keywords: *Buoi bung; Acronychia pedunculata; Morphological characteristics; Microscopic characteristics; Essential oil.*

1. Introduction

Acronychia pedunculata (L.) Miq., (synonym: *Acronychia laurifolia*) a medicinal plant in the Rutaceae family, is widely distributed in northern and southern Vietnam [1]. In traditional medicine, its roots, stems, leaves, and fruits are used to treat joint pain, rheumatism, hernias, indigestion, colds, coughs, and wounds [1],[2].

Previous studies on *A. pedunculata* primarily focus on the essential oil composition and bioactivity of its stems and leaves, such as studies on *A. pedunculata* by Lesueur [3], Phung Thi Tuyen [4] in Vinh Phuc Province, research at Phu Huong Nature Reserve [5], studies by Hong Thien in Ba Ria - Vung Tau [6], and Trinh Thi Diep in Lam Dong [7]. However, no comprehensive studies on the morphological, histological, and phytochemical characteristics of this species in Vietnam have been conducted to establish a scientific basis for the identification and pharmacognostic evaluation of this medicinal plant. To contribute to the accurate identification and standardization of *A. pedunculata* for future research on this species, we conducted a study on the morphological characteristics, microscopic features, and qualitative analysis of chemical groups in the stems, leaves, and fruits of *A. pedunculata* using chemical reactions. Additionally, we investigated the chemical composition and antimicrobial activity of the essential oil extracted from the fruits of *A. pedunculata* collected in Quang Ninh Province.

2. Materials and Methods

2.1. Subjects of Study

The research samples were collected in Thuong Cong Yen Commune, Uong Bi City, Quang Ninh Province, between August and October 2023. The samples were scientifically identified by Master Nghiem Duc Trong (Department of Botany, Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy) as *Acronychia pedunculata* (L.) Miq., belonging to the Rutaceae family. A voucher specimen was preserved at the Department of Botany, Hanoi University of Pharmacy, under the code HNIP32/2024.

Essential oils were extracted from the stems, leaves, and fruits of *A. pedunculata*. Subsequently, the fruit essential oil was analyzed at the Institute of Ecology and Biological Resources (IEBR).

2.2. Research Methods

Morphological Characteristics Research Method

Observations and detailed descriptions of the

morphological characteristics were conducted. The stems, leaves, flowers, fruits, and seeds of the research samples were analyzed, photographed, and documented.

Microscopic Characteristics Analysis Method

Microtomy of the stem, leaf, and fruit specimens was performed using the double staining method, mounted using the drop-squash technique, and observed and photographed under a microscope [8],[9].

Powder Characteristics Analysis Method

Microscopic observation of the microstructure and powder characteristics of the stems, leaves, and fruits were conducted. The features were described and digitally photographed [8],[9].

Qualitative Method of Determining Several Groups of Substances

Preliminary qualitative analysis of several organic compound groups in the stem, leaf, and fruit specimens was conducted using chemical reactions [8].

Essential Oil Research Method

Essential oil was extracted using the essential oil quantification apparatus according to the United States Pharmacopeia (USP 38-NF 33) (suitable for essential oils lighter than water). The composition of the essential oil were analyzed using a GC-MS system (Agilent Intuvo 9000 GC-SN: US2029M007), equipped with an Agilent 122-5532UI-INT column (30 m x 0.25 mm I.D. x 0.25 µm). The temperature program started at 50°C, increased to 200°C at 5°C/min (held for 0 minutes), then to 280°C at 8°C/min (held for 10 minutes). The injector temperature was 200°C, with the N_2 carrier gas flow rate of 1 mL/min. The ion source and quadrupole temperatures were 230°C and 150°C, respectively, with a scan range of 45-500 amu. Compounds were identified by comparing their spectra to the NIST library.

Evaluation Method of Antimicrobial Activity

The minimum inhibitory concentration (MIC) was determined using the microdilution method on 96-well plates according to the method of Le Thi Thuy [10]. The test was conducted on the following microorganisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 1023. Meropenem was used as the reference substance for *S. aureus* and *E. coli*, while Itraconazole was used for *C. albicans*. 100 µL of culture medium was added to each well, except for the negative control, which received 200 µL. The minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) was determined from the MIC plates. All wells showing no visible growth were subcultured onto TSA plates (for bacteria, incubated at 37°C) and SDA plates (for fungi, incubated at 30°C) to confirm the MBC/MFC values.

3. Results

3.1. Morphological characteristics

Shrubs or small trees, about 2-4 m tall. Simple, entire, opposite leaves (a-e). Petiole cylindrical, about 1.5-2.5 cm long, swollen at both ends, green, with very short, and scattered hairs on young petioles. Leaf blade oval, 6-12 cm long, 4-6 cm wide; upper surface dark green, lower surface gray-green, smooth; leaf base rounded, apex obtuse or slightly rounded, margin entire; pinnate venation with prominent midrib, sparsely short-haired in young leaves, and 5-6 pairs of lateral veins (Fig. 1).

Fig. 1. Morphological characteristics of vegetative organs *(a. Branch with leaves and leaf morphology; b. Surface of old branch; c. Leaf arrangement; d1. Upper leaf surface; d2. Lower leaf surface; e. Leaf margin; f. Petiole surface and venation of young leaf).*

Inflorescences are dichasial cymes located in leaf axils, with cylindrical peduncles about 4-5 cm long, smooth, green, bearing 9-15 small. Flowers are actinomorphic, bisexual, about 1.5 cm long, and yellowish green in color. The pedicel (c1) is cylindrical, approximately 1.0 cm long, yellowish green, with a smooth surface bearing two-minute scale-like bracts positioned oppositely. Calyx 4, connate, triangular scale-like, inconspicuous,

green, smooth (c1); Corolla 4, ribbon-shaped, 4-5 mm long, approximately 1 mm wide; margins curved inward, green, smooth on both surfaces (c2); Androecium 8, free, arranged in 2 whorls, the outer whorl longer than the inner whorl; filaments fleshy, approximately 0.8 mm in diameter, 3-5 mm long, green, smooth; anthers ovate, about 1.0 mm long, pale yellow, 2-locular, dehiscing longitudinally, dorsifixed (c3). Gynoecium composed of 4 connate carpels forming a superior ovary, four locules, axile placentation, typically one ovule per locule; ovary globular, 1.5-2 mm in diameter; style filiform, approximately 3 mm long, yellowish-green, smooth; stigma truncate; with a yellow nectary disc located beneath the ovary, inside the androecium, approximately 2-3 mm in diameter. Fruits are arranged in cymose clusters; the fruiting peduncle is 3-5 cm long, with individual fruit pedicels 1-2 cm long, showing scars of two fallen bracts. The fruit is a simple drupe, ovoid in shape, about 0.5-1.2 cm in size; immature fruits are spherical, green, and smooth, while mature fruits develop four shallow lobes at the apex and turn yellowish. The exocarp is smooth, the mesocarp is hard and brittle, and the endocarp forms a thin stone with four locules, each containing one dark brown seed (Fig. 2).

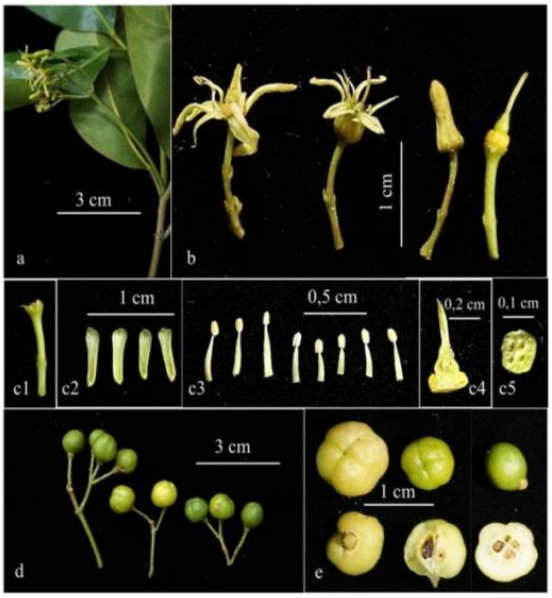

Fig. 2. Reproductive organ characteristics
(a. Flowering branch; b. Intact flower; c1. Pedicel, bracts, and calyx; c2. Corolla; c3. Androecium; c4. Longitudinal section of ovary; c5. Cross section of ovary; d. Fruit cluster; e. Intact fruit, longitudinal section, and cross section).

3.2. Microscopic Characteristics

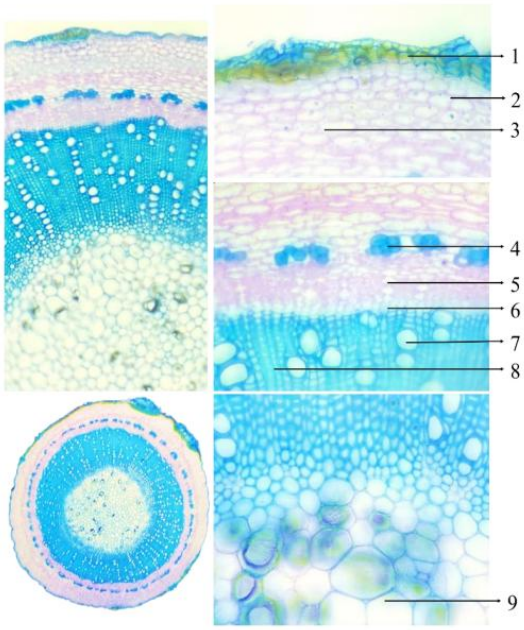

Fig. 3. Microscopic characteristics of the stem of the studied specimen
(1. Cork; 2. Collenchyma tissue; 3. Cortex parenchyma; 4. Phloem fibers; 5. Secondary phloem; 6. Cambium; 7. Secondary xylem vessels; 8. Medullary rays; 9. Pith parenchyma).

Microscopic Characteristics of the Stem: The stem of the studied specimen has a circular cross-section, composed from the outside to the inside as follows: The cork (1) (phellem) is composed of 5-10 layers of tangentially elongated cells, arranged radially, with suberized cell walls that appear yellow-brown or blue upon staining. Immediately beneath the cork are 2-3 layers of chlorenchyma, consisting of parenchymatous cells arranged in radial rows. The collenchyma tissue (2) consists of 3-5 layers of polygonal cells, increasing in size inwardly, tightly arranged, with angular thickening of the cell walls. The cell walls are intensely stained pink. The cortex parenchyma (3) consists of multiple layers of isodiametric to ovoid cells, varying in size, and loosely arranged with abundant intercellular spaces. The thin primary walls were stained pink. The phloem fibers (4) appear as compact clusters of sclerenchymatous fibers situated externally to the secondary phloem. The cells are rounded, with heavily lignified, thick secondary walls stained blue, often obscuring the cell lumens. The

secondary phloem (5) is composed of multiple layers of sieve elements and companion cells, aligned in radial rows, with thin, undulating primary walls-stained pink. The cambium (6) is a narrow meristematic zone located between the secondary phloem and secondary xylem, consisting of thin-walled, tangentially elongated cells. The secondary xylem vessels (7) comprise large, circular to oval xylem elements with thick lignified walls stained green and prominent lumens. Interspersed within the secondary xylem are the medullary rays (8), composed of radially arranged, parenchymatous cells forming continuous bands. The cells have thick, lignified walls stained green and narrow lumens. The pith parenchyma (9) occupies the central region, consisting of large, irregularly shaped, polygonal cells with thin primary walls and numerous intercellular spaces. Some cells undergo gradual lignification, staining green (Fig. 3).

Microscopic characteristics of the leaf: The transverse section of the *A. pedunculata* leaf shows a distinct convex shape on both surfaces of the midrib, with the lower surface being more prominent than the upper one. The thickness of the midrib is 3-4 times greater than that of the leaf blade. The detailed anatomical structure is described in Fig. 4.

Midrib Structure: The lower epidermis (1) consists of very small, closely packed cells with an outer wall cutinized and stained blue. The lower collenchyma (2) comprises 3-5 layers of polygonal cells that gradually increase in size, tightly packed, with thickened corners-stained deep pink. The parenchyma (3, 8) consists of large, irregularly shaped polygonal cells arranged loosely with many intercellular spaces, with thin walls stained light pink. The sclerenchyma arch (4) consists of 5-8 layers of polygonal cells, densely packed, with thick lignified walls stained blue, encircling the vascular bundle. The phloem (5), situated between the xylem and sclerenchyma arch, consists of many small, closely packed cells with thin cellulose walls-stained pink, divided into two crescent-shaped bundles at the top and bottom. The xylem (6) lies interior to the phloem, containing irregularly sized circular or polygonal cells aligned in radial rows, with thick lignified walls stained blue. The middle of the midrib consists of parenchyma (7) with polygonal cells, loosely arranged with numerous intercellular spaces, with thin walls-stained pink. The upper collenchyma (9) and the upper epidermis (11) share similar characteristics with the lower side of the midrib. Within the parenchyma, near the epidermis, occasional essential oil sacs (10) of large oval shape can be observed.

Leaf blade Structure: The lower epidermis (a) consists of very small cells tightly packed together, with the outer wall cutinized and stained blue. The spongy mesophyll (b) is composed of loosely arranged oval to round parenchyma cells of variable size, forming prominent intercellular spaces. The thin primary walls are stained pink. The palisade mesophyll (c) comprises 1-2 layers of rectangular parenchyma cells, closely packed in vertical columns and oriented perpendicularly to the epidermis. These cells also possess thin walls and are stained pink. The upper hypodermis (d) consists of a single layer of polygonal parenchyma cells, tightly arranged immediately above the palisade layer. The upper epidermis (e) exhibits a structural composition similar to the lower epidermis.

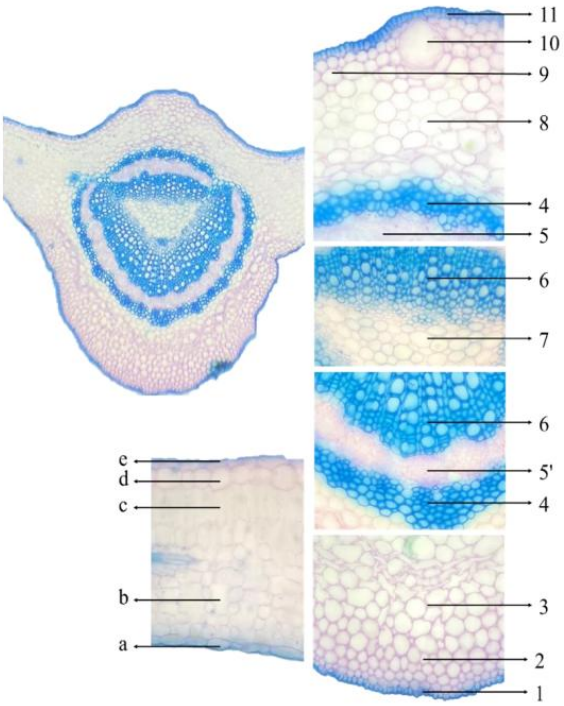

Fig. 4. Microscopic characteristics of the leaf of the studied specimen (*Midrib Structure: 1. Lower epidermis; 2. Lower collenchyma; 3,8. Cortex parenchyma; 4. Sclerenchymatous fiber sheath; 5. Phloem; 6. Xylem; 7. Pith parenchyma; 9. Upper collenchyma; 10. Essential oil secretory sac; 11. Upper epidermis;*
Leaf blade Structure: a. Lower epidermis; b. Spongy mesophyll; c. Palisade mesophyll; d. Upper hypodermis; e. Upper epidermis).

Microscopic characteristics of the fruit peel: The microscopic examination of the *A. pedunculata* fruit peel reveals that its cross-section is nearly square, clearly divided into four lobes and four locules (seed chambers). It exhibits a secondary structure.

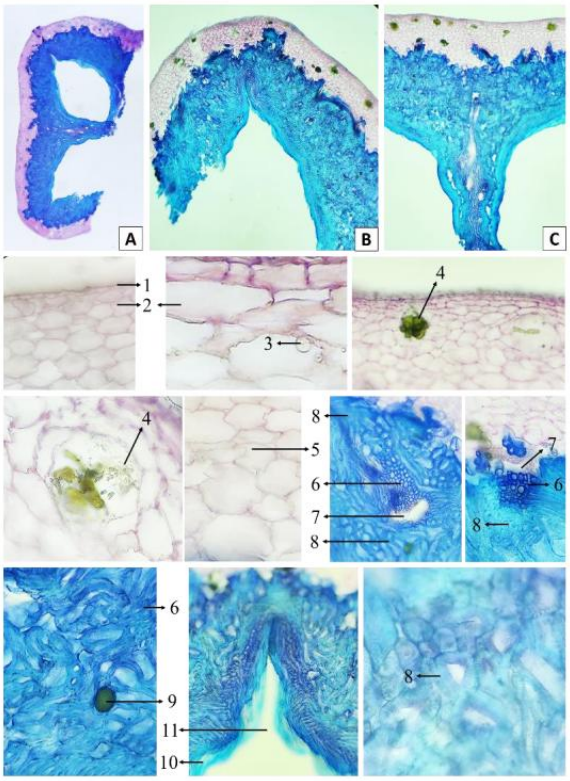

Fig. 5. Microscopic characteristics of the *A. pedunculata* fruit peel
(A) Transverse section of the fruit peel (half-section), revealing a nearly square shape, distinctly divided into four lobes and four locules (seed chambers), and exhibiting secondary structure; (B) The corner region of the section observed under a 4× objective lens; (C) The partition wall between fruit compartments observed under a 4× objective lens; 1. Epidermis; 2. Collenchyma; 3. Starch grains; 4. Essential oil secretory sac; 5. Parenchyma; 6. Xylem*; 7. Phloem; 8. Sclerenchyma (sclereids); 9. Lipid inclusions; 10. Sclereid cells; 11. Seed cavity.*

Epidermis (1) consists of very small, narrow cells tightly packed together. Collenchyma (2) comprises 1-3 layers of polygonal cells that gradually increase in size, arranged in an alternating pattern with the epidermis. These cells are about 4-5 times wider than epidermal cells, with corner-thickened walls staining a deep pink. Parenchyma (5) is composed of large, irregularly shaped polygonal cells loosely arranged. Within this tissue, one may occasionally observe essential oil secretory sacs (4) containing yellowish lipid inclusions (9) or spherical starch grains (3) with a clearly visible hilum. Sclerenchyma (8) consists of numerous thick-walled cells (sclereids) that stain blue. These sclerenchyma cells form a continuous arc, accounting for up to two-thirds of the peel's thickness in the transverse section. Scattered xylem-phloem bundles occur within the sclerenchyma and may be located along the partition walls between empty compartments in section (C) or at the four corners of section (B). The secondary xylem (6) comprises relatively uniform, circular vessels smaller than the surrounding sclereids, with thick, lignified walls staining blue and hollow lumens. Secondary phloem (7) comprises multiple layers of tightly packed, very small, thin-walled cells that stain pink and are difficult to discern. Adjacent to the seed cavity (11) lies a layer of sclereid cells (10) that are relatively long and narrow, densely arranged in a brush-like fashion (Fig. 5).

3.3. Characteristics of Medicinal Powder

Stem powder*:* The powder is greenish brown with a mild aroma. Under a 40× objective lens, the following features can be observed: (1) Sclerenchyma fibers: Long fibers (~20 μm in diameter), scattered, with thick cell walls and narrow lumens. (2) Pitted vessel fragments: Present either in bundles or singly. (3) Spiral vessel fragments: About 20-30 μm in diameter. (4) Collenchyma fragments: Polygonal, thick-walled cells, tightly packed. (5) Parenchyma fragments: Rectangular cells, approximately 30-40 μm in length. (6) Essential oil droplets: Oval or circular droplets, 50-70 μm in diameter, scattered throughout the sample. (7) Protective trichomes, usually 2-3 cells, pointed tip, thick-walled, 100-150 μm, often broken (Fig. 6).

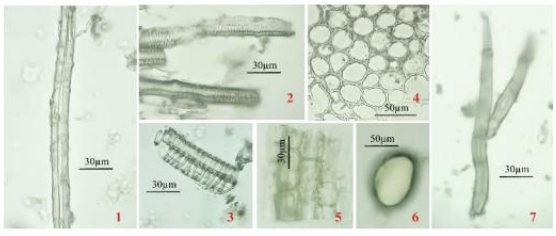

Fig. 6. Stem powder
(1. Sclerenchyma fibers; 2. Pitted vessel fragments; 3. Spiral vessel fragments; 4. Collenchyma fragments; 5. Parenchyma fragments; 6. Essential oil droplets; 7. Protective trichomes)

Leaf powder: The powder is dark green with a mild aroma. Under a 40× objective lens, the following features can be observed: Sclerenchyma fibers: Long fibers (~20 μm in diameter), scattered, with thick cell walls and narrow lumens. Epidermal fragments bearing stomata: Elliptical stomata measuring about 20 μm × 30 μm. Pitted vessel fragments and Spiral vessel fragments: Approximately 20-25 μm in diameter, scattered throughout. Collenchyma fragments: Polygonal cells with thick walls, tightly packed. Upper epidermis: Polygonal cells arranged closely together, lacking stomata. Essential oil droplets: Oval or circular droplets, 50-70 μm in diameter, scattered throughout. Protective trichomes: Characterized by a pointed tip, typically consisting of two cells; often broken, appearing single-celled, with relatively thick walls, about 100-150 μm in length.

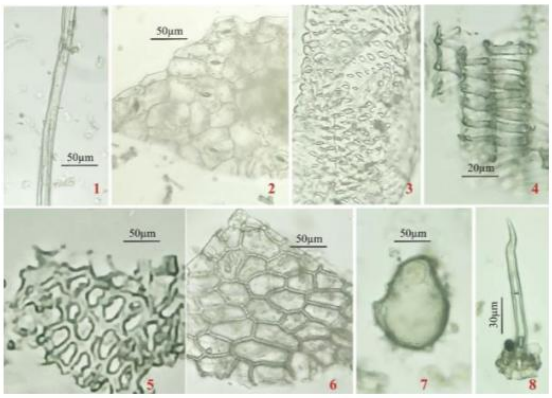

Fig. 7. Leaf powder
(1. Sclerenchyma fibers; 2. Epidermal fragments with stomata; 3. Pitted vessel fragments; 4. Spiral vessel fragments; 5. Collenchyma fragments; 6. Upper epidermis; 7. Essential oil droplets; 8. Protective trichomes).

Fruit Powder: The powder is gray with a mild aroma. Under a 40× objective lens, the following features can be observed: Collenchyma fragments (1) and parenchyma fragments (2), measuring about 20 μm × 30 μm. Sclerenchyma fibers (3): Long (~20 μm in diameter), scattered, with thick walls and narrow lumens. Protective trichomes (4): Pointed tips, typically consisting of two cells; often broken, appearing single-celled, with relatively thick walls, about 100-150 μm in length. Pitted vessel fragments (5), spiral vessel fragments (6), and scalariform vessel fragments (7) are scattered throughout. Sclerenchyma cells (8-9): Thick-walled, somewhat round to polygonal shape, about 30 × 50 μm. Starch grains (10-11): Usually solitary and scattered, ranging from spherical to sub-spherical with a distinct hilum, about 15-30 μm in diameter. Brown pigmented fragments (12): Measuring about 25-40 μm, observable in the field of view (Fig. 8).

Fig. 8. Fruit powder
(1. Collenchyma fragments; 2. Parenchyma fragments; 3. Sclerenchyma fibers; 4. Protective trichomes; 4. Pitted vessel fragments; 6. Spiral vessel fragments; 7. Scalariform vessel fragments; 8-9. Sclerenchyma cells; 10-11. Starch grains; 12. Brown pigmented fragments).

3.3. Qualitative determination of chemical composition

Preliminary qualitative analysis of several organic compound groups in the stems, leaves, and fruits of the studied samples using chemical reactions showed that both samples (stems, leaves, and fruits) tested positive for flavonoids, coumarins, saponins, organic acids, tannins, reducing sugars, fats, sterols, amino acids, and essential oils. Notably, the reactions for saponins, tannins, and reducing sugars were significantly more pronounced in the stem and leaf samples compared to the fruit samples. In contrast, amino acids exhibited a more substantial positive reaction in the fruits. The essential oil content was determined to be 0.08% in the stems and leaves, and 0.167% in the fruits. Alkaloids showed an unclear or weakly positive reaction.

The qualitative results are presented in Table 1.

Table 1. Qualitative results of organic groups in the research sample using chemical methods

No.	Compound groups (Extract)	Qualitative reaction	Stems and leaves	Fruits	Conclusion
1	Flavonoids (methanol extraction)	Cyanidin	(++)	(+)	Yes
		Ammonia	(+)	(+)	
		NaOH 10%	(+)	(+)	
		$FeCl_3$ 5%	(+++)	(+++)	
2	Alkaloids (chloroform extract after acidification with H_2SO_4)	Bouchardat reagent	(-/+)	(-/+)	Unclear
		Mayer reagent	(-/+)	(-/+)	
		Dragendorff reagent	(-/+)	(-/+)	
3	Cardiac Glycosides (chloroform fraction of 25% ethanol extract, tannin-free)	Liebermann reagent	(-)	(-)	No
		Legal reagent	(-)	(-)	
		Baljet reagent	(-)	(-)	
		Keller- Kiliani	(-)	(-)	
4	Anthranoids (chloroform extract)	Borntraeger	(-)	(-)	No
5	Coumarins (ethanol 90% extract)	Opening and Recyclization of the Lactone ring	(+)	(+)	Yes
		Fluorescence observation	(-)	(-)	
6	Saponins (aqueous extract)	Foaming phenomenon	(+)	(-)	Yes
		Salkowski	(++)	(++)	
7	Tannins (aqueous extract)	$FeCl_3$ 5%	(+++)	(+++)	Yes
		Lead acetate reagent	(+++)	(+)	
		Gelatin 1 % reagent	(+++)	(+)	
8	Reducing sugars (aqueous extract)	Fehling reagent	(++)	(+)	Yes
9	Polysaccharides (aqueous extract)	Lugol reagent	(-)	(-)	No
10	Organic acids (aqueous extract)	Na_2CO_3	(+)	(+)	Yes
11	Fats (*n*-hexane extract)	Filter paper blur reaction	(+)	(+)	Yes
12	Sterols (*n*-hexane extract)	H_2SO_4	(-)	(-)	Yes
13	Carotenes (*n*-hexane extract)	H_2SO_4	(-)	(-)	No
14	Amino acids (aqueous extract)	Ninhydrin reagent	(+)	(++)	Yes
15	Essential oils (stems, leaves, and fruits)	Steam distillation	(++) 0.08%	(+++) 0.167%	Yes

Note: (-): negative; (+): positive; (++): clearly positive; (+++): very clearly positive; (-/+): unclear.

3.4. Study on the essential oil of A. Pedunculata

The essential oil content of the fruits of *A. pedunculata* collected from Quang Ninh was determined to be 0.167% (calculated based on the dry weight of the sample). Using the Gas Chromatography-Mass Spectrometry (GC-MS) method, 23 compounds were identified in the essential oil of the fruits (Table 2). Among them, α-pinene (21.2%), caryophyllene (30.4%), and (-)-spathulenol (12.6%) were the most abundant constituents.

Table 2. GC-MS analysis results of the essential oil from *A. pedunculata* fruits

Retention (min)	Compound name	% (Peak area)
6.13	*α*-Pinene	21.2
7.19	*β*-Pinene	0.4
7.42	*β*-Myrcene	1.0
8.49	Limonene	1.9
8.62	*β*-Ocimene	2.2
8.91	1,3,7-Octatriene,3,7-dimethyl-	3.4
10.15	*p*-Cymene	1.0
10.38	cis-*p*-Mentha-2,8-dien-1-ol	2.2
10.72	1,3,8-*p*-Menthatriene	0.6
11.14	3,5-Dimethylanisole	2.7
11.70	Camphene hydrate	2.5
12.44	Terpinen-4-ol	0.5
13.07	*α*-Terpineol	1.3
13.89	*cis*-Sabinene hydrate acetate	1.4
14.67	*trans*-Sabinene hydrate acetate	1.2
17.93	Isoledene	2.3
19.08	Caryophyllene	30.4
19.97	Humulene	2.5
20.08	Alloaromadendren	1.1
20.58	*γ*-Thujaplicin	1.2
21.50	*β*-Cadiene	1.0
22.65	(*E*)-Nerolidol	1.4
23.15	(-)-Spathulenol	12.6

The results of antimicrobial testing on three microbial strains indicated no inhibitory effect against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* at the highest tested concentration (dilution 1/32).

Table 3. MIC & MBC results of the essential oil in the research sample

Microbial Strain	MIC			MBC		
	Dilution			Dilution		
	Trail 1	Trail 2	Result	Trail 1	Trail 2	Result
Staphylococcus aureus ATCC 25923	>1/32	>1/32	>1/32	nd	nd	**nd**
Escherichia coli ATCC 25922	>1/32	>1/32	>1/32	nd	nd	**nd**
Candida albicans ATCC 10231	>1/32	>1/32	>1/32	nd	nd	**nd**

Note: nd: not determined; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

4. Discussion

For the first time, the research results comprehensively described the morphological and micro-characteristics of the *A. pedunculata* species. Notably, this marks the first publication in Vietnam concerning its micromorphology and powder analysis. These results will contribute to the identification and establishment of quality standards for this medicinal plant in the future. Qualitative analysis of organic compounds by chemical reactions in the stems, leaves, and fruits of *A. pedunculata* in Quang Ninh indicated chemical groups such as flavonoids, coumarins, saponins, reducing sugars, fats, sterols, and essential oils. Excluding the alkaloid tests, which yielded ambiguous results (potentially due to active ingredient concentration or harvest season - requiring further investigation for confirmation), the obtained results align with the chemical composition studies of other *Acronychia* species [11]. The essential oil content in the stems and leaves of *A. pedunculata* in Quang Ninh was not

only qualitatively confirmed but also quantified at approximately 0.08%. This aligns with the essential oil content in the stems and leaves reported by other studies in Vietnam, such as in Me Linh - Vinh Phuc (0.08%) [3] and Tay Thien - Vinh Phuc (0.09%) [4]. This is higher than samples collected in Lam Dong (0.031% in stems and 0.016% in leaves) [7] and Binh Chau - Phuoc Buu (0.032% in leaves) [6], yet considerably lower than the sample from Pu Huong (0.42%) [5]. Meanwhile, the essential oil content in the fruits of *A. pedunculata* harvested in Quang Ninh reached 0.167%, exceeding the content determined by Trinh Thi Diep in Lam Dong (0.101 ± 0.002%) [7], consistent with Trinh Thi Diep's research indicating a significantly higher essential oil content in the fruits compared to the stems and leaves. However, variations exist in the composition of essential oil between the two studies. While caryophyllene (30.4%), α-pinene (21.2%), and (-)-spathulenol (12.6%) are the three most abundant constituents in the *A. pedunculata* fruits essential oil from Quang Ninh, in the Lam Dong samples, the dominant constituents were α-pinene (21.4%), (*E*)-β-ocimene (16.5%), β-caryophyllene (13%), linalool (12.5%), caryophyllene oxide (7.2%), and α-cubebene (7%). Given that the proportions and composition of essential oils in leaves and fruits are susceptible to seasonal and cultivation condition changes, further in-depth investigations are necessary to draw definitive conclusions about the essential oil composition of *A. pedunculata*.

The antimicrobial activity of the fruit essential oil from the studied sample was evaluated; however, no antimicrobial activity was observed at the diluted concentration. To date, there are no documented studies reporting the antimicrobial effects of *A. pedunculata* fruits in Quang Ninh province.

5. Conclusion

The study meticulously described the morphological characteristics (including stems, leaves, flowers, and reproductive structures), the microanatomy of stems and leaves, the powdered medicinal material (derived from stems, leaves, and fruits), and conducted preliminary qualitative analysis of the chemical constituents in the stems, leaves, and fruits using chemical reactions. Additionally, the study quantified the content, composition, and antimicrobial activity of the essential oil extracted from the fruits of *A. pedunculata* harvested in Quang Ninh. The findings of this research provide comprehensive and detailed data on the morphological and anatomical features, as well as the organic compound groups present in *A. pedunculata*. These results serve as valuable resources for academic education, scientific research, and the identification and standardization of this medicinal plant.

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