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Research Article

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Chemical composition and biological activities of acetone extract from the fruit of *Acronychia pedunculata*

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ABSTRACT

Acronychia pedunculata, a member of the Rutaceae family, has long been used in traditional medicine in South and Southeast Asia. This study provides the first comprehensive analysis of the phytochemical constituents, volatile compounds, antibacterial, and antioxidant properties of the acetone extract from A. *pedunculata* fruit. The results revealed that the extract contains several bioactive compounds, including phenolics, tannins, coumarins, terpenoids, steroids, and flavonoids. Additionally, 26 volatile compounds were identified, with 3-furaldehyde; 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; and 5-hydroxymethylfurfural being the major components. The extract demonstrated antibacterial activity against three of the six tested bacterial strains: *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. It also exhibited ABTS radical-scavenging activity with an IC_{50} value of 223.62±4.83 µg/mL.

KEYWORDS: *Acronychia pedunculata*, Antibacterial activity, Acetone extract, Antioxidant activity, Phytochemical constituents, Volatile compounds

INTRODUCTION

The genus *Acronychia* comprises about 50 small shrub species in the Rutaceae family, which are widely distributed across Southeast Asia, Sri Lanka, Australia, India, China, and the Pacific islands (Brophy *et al.*, 2004; Kouloura *et al.*, 2012; Chen *et al.*, 2018; Miyake *et al.*, 2019; Panyasawat *et al.*, 2022). Many species of *Acronychia* have been extensively used in traditional medicine among Australian and Asian populations to treat fungal and microbial infections and serve as anti-hemorrhagic, anti-pyretic, anti-spasmodic, and stomachic agents. Additionally, essential oils extracted from the leaves and flowers of certain *Acronychia* species are utilized in the cosmetic industry, while the aerial parts and fruits are used in food (Sillitoe, 1995). Studies have demonstrated that *Acronychia* species are rich in bioactive components such as coumarins, terpenoids, alkaloids, and acetophloroglucinols (Funayama & Cordell, 1984; Ali *et al.*, 2005; Wisetsai *et al.*, 2022; Nathabumroong *et al.*, 2023). Furthermore, various solvent extracts from Acronychia plants exhibit biological properties, including antioxidant (Su *et al.*, 2003; Kouloura *et al.*, 2012), anti-inflammatory (Pathmasiri *et al.*, 2005; Raju *et al.*, 2022), cytotoxicity (Cui *et al.*, 1999; Tran *et al.*, 2020; Yang *et al.*, 2015), and antibacterial activities (Ranaweera *et al.*, 2016).

Acronychia pedunculata, a shrub commonly found in Southern Asia including Vietnam, Malaysia, Indonesia, Sri Lanka, India, and Southern China (Hartley, 1974; Pham, 1999), has been traditionally used to treat various ailments such as itchy skin, asthma, rheumatism, and diarrhea (Ito *et al.*, 2016a, b; Tanjung *et al.*, 2018). This species has yielded several secondary metabolites, including prenylated acetophenone derivatives (Kumar *et al.*, 1989; Sy & Brown, 1999; Su *et al.*, 2003) and furoquinoline alkaloids (Waterman, 1975; de Silva *et al.*, 1979; Cui *et al.*, 1999). Solvent extracts from various parts of A. *pedunculata* have demonstrated antiplasmodial (Horgen *et al.*, 2001) properties. While some studies have reported on the chemical composition and biological activities of A. *pedunculata*

extracts, there is limited information on the acetone extract of this species. Therefore, this study investigates for the first time the phytochemical screening, volatile compounds, and antibacterial and antioxidant properties of the acetone extract from A. *pedunculata* fruit.

MATERIALS AND METHODS

Plant Sample

The fruits of *Acronychia pedunculata* were collected from Binh Chau-Phuoc Buu Nature Reserve, Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam (Figure 1). The vouchered specimen, Le VS 1125, was deposited at the Herbarium of Binh Chau-Phuoc Buu Nature Reserve.

Bacterial Strains

Six bacterial strains were used to determine the antibacterial properties of the studied sample: 4 Gram-negative strains (*Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13976, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853,) and 2 Gram-positive strains (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 13883).

Extraction Procedures

The fresh fruit of A. *pedunculata* was dried at 50 ℃ to constant weight, then ground into powder. Five hundred milliliters of acetone (99%, Thermo Fisher Scientific) was used to immerse 100 grams of the powder for 72 hours. The Whatman paper was used to collect the first filtrate. The procedure was repeated two more times, and the final extract was recovered by combining all the filtered fractions. The solvent was then eliminated in vacuum condition at a temperature of 45℃.

Detection of Major Phytochemical Compounds

The phytochemical screening of the studied extract was determined using the qualitative assays presented Table 1.

Gas Chromatography/Mass Spectrometry Assays

The volatile compounds of the acetone extract were identified using the TRACE 1310 Gas Chromatograph in conjunction with the ISQ 7000 mass spectrometer (Thermo Fisher Scientific, USA). The experimental procedure was performed according to prior our work (Nguyen *et al.*, 2023). An Agilent DB-5MS column (30 m × 0.25 mm × 0.25 μm) was used with helium as the carrier gas at a flow rate of 1.2 mL/min. The injection chamber was maintained at 270 ℃, and samples were introduced in split mode with a 30:1 split ratio and split flow rate of 36 mL/min. The column temperature was programmed at 80 ℃ for 5 minutes, then increase (20 ℃/min) to 280 ℃ for 10 minutes, and finally reaches 300 ℃ for 3 minutes. The ion source and transfer line were set at 250 ℃ and 280 ℃, respectively. Electron impact ionization (70 eV) was applied, with a mass range of 29-650 m/z and scan rate of two scans/sec. Chemical composition were identified based on the NIST 2017 library.

Determination of Antibacterial Activity

Disk diffusion method was used to determine the antibacterial property of the extract according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018) in which gentamicin disk (10 μg, Nam Khoa BioTek, Vietnam). The experiment was conducted in triplicate and Fisher's least significant difference (LSD) and one-way analysis of variance (ANOVA) were employed for statistical analysis.

ABTS Radical Scavenging Assay

Antioxidant activity was determined by ABTS radical scavenging assay described by Maeng *et al.* (2017) with slight modifications.

Figure 1: *Acronychia pedunculata*. a) The plant in habitat, b) Flowers and c) Fruits. (Photos: Van Son Le)

Table 1: The methods used in phytochemical screening of *Acronychia pedunculata*

Phytochemical	Reagent	Positive reaction	References
Phenolic and tannin	2 mL extract + 2 mL H_2O+2-3 drops $FeCl_3$ 5%	color change to brownish to blackish green	Deka *et al.*, 2017
Alkaloid	2 mL extract + 3-4 drops Wagner reagent	reddish-brown precipitate formation	Bodi *et al.*, 2014
Flavonoid	2 mL extract + 2 mL Pb $(COOH)_2$ 10%	yellow precipitate formation	Nguyen *et al.*, 2017
Saponin	2 mL extract + 10 mL H_2O + 2-minute ebullition	foam formation	Shaikh & Patil, 2020
Terpenoid and steroid	5 mL extract + 2 mL $CHCl_3$ + 3 mL H_2SO_4 concentrate	color change to brownish red	Llauradó *et al.*, 2013
Coumarin	2 mL extract + 3 mL NaOH 10%	color change to yellow or dark yellow	Vo *et al.*, 2017

Initially, solution A comprised of 7 mM ABTS and 2.45 mM $K_2S_2O_8$ was prepared and incubated at 37 °C for 18 hours in the dark. Subsequently, a mixture of 3 mL of solution A, 0.1 mL of the studied extract, and 1.9 mL of acetone was made, followed by 15-minute incubation in the dark. To assess the ABTS radical scavenging activity, the reaction was measured for absorbance at 734 nm using UVS 2800 spectrophotometer (Labome, USA). Ascorbic acid served as the reference standard and the sample concentration was deduced from the standard curve and expressed as μg/mL of ascorbic acid.

RESULTS AND DISCUSSION

Detection of Major Phytochemical Compounds

The phytochemical screening of the acetone extract obtained from *A. pedunculata* fruit was shown in Table 2. Accordingly, the extract contains four out of six major bioactive compounds, including phenolics and tannins; coumarins; terpenoids and steroids; and flavonoids. This aligns with previous study on *A. pedunculata* collected in India. All four extracts of ethyl acetate, petroleum ether, methanol, and chloroform contained sterols, triterpenes, carbohydrates, and glycosides. Saponins and alkaloids were exclusive to the methanol extracts, with tannins found only in the methanol stem extract. Additionally, flavonoids were present in the methanol extracts and the chloroform stem extracts (Gireesha & Raju, 2016). Additionally, the leaf ethanol extracts of *A. pedunculata* collected in Vietnam were also reported to contain polyphenol and flavonoid contents (Phung *et al.*, 2021).

The Volatile Compounds of the Fruit Acetone Extract

The chemical constituents of the acetone extract were presented in Table 3 with a total of 26 volatile compounds. According to the gas chromatograms presented in Figure 2, 3-Furaldehyde; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 5-Hydroxymethylfurfural; 3,4-Altrosan; D-Mannose; 9(E),11(E)-Conjugated linoleic acid; and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- were found to be major components. To date, no other phytochemical compositions of extracts from *A. pedunculata* have been reported, aside from those identified in its essential oil. The essential oil isolated from the aerial parts of *A. pedunculata* collected from Vinh Phuc province, Vietnam was found to be rich in α-pinene (57.4%), caryophyllene (13.6%), and caryophyllene oxide (3.0%) (Lesueur *et al.*, 2008). Recently, the major components of the essential oil from *A. pedunculata* leaf collected from Ba Ria-Vung Tau province, Vietnam included caryophyllene (57.63%), globulol (13.03%), and β-ocimene

Table 2: The phytochemical screening of *Acronychia pedunculata*

Phytochemical components	*A. pedunculata*
Phenolics and tannins	+
Alkaloids	-
Flavonoids	+
Saponins	-
Terpenoids and steroids	+
Coumarins	+

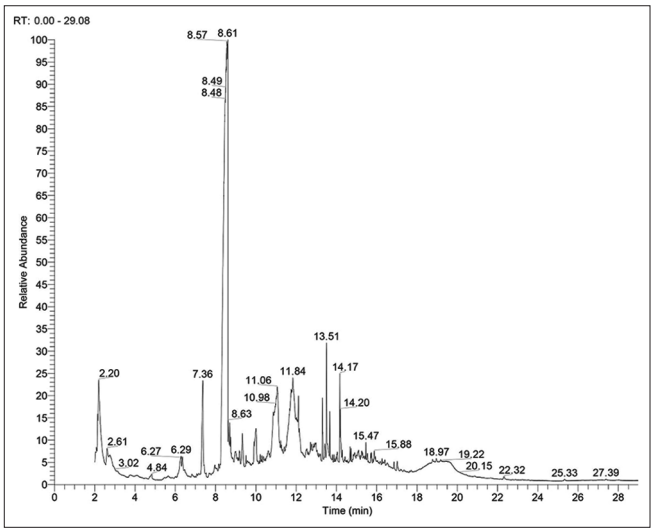

Figure 2: The GC chromatogram of acetone extract from *Acronychia pedunculata* fruit

(10.91%) (Van *et al.*, 2021). More recently, essential oils extracted from various parts of *A. pedunculata* grown in Lam Dong province, located in the Central Highlands of Vietnam, have also been studied with distinct compositions: the stem oil was rich in caryophyllene oxide (35.3%) and spathulenol (31.9%), the leaf oil contained caryophyllene oxide (51.3%) and linalool (21.0%), and the fruit oil featured α-pinene (21.4%), (E)-β-ocimene (16.5%), linalool (12.5%), and β-caryophyllene (13.0%) (Diep *et al.*, 2023).

Antibacterial Activity

Overalls, the acetone extract from *A. pedunculata* fruit was found to be effective against three out of six studied bacterial strains, including *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the inhibition zone diameter of 12.67±2.02, 10.75±0.66 and 7.5±0.87 mm respectively (Table 4). These findings suggest that the acetone extract is particularly effective against Gram-positive bacteria,

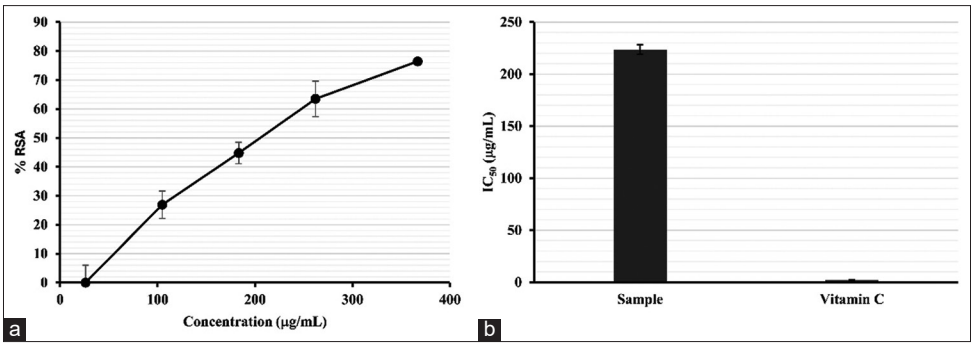

Figure 3: ABTS radical scavenging activity of acetone extract from *A. pedunculata* fruit in a) various concentration and b) IC_{50} value

Table 3: Chemical components of acetone extract from *Acronychia pedunculata* fruits

S. No.	RT	Compounds	Formula
1	2.20	3-Furaldehyde	$C_5H_4O_2$
2	6.23	3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl-	$C_6H_{10}N_2O$
3	7.36	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$
4	8.53	5-Hydroxymethylfurfural	$C_6H_6O_3$
5	8.71	6-Acetyl-b-d-mannose	$C_8H_{14}O_7$
6	8.75	2-Hexenal, 2-ethyl-	$C_8H_{14}O$
7	9.33	2H-Pyran-2-acetic acid, tetrahydro-	$C_7H_{12}O_3$
8	9.51	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	$C_{10}H_{18}O_2$
9	9.91	Vanillin	$C_8H_8O_3$
10	10.00	1,4-Dioxane, 2-ethyl-5-methyl-	$C_7H_{14}O_2$
11	11.06	3,4-Altrosan	$C_6H_{10}O_5$
12	11.84	D-Mannose	$C_6H_{12}O_6$
13	12.12	b-(4-Hydroxy-3-methoxyphenyl) propionic acid	$C_{10}H_{12}O_4$
14	13.31	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
15	13.33	Dibutyl phthalate	$C_{16}H_{22}O_4$
16	13.43	Scopoletin	$C_{10}H_8O_4$
17	13.51	Cirsiumaldehyde	$C_{12}H_{10}O_5$
18	13.67	2H,8H-Benzo[1,2-b: 5,4-b']dipyran-2-one, 8,8-dimethyl-	$C_{14}H_{12}O_3$
19	14.17	9(E),11(E)-Conjugated linoleic acid	$C_{18}H_{32}O_2$
20	14.20	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	$C_{18}H_{30}O_2$
21	14.29	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$
22	14.68	Brayelin	$C_{15}H_{14}O_4$
23	14.72	7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-	$C_{13}H_{10}O_5$
24	15.47	Trachyloban-18-oic acid	$C_{20}H_{30}O_2$
25	16.86	(E)-3,3'-Dimethoxy-4,4'-dihydroxystilbene	$C_{16}H_{16}O_4$
26	17.01	9,12-Octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis	$C_{28}H_{42}O_4$

Table 4: Antibacterial activity of acetone extract from *Acronychia pedunculata* fruits

Bacterial strains	Inhibition zone diameter (mm)	
	Studied sample	Gentamycin
Bacillus cereus	12.67 ± 2.02^a	16.5 ± 0.25^b
Escherichia coli	-	13.5 ± 0.50
Pseudomonas aeruginosa	7.5 ± 0.87^a	13.67 ± 1.18^b
Salmonella enteritidis	-	14.52 ± 0.71
Salmonella typhimurium	-	18.45 ± 0.67
Staphylococcus aureus	10.75 ± 0.66^a	17.42 ± 1.04^b

[a,b]Different superscript lower-case letters in the same row denote significant differences ($p<0.05$)

consistent with earlier studies on different methanol extracts of A. *pedunculata* grown in India (Gireesha & Raju, 2016), Vietnam (Van *et al.*, 2020), and Sri Lanka (Rodrigo, 2023). Meanwhile, essential oils from *A. pedunculata* aerial parts in Vinh Phuc province, Vietnam, inhibited various bacterial strains, including *B. subtilis*, *S. aureus*, *S. epidermidis*, *B. cereus*, *E. faecalis*, *E. cloacae*, *M. luteus*, *S. marcescens*, *E. coli*, *S. enterica*, and *P. aeruginosa* (Lesueur *et al.*, 2008). Similarly, essential oils from the stem, leaf, and fruit of A. *pedunculata* in Lam Dong province showed activity against *B. subtilis*, *S. aureus*, *L. fermentum*, *E. coli*, *S. enterica*, and *P. aeruginosa* (Diep *et al.*, 2023).

Antioxidant Activity

The ABTS radical scavenging activity of the studied sample was shown in Figure 3. Accordingly, the extract possessed antioxidant activity with IC_{50} value of 223.62 ± 4.83 µg/mL compared to ascorbic acid ($IC_{50}=2.02\pm0.13$ µg/mL). Previous studies on A. *pedunculata* extracts, such as those utilizing ethanol, reported similar antioxidant effects, albeit with approximately two-fold lower potency ($IC_{50}=612.9\pm12.9$ µg/mL), highlighting the critical role of phenolic and flavonoid compounds in free radical neutralization (Phung *et al.*, 2021).

CONCLUSION

This study presents the first comprehensive investigation on the chemical composition, antibacterial, and antioxidant properties of the acetone extract from A. *pedunculata* fruits. The findings provide valuable insights into the bioactive potential of this species, serving as a foundational reference for future research on medicinal materials and supporting its potential applications in the pharmaceutical and related industries.

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