Original articles

Phytochemical screening, total phenolic and flavonoid contents and antioxidant activity of *Alpinia pinnanensis* T.L.Wu & S.J.Chen (Zingiberaceae)

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Abstract

Background: *Alpinia pinnanensis* is commonly used in Vietnamese ethnomedicine for treating various ailments such as cough, fever, abdominal pain, flatulence and stomachaches. However, research on *A. pinnanensis* remains limited.

Objectives: This study was conducted to perform a preliminary phytochemical screening, quantify the total phenolic and flavonoid contents and assess the antioxidant potential of *A. pinnanensis*.

Materials and methods: The whole of *A. pinnanensis* was collected from A Luoi district, Thua Thien Hue province. Phytochemical analysis was conducted through specific chemical reactions. Total phenolic and flavonoid contents were quantified using the Folin-Ciocalteu and the aluminum chlorid assays, respectively. Antioxidant activity was assessed via the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays.

Results: Phytochemical analysis revealed the presence of flavonoids, coumarins, saponins and tannins. The aerial part extract showed higher total phenolic (132.94 ± 1.24 mg GAE/g) and flavonoid (13.50 ± 0.11 mg QE/g) contents compared to the underground part. Furthermore, the aerial part extract demonstrated significant antioxidant activity in both DPPH and ABTS radical scavenging assays with IC_{50} values of 19.03 ± 0.11 µg/mL and 2.63 ± 0.18 µg/mL, respectively.

Conclusion: This study provided the first detailed report on phytochemicals, total phenolic and flavonoid contents and antioxidant activity of *A. pinnanensis*, highlighting its potential for further exploration in pharmaceutical and neutraceutical applications.

Keywords: *Alpinia pinnanensis; total phenolic; total flavonoid; DPPH; ABTS.*

1. BACKGROUND

Free radicals are unstable molecules that can induce damage to cellular components, including lipids, proteins and DNA, thereby facilitating the pathogenesis of various serious diseases such as cancer, cardiovascular disorders, diabetes and aging. Natural antioxidants play a pivotal role in protecting human health by scavenging free radicals, thereby reducing oxidative stress and preventing the development of numerous pathological disorders. Consequently, the identification and evaluation of natural antioxidant sources, particularly plant-derived compounds such as phenolics and flavonoids, are essential for the development of effective and safe therapeutic agents aimed at disease prevention and health enhancement [1, 2].

Alpinia Roxb., a diverse genus within the Zingiberaceae family, includes around 230 species mostly found in tropical and subtropical regions [3]. The chemical composition of *Alpinia* species varies, but they generally contain bioactive compounds such as essential oils, phenolic compounds and flavonoids, which contribute to their medicinal properties, flavor, and fragrance. Traditionally, *Alpinia* species have been used to treat a variety of health issues, including digestive problems, inflammation and respiratory ailments. Extracts and compounds from these plants demonstrated anti-inflammatory, antioxidant, antimicrobial, analgesic, digestive and immune-boosting effects, highlighting their potential for future research in neutraceuticals and pharmaceuticals [3, 4].

Species of the *Alpinia* genus are widely used in the daily lives of Vietnamese communities [5]. *Alpinia pinnanensis* T.L.Wu & S.J.Chen is a herbaceous plant that grows up to 1.5 meters tall. It has lanceolate leaves with golden velvety hairs

along the midvein and margin, an attenuate and acuminate leaf base. The flowers are yellow and the fruit is a red, globose capsule [6]. This species has been recorded in Lao Cai, Son La, Phu Tho, Vinh Phuc, Nghe An and Thua Thien Hue provinces [5, 7]. *A. pinnanensis* is commonly used in Vietnamese ethnomedicine for treating various ailments such as cough, fever, abdominal pain, flatulence and stomachaches [5]. Previous phytochemical studies of this species have identified diarylheptanoids, flavonoids, and triterpenoids [7]. Extensive research has been conducted on the essential oils of this species, particularly focusing on its chemical constituents and biological activities [8, 9]. The findings of this study will provide valuable insights into the phytochemical composition, total phenolic and flavonoid contents and antioxidant potential of the ethanol extract from *A. pinnanensis*.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant material

The whole of *Alpinia pinnanensis* T.L.Wu & S.J.Chen (Zingiberaceae) was collected in A Luoi district, Thua Thien Hue province in July 2024 (Figure 1). The plant material was identified by Dr. Anh Tuan Le (Mientrung Institute for Scientific Research, Vietnam National Museum of Nature, VAST, Vietnam). The fresh medicinal herbs (1.0 kg) were washed, divided into aerial and underground portions, dried and ground into fine powder. The powdered herbs were stored in clean polyethylene bags under cool condition until extraction.

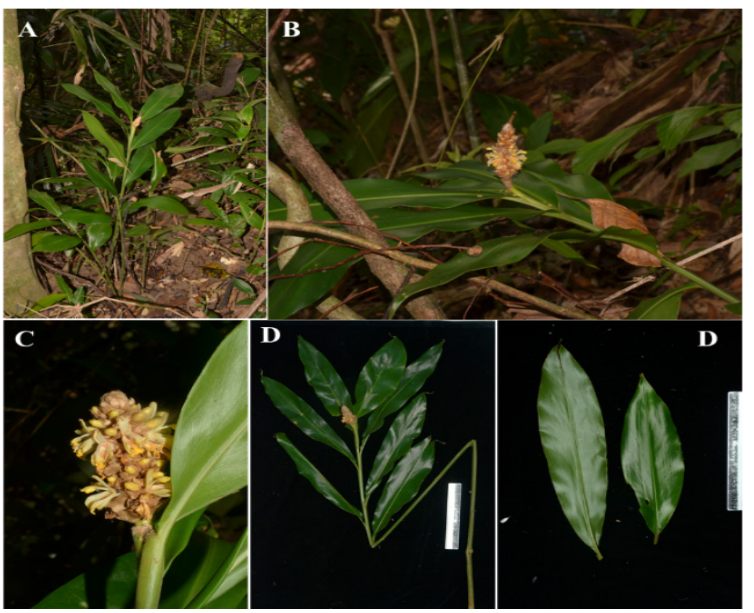

Figure 1. Some of the pictures of *Alpinia pinnanensis*
A: Plant; B: Leaves and inflorescence; C: Inflorescence; D: Leaves

2.1.2. Chemicals and reagents

Folin-Ciocalteu reagent and potassium persulfate were purchased from Shanghai Zhanyun Chemical. Quercetin was supplied by Biopurify Phytochemicals Ltd., while anhydrous aluminum chloride was obtained from Sigma-Aldrich. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were obtained from Tokyo Chemical Industry, VWR BDH Chemicals and Cool Chemical Science and Technology, respectively. Anhydrous sodium carbonate was purchased from Guangdong Guanghua Sci-Tech Co., Ltd. Methanol and absolute ethanol were purchased from Xilong Scientific Co., Ltd. and Vylab Company Limited, respectively. All the chemicals and reagents used in the present study were of analytical grade.

2.1.3. Apparatus and equipments

Drying oven (UF160, Memmert), electrical grinder (YB-2500A, China), refrigerator (Toshiba Inverter, Japan), rotary evaporator (Buchi Rotavapor R-100, Switzerland), UV-Visible spectrophotometer (V-730, Jasco, Japan), analytical balance (HR-250AZ, Japan), moisture analyzer (AND MX-50, Japan) were used in the study.

2.2. Methods

2.2.1. Preparation of the extract

The powder of dried aerial and underground parts of *A. pinnanensis* (10.0 g, each sample) were macerated with ethanol (100 mL × 3 times) at room temperature for three days with intermittent shaking and stirring. The ethanol extracts were subsequently filtered through cotton and filter paper. The evaporation was performed with a rotary evaporator. The obtained extracts were stored under refrigerated condition for further analysis.

2.2.2. Preliminary phytochemical screening

Phytochemical screening of the aerial and underground extracts of *Alpinia pinnanensis* was performed to detect the presence of alkaloids, anthranoids, coumarins, cardiac glycosides, flavonoids, saponins and tannins using specific chemical tests [10].

2.2.3. Determination of phenolic and flavonoid contents

2.2.3.1. Determination of total phenolic content

Total phenolic content was quantified via a slightly modified Folin-Ciocalteu colorimetric assay [11]. Briefly, 0.2 mL of each test sample was combined with 0.8 mL of distilled water and 1.0 mL of 10% Folin-Ciocalteu reagent. The mixture was shaken for 5 minutes, followed by the addition of 2.5 mL of 7.5% sodium carbonate. After incubation at room temperature in the dark for 30 minutes, absorbance was measured at 760 nm. A standard calibration curve was constructed using gallic acid. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry extract, according to the formula (1):

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

where TPC represents the total phenolic content expressed in mg/g (as gallic acid equivalents, GAE), C is the concentration of gallic acid obtained from the calibration curve (mg/mL), V is the volume of the extract (mL), k is the dilution factor and m is the dry mass of the plant extract (g).

2.2.3.2. Determination of total flavonoid content

The total flavonoid content (TFC) was assessed using the aluminum chloride colorimetric assay [12]. A 2.0 mL aliquot of each sample was mixed with 2.0 mL of 2% $AlCl_3$ solution and incubated at room temperature for 10 minutes. Absorbance was then recorded at 440 nm. The standard calibration curve was established using quercetin as a reference compound. The results were expressed as milligrams of quercetin equivalents per gram of dry extract, following formula (2):

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

where TFC represents the total flavonoid content expressed in mg/g (as quercetin equivalents, QE), C is the concentration of quercetin obtained from the calibration curve (mg/mL), V is the volume of the extract (mL), k is the dilution factor and m is the dry mass of the plant extract (g).

2.2.4. Evaluation of antioxidant activity

2.2.4.1. DPPH assay

Antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with slight modifications [13]. In each test tube, 2 mL of 0.135 mM DPPH solution was combined with 2 mL of the sample at varying concentrations and incubated for 30 minutes at room temperature. The absorbance was then measured at 517 nm. A solution containing 2 mL of DPPH and 2 mL of methanol was given as a control. Ascorbic acid was used as a standard. The scavenging activity was calculated using the following formula (3):

$$\mathbf{DPPH\ scavenging\ capacity} = \frac{\mathbf{A\ control - A\ sample}}{\mathbf{A\ control}} \times \mathbf{100} \quad (3)$$

where $A_{control}$ is the absorbance of DPPH solution and methanol, A_{sample} is the absorbance of DPPH solution containing the test sample. The IC_{50} value is the concentration of extract required to scavenge 50% of DPPH radicals, was calculated using Microsoft Excel.

2.2.4.2. ABTS assay

The 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) decolorization assay was conducted based on the method described by Arnao et al. [14, 15], with slight modifications. Stock solutions of 7 mM ABTS and 2.4 mM potassium persulfate were prepared and mixed in equal volumes, then incubated in the dark at room temperature for 14 hours. A working solution was obtained by diluting 1 mL of the resulting solution with 50 mL of methanol to reach an absorbance of 0.82 ± 0.01 at 744 nm. Fresh ABTS solution was prepared prior to each assay. For the test, 2 mL of the plant extract was mixed with 2 mL of the ABTS working solution. The absorbance was recorded at 744 nm after 7 minutes. The antioxidant activity of the extract was compared to the standard antioxidant, ascorbic acid. The radical scavenging activity was determined according to the formula below (4):

$$\mathbf{ABTS\ scavenging\ capacity} = \frac{\mathbf{A\ control - A\ sample}}{\mathbf{A\ control}} \mathbf{x\ 100} \quad (4)$$

where $A_{control}$ is the absorbance of ABTS solution and methanol, A_{sample} is the absorbance of ABTS

solution containing the test sample. The IC_{50} value is the concentration of extract required to scavenge 50% of ABTS radicals, was calculated using Microsoft Excel.

2.2.5. Statistics analysis

All experimental data were analyzed in triplicate using Microsoft Excel for Microsoft 365 MSO (Version 2604). Results are reported as the mean ± standard deviation (SD) derived from three independent replicates for each sample.

3. RESULTS

3.1. Extraction yield

The powdered aerial and underground parts exhibited moisture contents of 6.83% and 8.64%, respectively. The ethanol extraction yields of *Alpinia pinnanensis* were determined to be 12.25% for the underground part and 10.46% for the aerial part. The moisture contents of the extracts were found to be 3.57% for the aerial part and 2.73% for the underground part, respectively.

3.2. Phytochemical screening

The phytochemical screening results of *A. pinnanensis* revealed that coumarins, flavonoids, saponins and tannins were present in both extracts from the aerial and underground parts, as shown in Table 1.

Table 1. Phytochemical screening results of *Alpinia pinnanensis*

No.	Class of phytochemicals	Test/Reagent	Results	
			The underground part	The aerial part
1	**Alkaloids**	Mayer	–	–
		Dragendorff	–	–
		Bouchardat	–	–
2	**Anthranoids**	Borntraeger	–	–
3	**Coumarins**	Lactone ring-opening	++	++
4	**Cardiac glycosides**	Lieberman	–	–
		Legal	–	–
		Keller-Kiliani	–	–
5	**Flavonoids**	Shinoda	+	++
6	**Saponins**	Foam	+++	++
7	**Tannins**	10% $FeCl_3$	+++	+++

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

3.3. Determination of total phenolic (TPC) and total flavonoid contents (TFC)

The total phenolic and flavonoid contents were calculated from the calibration curve for standard gallic acid and quercetin, respectively (Fig. 2).

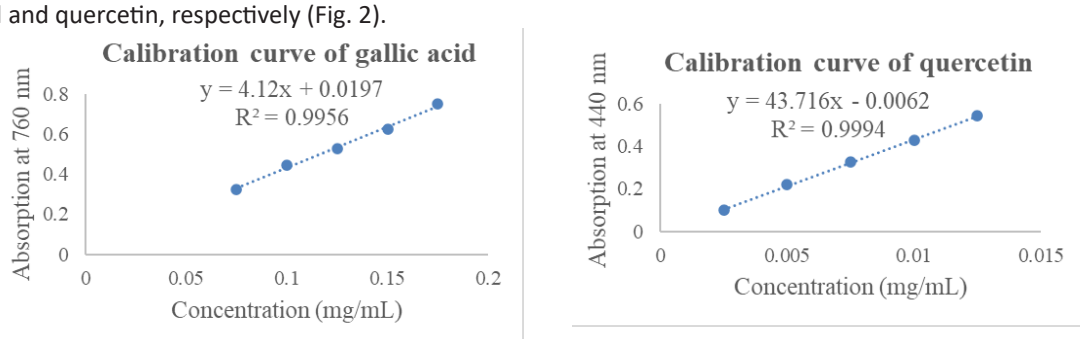

Figure 2. Calibration curve for standard gallic acid and quercetin

The ethanol extracts of *A. pinnanensis* demonstrated significantly high phenolic content, with the data presented in Table 2. The total phenolic content in the aerial and underground parts was determined to be 132.94 ± 1.24 mg GAE/g and 124.77 ± 1.53 mg GAE/g, respectively. The total flavonoid content was higher in the aerial part extract (13.50 ± 0.11 mg QE/g) compared to the underground part extract (5.65 ± 0.07 mg QE/g).

Table 2. Total phenolic and total flavonoid contents of *Alpinia pinnanensis*

No.	Sample	Equivalents per g dry extract	
		TPC ± SD (mg GAE/g)	TFC ± SD (mg QE/g)
1	**Aerial part**	132.94 ± 1.24	13.50 ± 0.11
2	**Underground part**	124.77 ± 1.53	5.65 ± 0.07

3.4. Evaluation of antioxidant activity

Table 3 presents a summary of the antioxidant activities of the ethanol extracts obtained from the aerial and underground parts of *A. pinnanensis*.

Table 3. Antioxidant activity of *Alpinia pinnanensis*

No.	Sample	DPPH	ABTS
		IC_{50} ± SD (µg/mL)	IC_{50} ± SD (µg/mL)
1	**Aerial part**	19.03 ± 0.11	2.63 ± 0.18
2	**Underground part**	32.75 ± 0.36	9.14 ± 0.09
	Ascorbic acid	2.69 ± 0.09	1.27 ± 0.08

As observed, the ethanol extracts of *A. pinnanensis* exhibited significant *in vitro* free radical scavenging activity in both DPPH and ABTS assays. The extract obtained from the aerial part displayed significantly higher antioxidant capacity than the underground part, with IC_{50} values of 19.03 ± 0.11 µg/mL and 2.63 ± 0.18 µg/mL for DPPH and ABTS, respectively. Nonetheless, both extracts exhibited weaker antioxidant activity than the reference antioxidant, ascorbic acid, which showed markedly lower IC_{50} values of 2.69 ± 0.09 µg/mL (DPPH) and 1.27 ± 0.08 µg/mL (ABTS).

4. DISCUSSION

Preliminary phytochemical screening of *A. pinnanensis* extracts revealed the presence of several major classes of secondary metabolites, notably tannins, flavonoids, saponins and coumarins. These compounds are also typical of the chemical composition found within the *Alpinia* genus. These bioactive constituents are likely contributors to the observed biological activities associated with *A. pinnanensis*.

Previous studies have reported the total phenolic and flavonoid contents of *Alpinia* species. In *A. breviligulata*, total phenolic content was measured at 34.3 ± 0.5 mg GAE/g extract in the aerial parts and 89.7 ± 0.9 mg GAE/g extract in the underground parts [16]. The methanolic extract of *A. galanga* rhizome was reported to contain 233.79 ± 2.18 mg GAE/g of total phenolic and 143.13 ± 2.44 mg QE/g of total flavonoid contents [17]. The methanol extract of *A. nigra* leaves were found to be 69.25 mg GAE/g extract and 78.84 mg QE/g extract [18]. Among the surveyed *Alpinia* species, *A. pinnanensis* exhibited relatively high total phenolic content, with 132.94 ± 1.24 mg GAE/g in the aerial part and 124.77 ± 1.53 mg GAE/g in the underground part. In contrast, the total flavonoid content in the aerial and underground parts was determined to be 13.50 ± 0.11 mg QE/g and 5.65 ± 0.07 mg QE/g, respectively, which is comparatively lower than that observed in other *Alpinia* species.

The antioxidant properties of *Alpinia* species have been previously reported in the literature. The aerial part of *A. breviligulata* showed greater DPPH radical scavenging activity (IC_{50} = 132.7 ± 2.6 µg/mL) compared to the underground part (IC_{50} = 219.2 ± 4.8 µg/mL) [16]. Extracts obtained from the rhizomes of *A. galanga* and the leaves of *A. nigra* displayed DPPH and ABTS radicals scavenging effects, with IC_{50} values of 79.34 ± 1.78 µg/mL and 88.94 ± 2.28 µg/mL for *A. galanga*, and 64.51 µg/mL and 28.32 µg/mL for *A. nigra*, respectively [17, 18]. Ethanolic extracts from the rhizome, stem, leaf, flower, pericarp, and seed of *A. zerumbet* demonstrated antioxidant activity in the DPPH assay with IC_{50} values ranging from 136.63 ± 0.33 to 580.38 ± 0.26 µg/mL. In the ABTS assay, these extracts exhibited IC_{50} values between 137.92 ± 1.74 and 293.90 ± 3.64 µg/mL [19]. The ethanol extract from the rhizome of *A. calcarata* showed antioxidant activity on DPPH and ABTS assays with IC_{50} values of 36 µg/mL and 141.75 µg/mL, respectively [20]. In this study, the ethanol extract from the aerial part of *A. pinnanensis* exhibited notable antioxidant activity,

with IC_{50} values of 19.03 ± 0.11 µg/mL (DPPH) and 2.63 ± 0.18 µg/mL (ABTS). The underground part also showed strong radical scavenging activity, with IC_{50} values of 32.75 ± 0.36 µg/mL and 9.14 ± 0.09 µg/mL, respectively. Overall, *A. pinnanensis* demonstrated significantly higher antioxidant capacity compared to other species within the *Alpinia* genus. The strong antioxidant activity observed in the present study may be attributed to the considerable phenolic content, which are known to contribute to free radical scavenging activity.

In this study, compared with the DPPH assay, the ABTS assay revealed stronger radical scavenging activity of the plant extracts may also be due to differences in the hydrophilic and lipophilic properties of the antioxidant compounds. The ABTS can detect both hydrophilic and lipophilic antioxidants, whereas the DPPH assay is limited mainly to lipophilic compounds, which may account for the relatively lower IC_{50} value observed in the ABTS assay.

5. CONCLUSION

This is the first report on the phytochemical profile, total phenolic and flavonoid contents and antioxidant activity of *Alpinia pinnanensis*, highlighting its ethnopharmacological significane and potential as a natural antioxidant source for pharmaceutical and nutraceutical applications. Further studies are required to isolate and characterize the bioactive compounds responsible for these activities, as well as to evaluate their toxicity and biological efficacy through *in vivo* investigations.

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