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RESEARCH COMMUNICATION

Comparative analysis of metabolites and bioactivities of *Alpinia nelumboides* leaves, pseudostems and rhizomes

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Abstract

Alpinia nelumboides Nob. Tanaka, T. T. K. Van & V. Hoang is a member of the family Zingiberaceae. *Alpinia* spp. are often used in traditional medicine. Limited data regarding phytochemicals and biological activities of this species are found in the available literature. This study aimed to identify pigments and phenolics in its leaves, pseudo-stems and rhizomes using high-performance liquid chromatography and spectrophotometry. Besides, free radical scavenging properties of acetonic extracts from the plant were assessed. The levels of chlorophyll a and b in the *A. nelumboides* leaves were approximately 3 time higher in the rhizomes. Among the phenolics examined, epicatechin was the most abundant compound, with an average concentrations ranging from 12.26—61.23 mg/g extract. The leaf extract exerted the strongest activity to scavenge ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radicals (IC_{50} = 339.46 ± 10.18 µg/mL) while the rhizome extract showed the most potent capacity to neutralize DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals (IC_{50} = 149.56 ± 2.37 µg/mL). These results provide a better understanding of the phenolic components of *A. nelumboides* and its potential health-promoting activities.

Keywords: *Alpinia nelumboides*; antioxidant activity; chlorophyll; epicatechin; phenolics

Introduction

Vietnam inhabits several species of the genus *Alpinia* (Chi Riềng in Vietnamese), belonging to the family Zingiberaceae. To date, more than 36 spp. of the genus *Alpinia* have been distributed Vietnam (1, 2). All of them are are characterized by small plant morphology, with solitary flowers or flower clusters that are either upright or drooping and long petals, rarely shorter than 3 cm. Bracts may or may not be present; when present, they nearly envelop the flower bud. One of the most distinctive features of *Alpinia* spp. is their long petals (3, 4). In traditional medicine, various parts of *Alpinia* spp are commonly used to treat a variety of ailments such as stomach disorders, vomiting and to aid digestion (5, 6). Given this long history of ethnomedicinal use, there is a growing need to validate these traditional claims through modern phytochemical analyses and bioactivity assessments, which can provide scientific evidence for their therapeutic potential. Numerous secondary metabolites have been identified from extracts of *Alpinia* spp. including terpenoids, phenylpropanoids, diarylheptanoids and flavonoids (7). In addition, many biological activities have been discovered, such as antibacterial (8), radical scavenging (9), anti-tumor (10-12), cardioprotective (13), gastroprotective (14), anti-inflammatory and analgesic effects (15). Free radical scavenging activity is important as biological functions such as antimutagenicity, anti-carcinogenicity and anti-aging arise from this property.

Alpinia nelumboides Nob. Tanaka, T. T. K. Van & V. Hoang was discovered by the research group led by Nobuyuki Tanaka and was published in 2022. This species has been found in several locations such as the Bolaven Plateau in Champasak Province, southern Laos, Lam Dong Province in southern Vietnam and it is likely distributed in the southeastern region of Indochina. *A. nelumboides* is a small herbaceous plant with long leaves and elongated flower clusters, resembling several other species such as *A. kwangsiensis*, *A. roxburghii* and *A. malaccensis* (2, 16). The non-volatile compound classes such as phenolics and flavonoids, along with their biological activities in *A. nelumboides*, have not yet been explored. However, the essential oils from the rhizomes and stems of this species have been studied, with the main chemical constituents identified as 1,8-cineole, β-pinene, (E)-citral, (Z)-citral, α-pinene and limonene, among which 1,8-cineole had the highest concentration at 20 %. These essential oils exhibited antioxidant, anti-α-glucosidase, anticancer and antimicrobial potentials which are associated with the presence of monoterpenoid and polyphenol compounds (17, 18). In *A. nelumboides*, leaves, pseudostems and rhizomes are medicinally important. The rhizomes are the most widely used part in traditional medicine, with leaves and other parts contributing secondary or complementary roles in traditional practices and modern phytochemical research. In this study, several phenolic and flavonoid compounds from this species were identified and quantified from different plant parts such as leaves,

pseudostems and rhizomes and their free radical scavenging abilities were also investigated.

Materials and Methods

Sample collection

The fresh *A. nelumboides* rhizomes, pseudo-stems and leaves (1 kg each part) were collected in Lam Dong province, Vietnam (11° 56'15.2" N, 108°23'18.1" E) in March 2023. A voucher specimen (NHTuan045) was kept in the Herbarium of Hanoi National University, Vietnam. The fresh samples were dried in a convection dryer until their moisture < 10 %.

Chemicals

Acetone (HPLC-grade, 99.5 %) was obtained from VWR Chemicals (France). Ferulic acid (> 99 %) was obtained from Sigma-Aldrich (USA). Chlorogenic acid (> 99 %) was purchased from the National Institute of Drug and Quality Control (Vietnam). Flavonoid analytical standards (purity > 99 %) were procured from Chengdu Biopurify Phytochemicals (China). 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Alfa Aesar and Sigma-Adrich (USA).

Crude extract preparation

To prepare acetonic crude extracts, each plant part (10 g) was separately mixed with 100 mL of acetone (100 %). After 24 hr of extraction at room temperature, the mixture was filtered through a filter paper. The obtained filtrate was evaporated at 35 °C in a rotary evaporator (DLAB, Beijing, China) to remove the solvent. The resulting crude extract was kept in a cold, dry place for further analysis.

Determination of pigments

Pigments including chlorophyll and total carotenoid content (TCC) were determined according to the standard method previously (19). The sample in powder form was extracted with acetone at a sample/solvent ratio of 1/20 g/mL and shaken thoroughly for 2 hr. The extract was then centrifuged at 60000 rpm for 15 min and the supernatant was used to measure absorbance at wavelengths of 470, 662 and 645 nm. The contents of chlorophyll a, b and carotenoids were calculated using the following formulae:

Chlorophyll a (CA) = (11,24 x A662 - 2,04 x A645) ´ V/W

Chlorophyll b (CB) = (20,13 x A645 - 4,19 x A662) ´ V/W

Total carotenoid content = [1000 ´ A470 - (1.9 ´ CA + 63.14 ´ CB)]/214 ´ V/W

Where V is the volume of solvent (mL) and W is the initial sample mass (g).

Determination of phenolic compounds

To determine the concentrations of certain phenolic acids and flavonoids present in the extracts of *A. nelumboides*, high-performance liquid chromatography (HPLC) was used (LC-2030C system) with a UV detector, an Agilent Zorbax Eclipse XDB C18 column and a mobile phase consisting of 1 % formic acid and 100 % acetonitrile (20). Quantification of the phenolics was carried out based on external standards.

Antioxidant activities

The DPPH free radical scavenging activity was evaluated according to previously reported methods (21, 22). A volume of 0.1 mL of each concentration of the extract or the positive control (ascorbic acid) was mixed with 0.5 mL of 0.1 mM DPPH solution. After incubating the mixture in the dark for 30 min, the absorbance (A) was measured at 517 nm using a UV-VIS spectrophotometer. The ABTS free radical scavenging activity was assessed according to methods previously published (23). A volume of 0.1 mL of each extract concentration was mixed with 3 mL of ABTS solution. The mixture was incubated in the dark for 30 min and the absorbance was measured at 734 nm using a UV-VIS spectrophotometer. The ABTS radical solution was prepared by mixing 50 mL of 7 mM ABTS solution with 50 mL of 2.45 mM. IC_{50} (µg/mL) values were used to express the antioxidant activity of the extracts.

Statistical analysis

The sample testing was carried out in triplicate and the results were shown as mean ± standard deviation. The statistical analysis was conducted by one-way ANOVA followed by Tukey's test at $p < 0.05$. The XLSTAT (version 2016) was employed for the statistical analysis. IC_{50} values were calculated using dose-response curve, which plots the percent inhibition against the concentration of the extracts or reference standard. Microsoft Excel 365 was used to construct the dose-response curves.

Results and Discussion

Pigments

The chlorophyll mainly consists of chlorophyll a (the primary pigment) and chlorophyll b (the accessory pigment). Both types of chlorophyll are key components of the photosynthetic membrane. The ratio of these 2 chlorophyll components depends on the natural growing conditions of the species. Typically, the chlorophyll a to b ratio is approximately 3; however, if the species grows in shaded areas, this ratio is lower, for example, between 2.3 and 2.9 (24). In this study, the chlorophyll a and b content in the dried leaf samples was less than 2, while in the dried pseudostems and rhizomes, it was about 2.2 (Table 1). The levels of chlorophyll a and b in the *A. nelumboides* leaves were about 3 times as much as those in the rhizomes Naturally, chlorophyll content in the leaves remains the highest among the 3 parts analyzed. The slightly lower chlorophyll content in this study compared to theoretical values may be due to losses of chlorophyll a and b during the drying process, under the influence of heat and light. The study revealed that this compound found in *A. zerumbet* and *A. oxyphylla*, the chlorophyll a to b ratio was found to be less than 2 (25, 26).

Carotenoids in green photosynthetic plant tissues are essential for the plant's photosynthetic function. However, some suggest that the primary role of carotenoids in plants is to protect chlorophyll a during photooxidation processes, indicating a close relationship between carotenoids and chlorophyll in plant tissues (27). In *A. nelumboides*, the carotenoid content was highest in the stems, reaching 5.08 mg/100 g of dry sample. The carotenoid content in all 3 parts of *A. nelumboides* was higher compared to that

Table 1. Chlorophyll and carotenoid contents (mg/100 g) of *A. nelumboides* parts

Pigments	Leaves	Pseudo-stems	Rhizomes
Chlorophyll a	29.50 ± 0.01 a	12.03 ± 0.06 b	11.01 ± 0.02 c
Chlorophyll b	15.94 ± 0.03 a	5.43 ± 0.02 b	4.87 ± 0.02 c
TCC	4.91 ± 0.03 b	5.08 ± 0.06 a	4.97 ± 0.02 b

Data are shown as mean ± standard deviation (n = 3). Different letters (a, b) indicate significant differences in pigment contents among the plant parts.

found in the fruit of *Renealmia alpinia* (28) and in the flowers of *A. labella* (29).

Phenolic compounds

Among the 8 compounds comprising two phenolic compounds and 6 flavonoid compounds their presence and concentrations were determined in 3 types of extracts from *A. nelumboides* using HPLC with a UV detector. The results showed the presence of all 8 compounds with varying concentrations in the analyzed samples, as presented in Table 2. Among them, epicatechin was found in all 3 extracts with the highest concentration (12.26—61.23 mg/g extract), with the highest amount in the pseudo-stems extract. In addition, the compounds chlorogenic acid, ferulic acid, rutin, quercetin and kaempferol were also detected in all 3 extracts but at lower concentrations than epicatechin. The least detected compound was EGCG (epigallocatechin gallate), found only in the leaf extract (1.54 ± 0.27 mg/g extract). As per the report, *A. pricei* Hayata, epicatechin and catechin were not detected in rhizome extracts (30). Meanwhile, chlorogenic acid was present in the highest concentration and was higher than in *A. nelumboides* (48.4 ± 0.2 mg/g extract). Ferulic acid and rutin were also present at slightly higher levels (30). Ferulic acid in the ethyl acetate extract from the leaves of *Alpinia* species was relatively high (30.4—32.3 mg/g extract). The ethyl acetate extract from *A. zerumbet* had a much higher content compared to the leaf, pseudo-stem and rhizome of *A. nelumboides* (31, 32). Furthermore, according to a study on the rhizomes of *A. galanga*, the compounds catechin (124.33 mg/100 g) and quercetin (105.34 mg/100 g) were found at lower concentrations than in *A. nelumboides* in this study (33). These findings indicate that species differences, origin of raw materials and the specific plant parts studied all contribute to variations in the content of phenolic and flavonoid compounds.

Table 2. Phenolic content (mg/g extract) of *A. nelumboides* extracts

Phenolics	Leaf	Pseudo-stem	Rhizome
Chlorogenic acid	0.14 ± 0.00 c	0.86 ± 0.00 a	0.19 ± 0.00 b
Ferulic acid	0.83 ± 0.01 a	0.37 ± 0.04 b	0.28 ± 0.00 c
Catechin	n.d.	1.92 ± 0.31 b	9.71 ± 0.69 a
Epicatechin	12.26 ± 1.20 c	61.23 ± 4.10 a	20.59 ± 2.73 b
EGCG	1.54 ± 0.27	n.d.	n.d.
Rutin	0.11 ± 0.00 c	0.28 ± 0.00 a	0.17 ± 0.00 b
Quercetin	1.60 ± 0.11 a	0.26 ± 0.00 b	0.33 ± 0.11 b
Kaempferol	1.33 ± 0.03 a	0.34 ± 0.03 c	0.57 ± 0.14 b

Data are shown as mean ± standard deviation (n = 3). Different letters (a, b, c) indicate significant differences among the extracts. n.d.: not detected.

Free radical scavenging activities

The antioxidant activity of the extracts from *A. nelumboides* was demonstrated through their ability to scavenge DPPH and ABTS free radicals. The results of this process are shown in Table 3, Fig. 1 and 2. The ABTS radical scavenging activity was found to be concentration-dependent within the range of 500—4000 µg/mL (Fig. 1). The DPPH radical scavenging activity was observed across concentrations ranging from 200—1000 µg/mL (Fig. 2). Accordingly, the extracts exhibited a faster scavenging effect on ABTS radicals compared to DPPH radicals. Among the extracts, the leaf extract demonstrated the highest scavenging capacity, achieving nearly 100 % ABTS radical removal at concentrations between 500—1000 µg/mL within 30 min. This was more effective than the rhizome extract in terms of efficiency and better than the pseudo-stems extract in terms of concentration. For DPPH radical scavenging, all three extracts achieved around 90 % effectiveness within 30 min, but this was only at a concentration of approximately 1000 µg/mL. At lower concentrations, the rhizome extract showed a higher scavenging efficiency and the correlation curve between concentration and percent of DPPH removed was steeper compared to the other 2 extracts.

In combination with IC_{50} data, for DPPH radical scavenging activity, the rhizome extract showed the highest activity > pseudo-stem extract > leaf extract. For ABTS radical scavenging activity, the leaf extract was the most effective > pseudo-stem extract > rhizome extract. The differences in radical scavenging activity among the extracts when tested against DPPH and ABTS radicals may be explained by the varying solubility and diffusion capacities of antioxidant compounds present in each extract. Natural compounds capable of scavenging DPPH radicals are typically soluble in less polar organic solvents and are generally ineffective in

Table 3. Free radical scavenging activities (IC_{50}, µg/mL) of the *A. nelumboides* extracts

Extracts	ABTS	DPPH
Leaf	339.46 ± 10.18 b	256.53 ± 13.93 a
Pseudo-stem	913.88 ± 22.33 a	251.10 ± 3.74 a
Rhizome	932.86 ± 9.77 a	149.56 ± 2.37 b
Ascorbic acid	60.52 ± 1.22 c	5.81 ± 0.07 c

Data are shown as mean ± standard deviation (n = 3). Different letters (a, b, c) denote significant differences among the extracts.

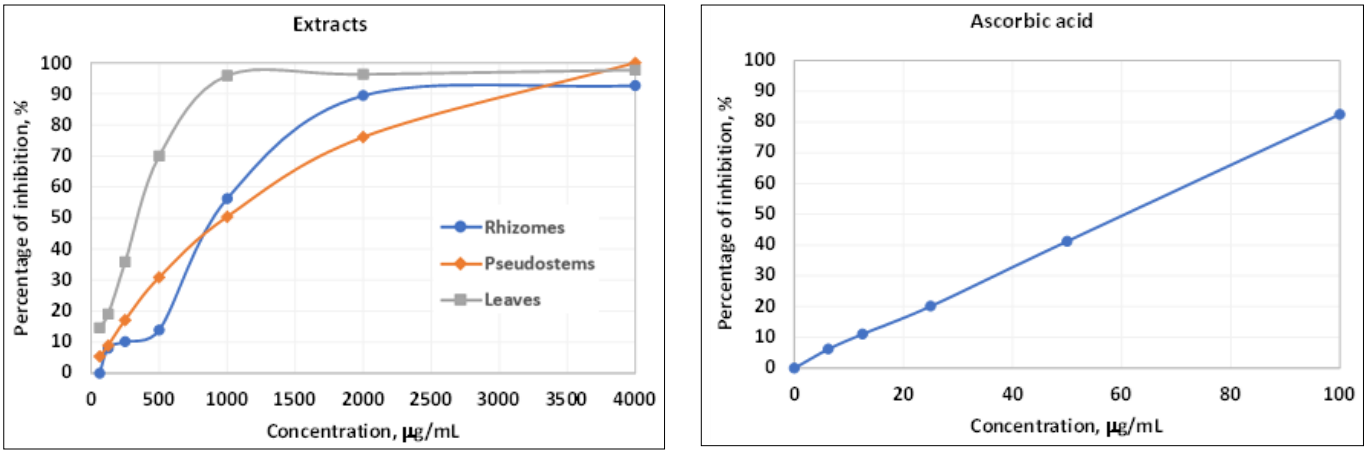

Fig. 1. ABTS radical scavenging activity of *A. nelumboides* extracts and ascorbic acid.

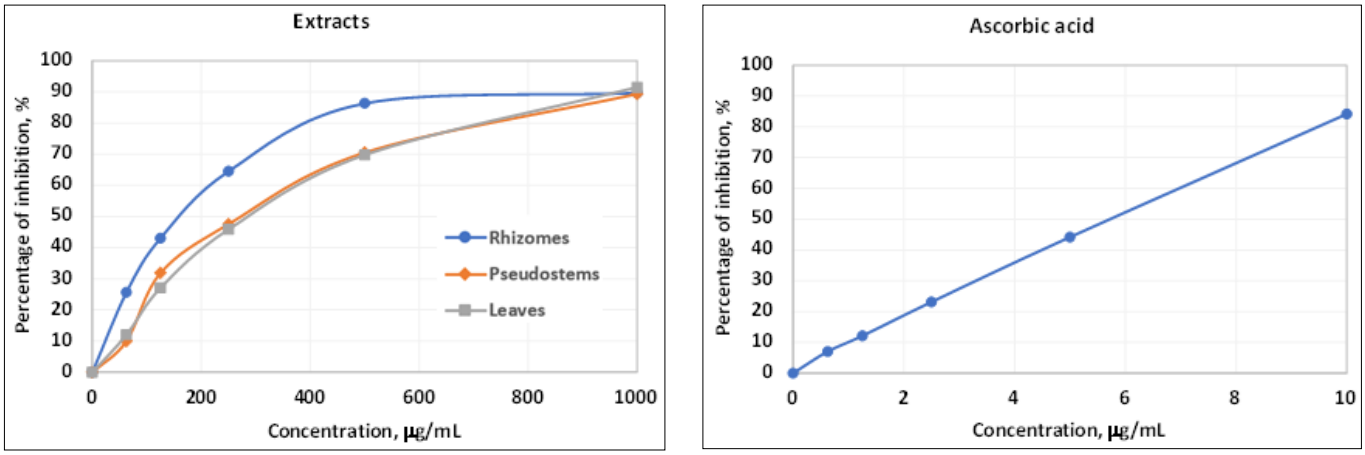

Fig. 2. DPPH radical scavenging activity of *A. nelumboides* extracts and ascorbic acid.

polar solvents (34, 35), whereas those soluble in polar solvents are more effective in ABTS radical scavenging (35). Among the extracts, the highest ABTS radical scavenging activity was observed in the rhizome extract with an IC_{50} of 149.56 ± 2.37 µg/mL, while the highest DPPH scavenging activity was shown by the leaf extract with an IC_{50} of 339.46 ± 10.18 µg/mL, both values being less effective than the positive control ascorbic acid. Compared to the leaf extract of *A. nigra*, the radical scavenging ability of *A. nelumboides* extracts was not as strong and specifically, *A. nigra* showed IC_{50} values of 65.51 µg/mL for DPPH and 28.32 µg/mL for ABTS (36). In a study on *A. mutica*, the DPPH radical scavenging activity of the rhizome extract from *A. nelumboides* was higher than in some *A. mutica* extracts, while the stem/branch extract also exhibited stronger activity than the ethyl acetate extract and other solvent extracts (80 % methanol, hexane, water) of *A. mutica* (37). For the rhizome part of *A. pahangensis*, the best DPPH scavenging activity was found in the ethyl acetate extract (0.35 ± 0.094 mg/mL), which is still lower in activity compared to extracts from various parts of the species in this study (38).

The findings of this study provide valuable insights into the phytochemical composition and antioxidant properties of *A. nelumboides*, which suggest potential pharmacological applications, particularly in the development of plant-derived therapeutics for disease prevention and management. The identification of epicatechin as the most abundant phenolic compound is noteworthy, given its well-documented biological activities, including anticancer and anti-inflammatory potentials, which highlight the relevance of *A. nelumboides* as a promising source of bioactive compounds. However, it is important to acknowledge the limitations of this study. The pharmacological significance of these results cannot be fully established without *in-vivo* validation, as antioxidant capacity measured *in-vitro* may not directly translate to biological efficacy in living systems. In addition, the outcomes may be influenced by the choice of extraction method, which can alter both yield and compound profiles, as well as by the purity and stability of the identified compounds.

Conclusion

This study provides the first analysis of chlorophyll, total carotenoid phenolic content in the leaves, pseudo-stems and rhizomes of *A. nelumboides*. It identified epicatechin as the predominant phenolic compound. The antioxidant activity of the plant's acetonic extracts was also demonstrated, highlighting its potential as a source of bioactive compounds with health-promoting properties. These findings not only expand current knowledge on the chemical composition of *A. nelumboides* but also suggest its promise in the development of plant-derived therapeutics for disease prevention and treatment. However, the study is limited to *in vitro* assays and chemical profiling. Further *in vivo* studies and mechanistic investigations are needed to validate the biological relevance and therapeutic applicability of these compounds.

Authors' contributions

NTN carried out the bioassays and contributed to drafting the manuscript. NTT was involved in experimental design, performed statistical analyses and also contributed to manuscript drafting. Both the authors reviewed and approved the final version of the manuscript.

Compliance with ethical standards

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

Ethical issues: None

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