Natural Product Communications
Volume 20(1): 1–9

DOI: 10.1177/1934578X251314080
journals.sagepub.com/home/npx

Chemical Composition, Antioxidant Activity, and Enzyme Inhibitory Effects of Essential Oils of *Alpinia gagnepainii* K.Schum Wild-Grown in Ha Tinh Province, Vietnam

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Abstract

Objective/Background: *Alpinia gagnepainii* K.Schum, a member of the ginger family, is predominantly found in northern Vietnam. **Methods:** Essential oils (EOs) of *A. gagnepainii* leaves and rhizomes were extracted through hydrodistillation and their chemical composition was analyzed using gas chromatography-mass spectrometry. The antioxidant activity of the EOs was assessed via DPPH and ABTS radical scavenging assays, and their *in vitro* inhibitory effects on α-amylase and tyrosinase were also evaluated. **Results:** The leaf EO comprised 46 compounds (95.83% of the total composition), while the rhizome EO contained 49 compounds (98.37%). β-Pinene was identified as the most abundant component, accounting for 27.09% in the leaf EO and 20.45% in the rhizome EO. The former demonstrated a stronger DPPH radical scavenging ability ($IC_{50} = 1070.35 \pm 9.17$ μg/mL), whereas the latter was more effective against ABTS radicals ($IC_{50} = 1519.18 \pm 112.58$ μg/mL). No significant difference in antityrosinase activity was observed between the two EOs; however, the rhizome EO ($IC_{50} = 195.14 \pm 6.49$ μg/mL) exhibited a more potent anti-α-amylase activity than the leaf EO. **Conclusion:** This study reported the chemical composition, antioxidant, anti-α-amylase, and antityrosinase activities of *A. gagnepainii* EOs for the first time. The bioactivities of the plant indicate their potential applications in the nutraceutical and cosmeceutical industries.

Keywords

Alpinia gagnepainii, essential oil, antioxidant, α-amylase, tyrosinase

Received: October 14th, 2024; Accepted: January 3rd, 2025.

Introduction

The *Alpinia* genus, also known as the galangal genus, belongs to the ginger family (Zingiberaceae) and consists of about 230 species. Most of these are perennial herbs, primarily growing in wet, well-lit regions, especially in South and Southeast Asia, as well as Australia.[1,2] In Vietnam, *Alpinia*, commonly referred to as "cao lương khương," "tiểu lương khương," "lương khương," or "galanga" encompasses around 31 species, mostly found under forest canopies, along streams, in humid areas, and distributed throughout the northern, central, and southern regions of the country.[2,3] Rhizomes of *Alpinia* are characterized by their strong aroma, spicy flavor, and warming properties, and they are commonly used as both a spice and a component in traditional medicine throughout several Asian countries, including Vietnam, China, and Indonesia.[4] The most used species of *Alpinia* for culinary

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purposes is *Alpinia officinarum*, which is widely cultivated in several Asian countries. Products such as ground rhizome powder, galangal essential oil, and red galangal root powder are commonly available on the market.

Numerous studies have been conducted on essential oils (EOs) of species within the *Alpinia* genus, showing that the chemical composition of the oils depends on the species and the region where the samples were collected. For example, *Alpinia galanga* collected in Indonesia contains cineole, 4-allylphenyl acetate, α-farnesene, (2,6-dimethylphenyl)borate, and α-pinene as the main components.[5] Meanwhile, in India, the primary component is 1,8-cineole (47.5-67.3%), with other components present in much smaller quantities. For *Alpinia calcarata*, the main components are 1,8-cineole, α-fenchyl acetate (26.3-38.7%), camphor (3.3-4.7%), camphene (0.7-6.6%), and α-terpineol.[6] For *Alpinia malaccensis* collected in India, the primary components are α-phellandrene, β-cymene, and β-pinene,[7] while *Alpinia macroura* collected in Vietnam contains β-pinene, 1,8-cineole, γ-terpinene, and α-pinene.[8] Research has also shown that different parts of the plant or different extraction methods can result in variations in the chemical composition of EOs. Specifically, for *Alpinia zerumbet*, EO extracted using simultaneous distillation-extraction and hydrodistillation methods from the leaves and flowers showed differences in chemical aspects. The leaf oil extracted using the former technique had terpinen-4-ol, 1,8-cineole, sabinene, and γ-terpinene as the main components, along with caryophyllene and caryophyllene oxide sesquiterpenes. When extracted using the hydrodistillation, the main components were sabinene, 1,8-cineole, and γ-terpinene. For the flowers, when extracted using the hydrodistillation method, the main components were 1,8-cineole, γ-terpinene, and terpinen-4-ol.[9]

Galangal EOs have a spicy and pungent aroma, with the intensity ranging from mild to strong depending on the extraction method and species. In addition to its fragrance, galangal EOs possess notable biological activities, such as insecticidal effects, antibacterial, antioxidant, antitumor, antidiabetic, antifungal, antiviral, anti-inflammatory, and analgesic properties.[10-14] Therefore, it can be used in aromatherapy to reduce stress, combat inflammation, and aid digestion.

Alpinia gagnepainii K.Schum. is found only in Vietnam, specifically in Ha Tinh, Ninh Binh, Thanh Hoa, and Lai Chau.[15] Though it belongs to the *Alpinia* genus, this species has not been extensively studied. Some phenolic compounds and bioactivities of methanol extracts from this species in Lai Chau, Vietnam, were reported.[16] However, no studies on EOs and their pharmacological activities of this plant species have been published. The aims of the present study were to compare the chemical composition of the EOs of *A. gagnepainii* leaves and rhizomes, their antioxidant potential and inhibitory effects against α-amylase and tyrosinase. The findings of the study will provide valuable insights into the chemical composition and potential use of this plant's EOs in managing diabetes and treating skin pigmentation disorders, offering opportunities for its application in the nutraceutical and cosmeceutical industries.

Experimental

Sample Collection

The plant used in this study was collected in Can Loc district, Ha Tinh province (18°31′24.9″N 105°47′58.2″E), Vietnam in January 2024, and authenticated by Assoc. Prof. Dr Nguyen Hoang Tuan at Hanoi University of Pharmacy. A voucher specimen (HC.52-AG.HTT) was deposited at the Department of Pharmacy, Da Nang University of Medical Technology and Pharmacy, Vietnam.

Chemicals and Reagents

A mixture of *n*-alkanes (C_7 – C_{30}), acarbose, kojic acid, L-DOPA (3,4-dihydroxy-L-phenylalanine), α-amylase from *Bacillus* sp., and tyrosinase from mushroom were obtained from MilliporeSigma (Burlington, Massachusetts, USA). Ascorbic acid was purchased from Duchefa Biochemie (Haarlem, The Netherlands).

Isolation of Essential Oils

The fresh leaves and rhizomes of *A. gagnepainii* were hydrodistilled for 4 h using a Clevenger-type apparatus as previously reported with minor modifications.[17] The EOs were dried with anhydrous sodium sulfate, transferred to 1.5 mL vials, and stored at 4 °C until analysis. The experiments were performed in triplicate.

Determination of Essential oil Composition

The analysis of *A. gagnepainii* EO was conducted on a gas chromatography system (7890B GC) connected with a mass spectrometer (5977B MSD) (GC-MS). The GC-MS parameters are shown in Table S1 (Supplemental material). Chemical composition of the EO was identified by comparing their mass spectral and retention indices (RIs) in the National Institute of Standards and Technology (NIST17) and Adams books,[18] as well as referencing the retention indices (RI) to a homologous series of n-alkanes (C_7-C_{30}).

Antioxidant Activity

DPPH. The antioxidant activity of the EOs was demonstrated by its ability to scavenge DPPH radicals. In this method, DPPH was prepared at a concentration of 0.08 mM (3.9 mg DPPH dissolved in 100 mL of methanol, diluted 10 times). Two mL of the EO solution were mixed with 2 mL of the 0.08 mM DPPH solution, and the mixture was incubated at room temperature in the dark for 30 min. The optical density was recorded at a wavelength of 517 nm using a UV-VIS spectrophotometer after incubation. Ascorbic acid was used as a positive control.[19]

ABTS. The antioxidant effect of the EOs was also evaluated by its ability to remove ABTS radicals. The ABTS solution was generated through the reaction between ABTS and $K_2S_2O_8$, incubated for 12 h in the dark, and the resulting solution was diluted with methanol to achieve an absorbance of $A = 0.7 \pm 0.02$. One mL of the sample was mixed with 2.5 mL of the ABTS solution, incubated in the dark for 30 min, and the optical density (A) was recorded using a UV-VIS spectrophotometer at a wavelength of 734 nm. Ascorbic acid was used as a positive control.[20]

α-Amylase Inhibitory Activity. The diluted EOs (62.5-500 μg/mL) and acarbose (25-200 μg/mL) were tested for their ability to inhibit α-amylase. In brief, a tube containing the sample (750 μL) and 150 μL of α-amylase (0.14 U/mL) was incubated at 37 °C for 15 min. Afterwards, 225 μL of 0.25% starch solution were added to the tube, followed by another incubation (37 °C, 15 min). the reaction was terminated by adding 750 μL of HCl (1 M) and 1.5 mL of a KI_3 solution. Changes in absorbance were determined at 540 nm.[21]

Tyrosinase Inhibitory Activity. The inhibition of tyrosinase was tested using L-DOPA as a substrate. The reaction mixture consisted of 100 μL of the diluted EO (62.5-500 μg/mL), 40 μL of 0.5 mM L-DOPA, and 40 μL of tyrosinase (80 U/mL). The mixture was incubated at 37 °C for 20 min. The formation of dopachrome was determined at 490 nm. Kojic acid was employed as a positive control. Each measurement was carried out in triplicate. IC_{50} values were calculated using concentration-response curves prepared by Microsoft Excel.[22]

Statistical Analysis

All the measurements were carried out in triplicate. The data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to evaluate differences in bioactivities among the studied EOs, followed by Tukey's Studentized Range HSD test at a significant level of $p < 0.05$.

Results

Chemical Composition of the EOs

The yields of the hydrodistillation process of *A. gagnepainii* leaves and rhizomes were 0.02 and 0.01% (w/w) essential oil, respectively, calculated on a fresh weight basis. The chemical composition of the EOs from *A. gagnepainii* leaves and rhizomes have been revealed using GC-MS. Names of the compounds and their percentage contents are shown in Table 1, Figures S1 and S2 (Supplemental material). The analysis showed the presence of 56 volatile organic compounds, with 46 compounds identified in the leaf EO, accounting for 95.83% on average, and 49 compounds in the rhizome EO, representing 98.37%. For both EOs from the leaves and rhizomes of *A. gagnepainii*, the major class of compounds was monoterpene hydrocarbons, making up 72.87% and 54.24% of the EOs (leaves and rhizomes, respectively). This class contains 14 compounds, with the largest being β-pinene, accounting for 27.09% and 20.45% of the EO contents, followed by α-pinene at 17.61% and 13.63% of the EO contents (leaves and rhizomes, respectively). The leaf EO also contained significant amounts of α-limonene at 6.84% and sabinene at 5.72%. In contrast, the rhizome EO had a significant amount of fenchone at 13.02% of its contents. The next most abundant class of compounds was oxygenated monoterpenes, comprising 20 compounds, with the most abundant being 1,8-cineole, at 14.46% of the rhizome EO and 3.40% of the leaf EO. Sesquiterpenes were also detected in the EOs, though their concentration was relatively low compared to monoterpenes. The sesquiterpene hydrocarbons accounted for 2.32% (rhizome) and 9.12% (leaf) of the EOs with 12 compounds, and oxygenated sesquiterpenes made up 2.54% and 3.95% (rhizomes and leaves, respectively), consisting of 8 compounds. The compounds in the sesquiterpene class were present in minor amounts.

Radical Scavenging Activity of the EOs

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging ability of the EOs from the leaves and rhizomes of *A. gagnepainii* are presented in Figure 1 and Table 2. From these results, it can be seen that the radical scavenging ability of the leaf EO is higher compared to the rhizome EO (Figure 1). The ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity of the *A. gagnepainii* EOs is shown in Figure 2 and Table 2. The rhizome EO exhibited significantly stronger scavenging activity compared to the leaf EO. When evaluating the radical scavenging capacity of the EOs at concentrations ranging from 500-3000 μg/mL, the rhizome EO showed an ABTS scavenging percentage of 18.7-84.9%, while the leaves ranged from 11.1-63.8%. The IC_{50} value for the rhizome EO was also lower than the leaf EO (1519.18 ± 112.58 and 2446.53 ± 22.30 μg/mL, respectively). These results indicated that the ABTS radical scavenging and antioxidant potential of the EO of the rhizomes is higher than that of the leaves.

Tyrosinase Inhibitory Effect of the EOs

As presented in Figure 3A, in the examined concentration range (62.5-500 μg/mL), consistent increases in the percentage of enzyme inhibition were observed for both EOs as the concentration values rose. This indicates that the inhibitory activity of the leaf and rhizome EOs against tyrosinase demonstrated a dose-dependent manner. No significant differences in the inhibitory effect between the EOs were observed at each tested concentration. That could explain the IC_{50} values of the leaf and rhizome EOs (164.73 ± 14.77 and 180.11 ± 21.85 μg/mL, respectively) did not significantly differ (Figure 3C). In other

Table 1. Chemical Constituents of the Alpinia gagnepainii Essential Oils.

No	RT (min)	RI (cal.)	RI (lit.)	Compounds	% Leaf EO	% Rhizome EO
1	6.10	925	925	Tricyclene	0.05 ± 0.01	–
2	6.24	930	929	α-Thujene	0.51 ± 0.02	0.35 ± 0.03
3	6.47	938	937	α-Pinene	17.61 ± 0.14	13.63 ± 0.09
4	6.93	952	952	Camphene	2.29 ± 0.02	1.71 ± 0.09
5	7.78	977	974	Sabinene	5.72 ± 0.03	2.20 ± 0.02
6	7.92	980	979	β-Pinene	27.09 ± 0.05	20.45 ± 0.07
7	8.39	993	991	β-Myrcene	1.80 ± 0.04	1.33 ± 0.03
8	8.87	1005	1005	α-Phellandrene	4.19 ± 0.05	2.25 ± 0.04
9	9.10	1012	1011	3-Carene	1.82 ± 0.04	0.44 ± 0.06
10	9.34	1019	1017	α-Terpinene	0.52 ± 0.04	0.26 ± 0.02
11	9.66	1027	1025	p-Cymene	2.97 ± 0.05	4.31 ± 0.03
12	9.82	1031	1030	α-Limonene	6.84 ± 0.02	6.64 ± 0.05
13	9.94	1034	1032	1,8-Cineole	3.40 ± 0.03	14.46 ± 0.08
14	10.63	1052	1049	β-trans-Ocimene	0.28 ± 0.02	0.08 ± 0.03
15	11.04	1062	1060	γ-Terpinene	1.17 ± 0.02	0.60 ± 0.05
16	12.27	1089	1087	Fenchone	4.19 ± 0.03	13.02 ± 0.13
17	12.80	1100	1099	β-Linalool	0.17 ± 0.01	0.32 ± 0.04
18	13.33	1114	1113	Fenchol	0.40 ± 0.03	1.90 ± 0.04
19	13.71	1123	1122	cis-2-p-Menthen-1-ol	–	0.19 ± 0.03
20	14.43	1140	1139	trans-Pinocarveol	–	0.17 ± 0.02
21	14.68	1146	1143	2-Bornanone	0.35 ± 0.02	0.90 ± 0.02
22	14.84	1150	1146	α-Fenchene hydrate	–	0.07 ± 0.01
23	15.50	1164	1164	α-Pinocarvone	–	0.09 ± 0.01
24	15.62	1167	1166	Borneol	–	0.62 ± 0.04
25	16.16	1178	1177	Terpinen-4-ol	0.35 ± 0.01	1.13 ± 0.02
26	16.76	1191	1189	α-Terpineol	0.29 ± 0.01	1.42 ± 0.03
27	17.00	1195	1193	Myrtenal	0.08 ± 0.02	0.31 ± 0.03
28	18.10	1221	1223	Fenchyl acetate	0.57 ± 0.02	4.01 ± 0.03
29	18.49	1231	1228	β-Citronellol	–	0.15 ± 0.02
30	18.76	1237	1235	Methyl thymyl ether	–	0.06 ± 0.01
31	19.65	1257	1255	trans-Geraniol	–	0.24 ± 0.03
32	20.35	1273	1270	(E)-Citral	–	0.09 ± 0.02
33	21.70	1301	1299	Carvacrol	–	0.12 ± 0.02
34	22.72	1327	1327	Myrtenyl acetate	0.08 ± 0.01	–
35	23.73	1351	1351	α-Cubebene	0.09 ± 0.01	0.14 ± 0.02
36	24.83	1376	1376	α-Copaene	0.22 ± 0.02	0.14 ± 0.02
37	25.54	1392	1391	β-Elemene	0.14 ± 0.01	–
38	26.63	1418	1419	β-Caryophyllene	4.08 ± 0.02	0.81 ± 0.06
39	27.25	1434	1433	γ-Elemene	0.17 ± 0.01	–
40	28.02	1453	1454	α-Caryophyllene	1.01 ± 0.03	0.20 ± 0.01
41	28.32	1460	1461	Alloaromadendrene	0.16 ± 0.01	0.07 ± 0.02
42	29.35	1485	1486	β-Eudesmene	0.57 ± 0.01	0.07 ± 0.01
43	29.71	1493	1494	α-Selinene	0.16 ± 0.01	0.10 ± 0.01
44	30.48	1513	1513	γ-Cadinene	1.48 ± 0.03	0.35 ± 0.02
45	30.59	1516	1517	7-epi-α-Selinene	0.07 ± 0.01	–
46	30.87	1523	1524	δ-Cadinene	0.86 ± 0.01	0.43 ± 0.03
47	31.60	1543	1542	α-Calacorene	0.10 ± 0.03	–
48	32.47	1565	1564	Nerolidol	0.17 ± 0.02	–
49	33.13	1582	1581	β-Caryophyllene epoxide	1.42 ± 0.02	0.90 ± 0.04
50	34.12	1607	1606	Humulene epoxide 2	0.14 ± 0.01	0.08 ± 0.02
51	34.36	1614	1614	1,10-di-epi-Cubenol	0.22 ± 0.01	0.07 ± 0.02
52	35.12	1635	1637	Caryophylladienol II	0.21 ± 0.01	0.09 ± 0.01
53	35.36	1641	1640	α-epi-Cadinol	0.40 ± 0.02	0.14 ± 0.02
54	35.64	1649	1649	β-Eudesmol	0.22 ± 0.01	0.09 ± 0.01
55	35.82	1654	1653	α-Cadinol	0.47 ± 0.02	0.20 ± 0.02
56	40.89	1795	1809	Ambrial	0.70 ± 0.01	0.97 ± 0.02

(Continued)

Table 1. Continued

No	RT (min)	RI (cal.)	RI (lit.)	Compounds	% Leaf EO	% Rhizome EO
	Monoterpene hydrocarbons (No. 1-12, 14, 15)				72.87 ± 0.18	54.24 ± 0.12
	Oxygenated monoterpenes (No. 13, 16-34)				9.89 ± 0.10	39.27 ± 0.24
	Sesquiterpene hydrocarbons (No. 35-47)				9.12 ± 0.12	2.32 ± 0.12
	Oxygenated sesquiterpenes (No. 48-56)				3.95 ± 0.02	2.54 ± 0.05
	TOTAL				**95.83 ± 0.21**	**98.37 ± 0.26**

RT (min): Retention time (minute); RI (cal.): Experimentally determined retention indices; RI (Lit.): Retention indices from literature; EO: Essential oil; "-": Not identified.

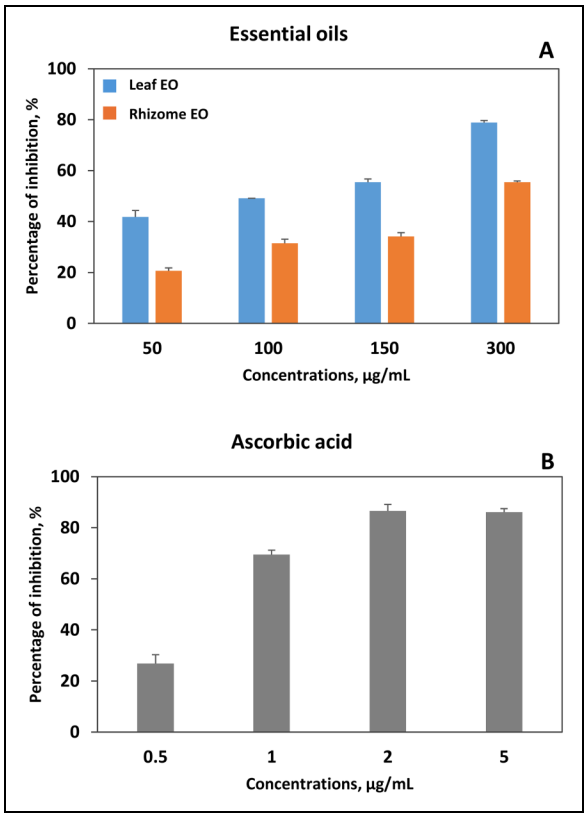

Figure 1. DPPH radical scavenging activity of the *Alpinia gagnepainii* EOs (figure 1A) and ascorbic acid (figure 1B).

words, the two EOs exhibited comparable antityrosinase activity. However, in comparison with kojic acid, the two EOs showed a weaker inhibitory effect against the enzyme.

α-Amylase Inhibitory Effect of the EOs

The inhibition percentage of α-amylase by the two EO samples at various concentrations is presented in Figure 4A. The inhibitory effect followed a concentration-dependent manner, with no significant difference observed between the two EOs at 62.5 µg/mL. However, from 125 to 500 µg/mL, the rhizome EO demonstrated higher inhibition percentages compared to the leaf EO. As shown in Figure 4C, the IC_{50} value for the rhizome EO (195.14 ± 6.49 µg/mL) was significantly lower than that of the leaf EO (343.65 ± 8.35 µg/mL), implying stronger inhibitory activity in the rhizome EO. In comparison to acarbose, a standard antidiabetic medication, both EOs exhibited lower anti-α-amylase activity (Figure 4C).

Table 2. Antioxidant Activity of *Alpinia gagnepainii* EOs.

	IC_{50} values (µg/mL) Rhizome EO	Leaf EO	Ascorbic acid
DPPH	295.01 ± 1.78 a	107.35 ± 9.17 b	0.81 ± 0.01 c
ABTS	1519.18 ± 112.58 b	2446.53 ± 22.30 a	7.09 ± 0.71 c

Different letters (a, b, c) denote significant differences in antioxidant activity among the test samples ($p < .05$).

Discussion

Volatile Composition of the EOs

Similarities and differences were noted when comparing the major components of the *A. gagnepainii* EOs with several other species collected in Vietnam. For example, in the study of the chemical composition of *A. pinnanensis* leaf EO collected in Central Vietnam, the composition included both monoterpene and sesquiterpene classes, similar to *A. gagnepainii*. However, the major compound was 1,8-cineole (20.5%), followed by 4-phenyl-2-butanol (19.5%) and α-phellandrene (10.8%), which differs from the species in the present study.[23] The EO from the rhizomes of *A. officinarum*, collected in Northern Vietnam, was also primarily composed of monoterpenes and sesquiterpenes, similar to the composition of *A. gagnepainii*. However, the dominant compounds were 1,8-cineole (50.0%) and exo-2-hydroxy-1,8-cineole acetate (11.2%), while both β-pinene and α-pinene were present in trace amounts.[24] For *A. galanga*, the leaves, stems, and roots were mainly composed of sesquiterpenes, while the rhizomes were predominantly monoterpenes, with the major compound in the rhizomes being 1,8-cineole (42.5%). The major compounds in the leaf EO were (E)-β-caryophyllene (15.8%) and caryophyllene oxide (7.4%), while β- and α-pinene were present in very low amounts.[25] For *A. calcicola*, collected in Central Vietnam, its EOs from both the leaves and rhizomes mainly contained sesquiterpenes, which differed from *A. pinnanensis*.

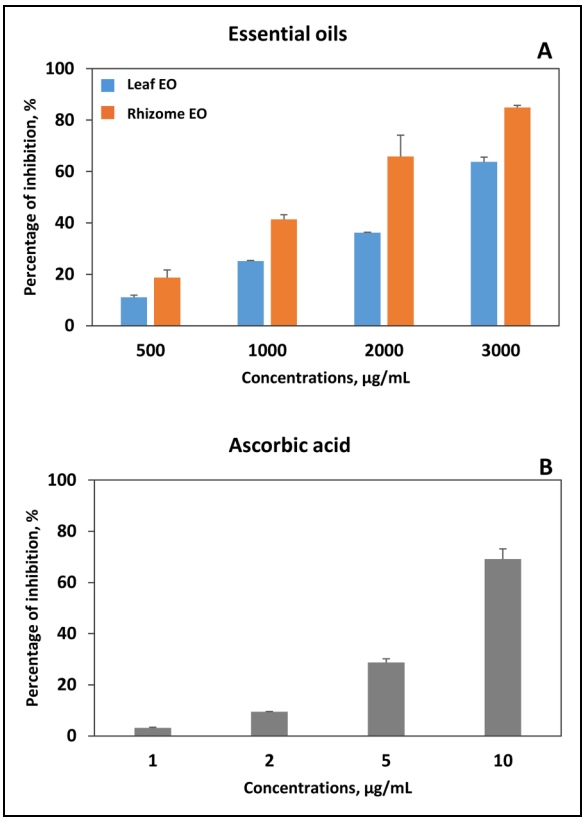

Figure 2. ABTS radical scavenging activity of the *Alpinia gagnepainii* EOs (figure 2A) and ascorbic acid (figure 2B).

The major compounds in the leaf EO were β-pinene (14.4%), δ-cadinene (9.7%), β-caryophyllene (7.7%), and α-cadinol (7.0%), while in the rhizome EO, there were α-cadinol (19.1%), β-pinene (15.3%), δ-cadinene (10.1%), and epi-α-muurolol (9.4%).[26]

Antioxidant Activity of the EOs

The major components in the EOs of both the leaves and rhizomes are α-pinene and β-pinene (Table 1), with higher concentrations in the leaves than in the rhizomes. According to previous research, these two compounds had very strong DPPH antioxidant properties.[27] This may be a contributing factor to the higher DPPH antioxidant activity observed in the leaf EO compared to its rhizome counterpart. Notably, when evaluating the radical scavenging efficiency of the leaf and rhizome EOs at concentrations ranging from 50 to 300 µg/mL, the former demonstrated a scavenging percentage of 41.8-78.9%, whereas the latter showed a percentage ranging from 20.7-55.4%. Combined with the IC_{50} values presented in Table 2, the results further confirm this, with the IC_{50} for the leaf EO being 1070.35 ± 9.17, and for the rhizome EO being 295.01 ± 1.78 µg/mL. This indicates that the antioxidant potential of the leaf EO measured by DPPH assay may be weaker than that of the rhizome EO in *A. gagnepainii*, albeit weaker than the positive control, ascorbic acid (Figure 1, Table 2). When comparing the antioxidant capacity of this species' EOs to those of other species within the *Alpinia* genus, the *A. gagnepainii* leaves possess a higher antioxidant capacity than the *A. galanga* flowers (IC_{50} = 138.6 µg/mL), while the rhizomes show a lower capacity.[28] In comparison with the EO of *A. malaccensis* (IC_{50} = 18.3 µg/mL), the EOs from both the leaves and rhizomes of *A. gagnepainii* show lower DPPH antioxidant activity.[7] With respect to the ABTS radical scavenging ability of the EO, previous research by Ling et al reported the activity was correlated with the presence of oxygenated monoterpenes.[29] This could explain the higher antioxidant capacity of the rhizome EO compared to the leaf EO in the present study. In comparison with EOs from other species in the genus *Alpinia,* such as *A. zerumbet, A. galanga,* and *A. malaccensis*, the EOs from *A. gagnepainii* might exert lower ABTS.[7,30]

Enzyme Inhibitory Activities of the EOs

In a review commenting on tyrosinase inhibition, Zolghadri et al (2019) showed that potent tyrosinase inhibitors are often phenolic compounds and their derivatives.[31] These compounds are effective due to their structural characteristics, such as hydroxyl groups that interact with the active site of the enzyme or chelate the copper ions necessary for tyrosinase activity. In the composition of the *A. gagnepainii* EOs, carvacrol (No. 33) was the only phenolic derivative. Furthermore, it was found at a trace concentration in the rhizome EO and not detected in the leaf EO. In other words, the EOs were not rich in phenolics, perhaps explaining why the tyrosinase inhibitory activity was not as strong as kojic acid. Several volatile compounds, including pinenes, limonene, myrcene, and β-caryophyllene, identified in the EOs of *A. gagnepainii*, have been previously reported to possess tyrosinase inhibitory activity.[32-34] Moreover, the antityrosinase properties of EOs from various *Alpinia* species have been extensively documented in the literature. For example, EO extracted from leaves of *A. zerumbet* has demonstrated strong tyrosinase inhibition, with an IC_{50} value of 25 µg/mL.[30] Similarly, flower EO of *A. galanga* displayed moderate tyrosinase inhibitory activity, with an IC_{50} value of 620 µg/mL.[28] Numerous other *Alpinia* species, such as *A. aquatica*, *A. nantoensis*, *A. nigra*, and *A. speciosa*, have been shown to inhibit tyrosinase.[35-38] The wide range of *Alpinia* species that exhibit tyrosinase inhibitory activity highlights the potential of this genus as a source of natural skin-whitening agents, which could be further developed for cosmetic and pharmaceutical applications. Tyrosinase, a key enzyme in melanin biosynthesis, is a well-known target for the treatment of hyperpigmentation disorders. In this regard, the potent inhibition observed in *A. gagnepainii* EOs suggests it could serve as an effective alternative to synthetic inhibitors.

As presented earlier, while the EOs have inhibitory effects on α-amylase, they may lack the targeted, high-affinity binding properties of inhibitors like acarbose. The latter can interact with the enzyme's active site, resulting in much stronger and more consistent inhibition. Besides, the active inhibitory

components in the EOs may be present at relatively low concentrations compared to acarbose. As a result, their collective inhibitory impact can be weaker. Previous studies have highlighted the inhibitory potential of species in the *Alpinia* genus on carbohydrate-hydrolyzing enzymes. For instance, molecular docking studies have shown that volatile compounds in *Alpinia nigra* EO have promising inhibitory effects on α-amylase.[39] Similarly, *in vitro* assays demonstrated that extracts from *A. nigra* strongly inhibit α-amylase, with effects comparable to acarbose.[40] Essential oils from other species within the Zingiberaceae family, such as *Zingiber atroporphyreus*, *Z. officinale*, and *Curcuma caesia*, have also been reported to inhibit α-amylase, though their efficacy was lower than that of acarbose.[41-43] The inhibitory activity of *Alpinia gagnepainii* EOs on α-amylase may be attributed to the presence of monoterpenes in their composition. Compounds such as limonene, pinenes, and 1,8-cineole, which were found as major components in this study, have been previously reported to inhibit α-amylase.[34,44] Additionally, the observed anti-α-amylase activity could result from additive or synergistic interactions among the various constituents of the EOs.

Limitations of the Study

As described above, *A. gagnepainii* was collected from a single geographic location (Can Loc district, Ha Tinh province, Vietnam), which may not account for regional variations in essential oil composition influenced by environmental factors. Another limitation is that the study does not include analyses of the key compounds while the chemical composition of the EOs were analyzed, and their bioactivities evaluated. Furthermore, only two antioxidant assays (DPPH and ABTS) were employed, and incorporating additional methods such as ferric reducing antioxidant power (FRAP) or oxygen radical absorbance capacity (ORAC) could have provided a more comprehensive understanding of the antioxidant potential of the EOs. The study also does not explore the potential synergistic or antagonistic effects among the identified compounds, which may significantly influence the observed bioactivities. Although enzyme inhibitory activities were demonstrated, the study does not investigate the molecular mechanisms or binding interactions using advanced techniques such as molecular docking or kinetic studies. By addressing these limitations in future studies, a more robust understanding of the potential applications of *A. gagnepainii* EOs can be achieved.

Conclusion

This study revealed that the EOs from the *A. gagnepainii* leaves and rhizomes collected in Ha Tinh province, Vietnam, are predominantly composed of monoterpenes. This is the first study to report the antioxidant potential, as well as the *in vitro* anti-α-amylase and antityrosinase activities of these EOs. The demonstrated bioactivities highlight the potential of these EOs as natural ingredients in nutraceuticals aimed at managing metabolic conditions such as diabetes, where the regulation of carbohydrate metabolism and oxidative stress is critical. Moreover, their tyrosinase inhibition suggests valuable cosmeceutical applications for skin care products targeting hyperpigmentation and signs of aging, capitalizing on their skin-protective and skin-brightening properties. To bridge these promising findings to practical applications, additional research is essential to elucidate the specific mechanisms of action underlying these bioactivities and to assess the safety and efficacy of *A. gagnepainii* EOs in formulation studies.

Acknowledgments

We are grateful to Assoc. Prof. Dr Nguyen Hoang Tuan (Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy) for his valuable contribution in determining the plant's scientific name.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

References

1. Burkill IH. *A Dictionary of the Economic products of the Malay Peninsula*. 2nd ed. Vol. 2. Ministry of Agriculture and Co-operatives; 1996:2444.
2. Thi L, Trinh H, Dau TH, et al. Diversity of Zingiberaceae family in Pu Mat national park, Nghe An province. *VNU J Sci: Nat Sci Technol*. 2018;34(1):84-89. doi.org/10.25073/2588-1140/vnunst.4722
3. Pham HH. *An Illustrated Flora of Vietnam*. 2nd ed. Vol. 2. Tre Publishing House; 1999.
4. Zhang W-J, Luo J-G, Kong L-Y. The genus Alpinia: a review of its phytochemistry and pharmacology. *World J Trad Chin Med*. 2016;2(1):26-41.
5. Hamad A, Alifah A, Permadi A, et al. Chemical constituents and antibacterial activities of crude extract and essential oils of *Alpinia galanga* and *Zingiber officinale*. *Int Food Res J*. 2016;23(2):837-841.
6. Raina AP, Abraham Z. Essential oil profiling of *Alpinia* species from southern India. *Indian J Exp Biol*. 2017;55(11):776-781.
7. Sahoo S, Singh S, Nayak S. Chemical composition, antioxidant and antimicrobial activity of essential oil and extract of *Alpinia malaccensis* Roscoe (Zingiberaceae). *Int J Pharm Pharm Sci*. 2014;6(7):183-188.
8. Huong LT, Dai DN, Chung MV, et al. Constituents of essential oils from the leaf, stem, root, fruit and flower of *Alpinia macroura* K. Schum. *Bol Latinoam Caribe Plantas Med Aromat*. 2017;16(1):26-33.

9. Victório CP, Riehl C, Lage CLS. Simultaneous distillation-extraction, hydrodistillation and static headspace methods for the analysis of volatile secondary metabolites of *Alpinia zerumbet* (Pers.) Burtt et Smith. from Southeast Brazil. *J Essent Oil-Bear Plants*. 2009;12(2):137-143.
10. Wang Y, You CX, Yang K, et al. Chemical constituents and insecticidal activities of the essential oil from *Alpinia blepharocalyx* rhizomes against *Lasioderma serricorne*. *J Serb Chem Soc*. 2015;80(2): 171-178.
11. Raut JS, Karuppayil SM. A status review on the medicinal properties of essential oils. *Ind Crops Prod*. 2014;62:250-264.
12. Bayala B, Bassole IHN, Scifo R, et al. Anticancer activity of essential oils and their chemical components-a review. *Am J Cancer Res*. 2014;4(6):591-607.
13. Hong Y, Liu X, Wang H, et al. Chemical composition, antibacterial, enzyme-inhibitory, and anti-inflammatory activities of essential oil from *Hedychium puerense* rhizome. *Agronomy*. 2021;11(12):2506.
14. Buchbauer G, Bohusch R. Biological activities of essential oils: an update. In: Baser KHC, Buchbauer G eds. *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press/Taylor & Francis Group; 2010:281-321.
15. Bân NT. *Danh lục các loài thực vật Việt Nam*. Vol. 3. Nhà xuất bản Nông Nghiệp; 2003:488.
16. Giang PM, Son PT. Biologically active phenolic constituents from Alpinia gagnepainii K. Schum.(Zingiberaceae). *Vietnam J Chem*. 2007;45(1):126-126.
17. Tran-Trung H, Giang LD, Duc DX, et al. Volatile constituents and in vitro antimicrobial activities of essential oils from leaves of Siliquamomum oreodoxa NS Lý & Škorničk and Curcuma thorelii Gagnep.(Zingiberaceae) growing in Vietnam. *J Biol Act Prod Nat*. 2023;13(2):145-155.
18. Adams R. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. 4th ed. Allured Publishing Corporation; 2007.
19. Tian M, Wu X, Lu T, et al. Phytochemical analysis, antioxidant, antibacterial, cytotoxic, and enzyme inhibitory activities of Hedychium flavum rhizome. *Front Pharmacol*. 2020;11:572659.
20. Vejayan J, Selladuri NE, Ibrahim H, et al. Biological activities of essential oils hydrodistillated from two closely related ginger species: alpinia malaccensis var. nobilis and Alpinia latilabris leaves. *J Essent Oil-Bear Plants*. 2017;20(4):959-971.
21. Ngan NT, Nguyen THD, Vu DC, et al. Chemical composition, enzyme inhibitory activities, and molecular docking studies of essential oil of Knema globularia leaves from Vietnam. *J Essent Oil-Bear Plants*. 2024;27(2):584-593.
22. Tran-Trung H, Vu DC, Van Trung H, et al. Assessing enzymatic inhibitory effects of essential oil from Distichochlamys orlowii K. Larsen & MF Newman rhizomes. *J Biol Act Prod Nat*. 2023;13(6):562-572.
23. Huong LT, Dai DN, Thang TD, et al. Analysis of the volatile constituents of alpinia pinnanensis. *J Essent Oil-Bear Plants*. 2017;20(1):264-271.
24. Ly TN, Yamauchi R, Kato K. Volatile components of the essential oils in galanga (Alpinia officinarum Hance) from Vietnam. *Food Sci Technol Res*. 2001;7(4):303-306.
25. Nguyen BV, Hung NH, Satyal P, et al. Chemical composition and pesticidal activity of Alpinia galanga (L.) willd. essential oils in Vietnam. *Rec Nat Prod*. 2022;16(2):182-187.
26. Huong LT, Linh LD, Dai DN, et al. Essential oil compositions and antimicrobial activity of the leaves and rhizomes of Alpinia calcicola from Vietnam. *Chem Nat Compd*. 2022;58(5):939-942.
27. Salehi B, Upadhyay S, Erdogan Orhan I, et al. Therapeutic potential of α-and β-pinene: a miracle gift of nature. *Biomolecules*. 2019;9(11):738.
28. Tian Y, Jia X, Wang Q, et al. Antioxidant, antibacterial, enzyme inhibitory, and anticancer activities and chemical composition of Alpinia galanga flower essential oil. *Pharmaceuticals*. 2022;15(9):1069.
29. Ling Q, Zhang B, Wang Y, et al. Chemical composition and antioxidant activity of the essential oils of citral-rich chemotype Cinnamomum Camphora and Cinnamomum Bodinieri. *Molecules*. 2022;27(21):7356.
30. Be Tu PT, Tawata S. Anti-oxidant, anti-aging, and anti-melanogenic properties of the essential oils from two varieties of Alpinia zerumbet. *Molecules*. 2015;20(9):16723-16740.
31. Zolghadri S, Bahrami A, Hassan Khan MT, et al. A comprehensive review on tyrosinase inhibitors. *J Enzyme Inhib Med Chem*. 2019;34(1):279-309.
32. Yang CH, Huang YC, Tsai ML, et al. Inhibition of melanogenesis by β-caryophyllene from lime mint essential oil in mouse B16 melanoma cells. *Int J Cosmet Sci*. 2015;37(5):550-554.
33. Capetti F, Tacchini M, Marengo A, et al. Citral-containing essential oils as potential tyrosinase inhibitors: a bio-guided fractionation approach. *Plants*. 2021;10(5):969.
34. El Omari N, Mrabti HN, Benali T, et al. Expediting multiple biological properties of Limonene and α-Pinene: main bioactive compounds of Pistacia lentiscus L., essential oils. *Front Biosci (Landmark Ed)*. 2023;28(9):229.
35. Lima RM, Polonini HC, Souza KC, et al. Assessment of different biological capacities of Alpinia speciosa (Pers.) BL Burtt and RM Sm. *J Young Pharm*. 2015;7(4s):500.
36. Romes NB, Basar N, Sirat HM, et al. Chemical compositions and tyrosinase activity of the essential oils of Alpinia aquatica. *Nat Prod Commun*. 2018;13(6):1934578X1801300635.
37. Kumar KJS, Vani MG, Wu P-C, et al. Essential oils of Alpinia nantoensis retard forskolin-induced melanogenesis via ERK1/2-mediated proteasomal degradation of MITF. *Plants*. 2020;9(12):1672.
38. Gupta MK, Senthilkumar S, Chiranjivi AK, et al. Antioxidant, anti-tyrosinase and anti-inflammatory activities of 3, 5-dihydroxy-4′, 7-dimethoxyflavone isolated from the leaves of Alpinia nigra. *Phytomed Plus*. 2021;1(3):100097.
39. Saikia J, Washmin N, Borah T, et al. Physicochemical properties, chemical composition and sensory attributes of Alpinia nigra (gaertn.) BL burtt rhizome: an underutilized spice source. *Eur Food Res Technol*. 2023;249(4):1097-1112.
40. Ghosh S, Rangan L. Molecular docking and inhibition studies of α-amylase activity by labdane diterpenes from Alpinia nigra seeds. *Med Chem Res*. 2014;23:4836-4852.
41. Nickavar B, Joshaghani RA. An effect-based approach to identify α-amylase inhibitors from the essential oil of Zingiber officinale rhizome: experimental and computational studies. *Food Biosci*. 2023;56:103345.

42. Tran-Trung H, Le DG, Hoang VT, et al. Essential oils of two Species of Zingiberaceae family from Vietnam: chemical compositions and α-glucosidase, α-amylase inhibitory effects. *Nat Prod Commun*. 2024;19(2):1934578X241232281.
43. Gangal A, Duseja M, Sethiya NK. Chemical composition, in vitro antioxidant and α-amylase inhibitory activities of rhizomes essential oil and nutrient components from rhizomes powder of Curcuma caesia roxb.(black turmeric) collected from garhwal region of uttrakhand, India. *J Essent Oil-Bear Plants*. 2023;26(6):1473-1486.
44. Jelenkovic L, Jovanovic VS, Palic I, et al. In vitro screening of α-amylase inhibition by selected terpenes from essential oils. *Trop J Pharm Res*. 2014;13(9):1421-1428.