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Research Article

Antioxidant activities and chemical composition of rhizome essential oil from *Zingiber plicatum* Škorničk. & Q.B.Nguyen from Vietnam

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Abstract
The *Zingiber* genus has been widely applied in food and pharmaceuticals due to its varied chemical composition and significant benefits. *Zingiber plicatum* Škorničk. & Q.B. Nguyễn was described in Vietnam in 2015, yet there has been no report on its phytochemicals and bioactivity. In this study, we first analyzed the chemical composition of essential oil and its antioxidant activities. Isolation of the rhizome essential oil was performed through hydrodistillation using a Clevenger apparatus for 3.5 h, then volatile compositions were determined using gas chromatography-mass spectroscopy (GC-MS). Additionally, the antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing power assays. The GC-MS analysis showed a total of 54 volatile components, mainly belonging to monoterpene hydrocarbons (57.69%) and sesquiterpene hydrocarbons (28.98%). The main compounds were β-pinene (26.85%), α-pinene (11.73%), limonene (10.03%), and germacrene D (5.26%). The essential oil also showed antioxidant activities, with an IC_{50} of 84.53 mg/mL for DPPH scavenging activity and an EC_{50} of 17.3 mg/mL for ferric reducing power. These findings first provided data on the chemical components and antioxidant properties of *Z. plicatum* essential oil.

Keywords
Zingiber plicatum, Essential oil, GC-MS, Chemical composition, Antioxidant activity

INTRODUCTION

Zingiber Mill., which belongs to the family Zingiberaceae, is a genus of flowering plants comprising approximately 144 species. These plants mainly grow in India, Japan, China, and Southeast Asian countries[1,2]. Over the years, species of the *Zingiber* genus have been extensively utilized in both food and pharmaceutical applications, providing significant economic value[3]. For example, *Z. officinale*, known as ginger, is recognized for its diverse biological activities such as antioxidant, antimicrobial, anti-cancer, anti-inflammatory, anti-diabetic, anti-platelet aggregation, analgesic, larvicidal, and anti-atherosclerotic properties[4]. *Z.*

zerumbet, has been used to treat stomach pain, toothache, fever, sprains, and indigestion[5]. *Z. striolatum* has been used to treat diabetes and constipation in Chinese folklore[6], etc.

One notable aspect of Zingiberaceae is its potential as a rich source of essential oils, with species like are *Zingiber* known for yielding abundant essential oils. Numerous studies have focused on the chemical composition of *Zinger* oils. For instance, the essential oil from *Z. pellitum* leaves is rich in β-caryophyllene (51.7%), β-pinene (12.0%), and α-pinene (10.0%). Meanwhile, the essential oil from the stems contains β-caryophyllene (24.9%), β-pinene (19.8%), and α-humulene (18.8%)[7]. Additionally, the root oil of this species comprises compounds such as 9-epi-(E)-caryophyllene (7.5%), humulene epoxide II (7.4%), α-humulene (6.4%), caryophyllene oxide (5.9%), β-caryophyllene (4.9%), camphene (4.3%), epi-α-cadinol (4.0%), and cyperotundone (4.0%). GC-MS analysis of *Z. striolatum* rhizomes oil identified predominant compounds, including β-phellandrene (24.0%), sabinene (17.3%), β-pinene (11.4%), geranyl linalool (8.6%), terpinen-4-ol (8.3%), α-pinene (5.6%), and crypton (4.5%)[6]. *Z. ottensii* demonstrated high levels of compounds in its rhizomes essential oil, such as zerumbone (25.21%), sabinene (23.35%), and terpene-4-ol (15.97%)[8]. In addition, the essential oils of *Zingiber* species have demonstrated various biological activities. Among these, studies on the antioxidant activities of *Zingiber* species have yielded noteworthy results. The essential oil of *Z. officinale* rhizomes exhibited remarkable antioxidant activities, including $OH^{\bullet}$ scavenging (IC_{50} = 0.0065 µg/mL), chelating capacity (IC_{50} = 0.822 µg/mL), $ABTS^{\bullet+}$ scavenging (IC_{50} = 3.94 µg/mL), xanthine oxidase inhibition (IC_{50} = 138.0 µg/mL), superoxide anion scavenging (IC_{50} = 404.0 µg/mL) and $DPPH^{\bullet}$ scavenging (IC_{50} = 675 µg/mL)[9].

Zingiber plicatum Škorničk. & Q.B.Nguyễn, an endemic species of Vietnam described in 2015, can be found in Phutho province[10]. *Z. plicatum* is characterized by having an elliptic to broadly elliptic to obovate lamina, open flowers, a long ligule, and a peach-purple to purple labellum with an incised apex. It is one of the only terminally flowering species with prominently plicate leaves. To date, no report is available on the phytochemical and bioactivity studies of *Z. plicatum*. This study was therefore undertaken to evaluate the chemical composition and the antioxidant activities of the essential oil of the *Z. plicatum* rhizomes for the first time.

Material and Methods

Chemicals

All reagents and solvents used were of analytical grade. Hydrocarbon mixtures (C_7-C_{30} *n*-alkanes), sodium sulfate, dichloromethane (CH_2Cl_2), and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant materials

Rhizomes of *Z. plicatum* were collected in September 2023 from Phutho province, Vietnam (latitude 21°07'21.5" N and longitude 104°57'11.4" E) and identified by Assoc. Prof. Dr. Nguyen Hoang Tuan (Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, Vietnam). A voucher specimen (ZP-12092023) was deposited at the Department of Pharmacy, Da Nang University of Medical Technology and Pharmacy, Vietnam.

Hydrodistillation of the essential oil

The fresh rhizomes of *Z. plicatum* were cut into small pieces and subjected to hydrodistillation using a Clevenger-type apparatus. 450 g of samples were added to 2 L of water and hydrodistillated for approximately 3.5 h. This process is repeated three times. The essential oil was separated and dried over anhydrous sodium sulfate before being stored at a low temperature (4°C).

Analysis and identification of the essential oil

The chemical composition of the rhizome essential oil of *Z. plicatum* was determined via

Gas Chromatography-Mass Spectrometry (GC-MS). The used instrument was GC 7890B linked with MS Detector 5977B with HP-5MS UI column (Agilent Technologies)[11-15]. In brief, 30 µL of obtained essential oil was diluted in 3.0 mL of CH_2Cl_2. The carrier gas was helium (flow rate of 1.0 mL/min). Injection volume of 1.0 µL with a split ratio of 25:1. Inlet-F temperature and MS source were set at 300°C and 230°C, respectively. Oven temperature progress included an initial hold at 60°C for 1 min, then increased by 4°C/min to 240°C and finally held at this temperature for 4 min. The scanning range was 50 to 550 amu at 2 scans per second. The calculation of retention indices (RIs) was carried out using a homologous series of *n*-hydrocarbons (C_7-C_{30}) under the same operating conditions.

The individual peaks and components were identified by comparison of their RI and mass spectra with those reported in the literature (NIST17 and Adams book[16]). The relative percentages of components were calculated based on the GC peak area without correction. The data processing software was Qualitative Navigator (Version B.08.00).

Antioxidant activity

DPPH free radical scavenging activity

The essential oil's scavenging activity was determined by the DPPH assay[17]. The essential oil was dissolved in DMSO at the investigated concentrations and subsequently mixed with the 3 mM DPPH solution (1:1, v/v). The reaction occurred in the dark for 30 min, after which the mixture was spectrophotometrically measured at 517 nm. DMSO and Trolox (0-100 µg/mL) were utilized as the negative control (NC) and the positive control, respectively. The DPPH scavenging activity (%) was calculated using the following equation:

DPPH Scavenging Activity (%) = $[(A_{NC}- A_t)/A_{NC}] \times 100\%$.

Where A_{NC} denoted the absorbance of the negative control and A_t represented the absorbance of the tested samples. The linear regression models for the essential oil were performed by Microsoft Excel (Microsoft Corporation, 2018). Based on the fitted lines between concentrations and scavenging activity, the IC_{50} values were determined.

Ferric reducing antioxidant power (FRAP)

The ferric reducing power was determined using the FRAP method as in the previous studies[18,19,] with modifications. The essential oil was prepared at serial dilution using DMSO and reacted with freshly prepared FRAP working solution, containing 10 mM TPTZ solution mixed with 20 mM ferric chloride solution in acetate buffer, for 15 min in the dark. After that, the absorbance at 593 nm was spectroscopically measured. The mixture changed color because ferric (Fe^{3+}) complexes were reduced to ferrous (Fe^{2+}) forms. The higher reducing power was represented by the larger absorbance value. The concentration having an absorbance of 0.5 was stated as EC_{50} (mg/mL)[13].

Statistical analysis

All the experiments were conducted in triplicate. The results were represented as mean ± SD.

Results and Discussion

Chemical composition of the essential oil

The hydrodistillation of the fresh rhizomes of *Z. plicatum* produced a light, yellow-colored essential oil. The obtained essential oil yield was 0.24% (v/w), calculated on a fresh weight basis. A total of 54 components (Table 1) from the essential oil of *Z. plicatum* rhizomes were identified by the GC-MS analysis. The GC chromatogram is shown in Fig. 1.

The major classes found in this essential oil were monoterpene hydrocarbons (57.69%) and sesquiterpene hydrocarbons (28.98%). The main compounds (> 5%) found in the essential oil were β-pinene (26.85%), α-pinene (11.73%), limonene (10.03%), and germacrene D (5.26%). Besides, several prominent components (> 2%) were observed, including bicyclogermacrene (4.53%), δ-elemene (3.86%), *p*-cymene (2.86%), and β-bisabolene (2.05%).

In comparison to other studies on the essential oils of *Zingiber* from Vietnam, the essential oil obtained in the current study demonstrated significant compositional differences. The

Table 1. Chemical constituents identified in the essential oil from *Z. plicatum* rhizomes

No.	RT (min)	Compounds	RI (cal.)	RI (lit.)	Content (%)
1	5.290	Tricyclene	928	925	0.08
2	5.376	α-Thujene	932	929	0.38
3	5.553	α-Pinene	940	937	11.73
4	5.868	α-Fenchene	953	950	0.53
5	5.908	Camphene	955	952	0.85
6	6.039	2,4(10)-Thujadiene	960	956	0.06
7	6.526	Sabinene	978	974	0.58
8	6.646	β-Pinene	983	979	26.85
9	6.938	β-Myrcene	993	991	0.58
10	7.333	α-Phellandrene	1007	1005	0.39
11	7.498	3-Carene	1013	1011	1.70
12	7.676	α-Terpinene	1020	1017	0.05
13	7.905	p-Cymene	1029	1025	2.86
14	8.036	Limonene	1033	1030	10.03
15	8.580	β-(E)-Ocimene	1052	1049	0.23
16	8.918	γ-Terpinene	1063	1060	0.14
17	9.856	Terpinolene	1091	1088	0.65
18	12.248	Pinocarvone	1167	1164	0.07
19	13.352	Myrtenal	1198	1193	0.11
20	14.136	Fenchyl acetate	1223	1223	1.21
21	16.305	Bornyl acetate	1288	1285	0.21
22	17.524	Methyl geranoate	1327	1324	0.10
23	17.976	δ-Elemene	1341	1338	3.86
24	19.217	α-Copaene	1379	1376	0.06
25	19.726	β-Elemene	1394	1391	0.68
26	19.978	Cyperene	1402	1399	0.37
27	20.442	*cis*-α-Bergamotene	1418	1415	0.30
28	20.591	α-Santalene	1423	1420	1.90
29	20.808	*cis*-Thujopsene	1430	1429	0.56
30	20.888	β-Copaene	1433	1432	0.34
31	21.077	*trans*-α-Bergamotene	1439	1435	1.04
32	21.191	Aromandendrene	1443	1440	0.10
33	21.363	Isogermacrene D	1449	1448	0.31
34	21.643	Humulene	1458	1454	0.83
35	21.712	(E)-β-Famesene	1460	1457	0.39
36	21.867	Alloaromadendrene	1465	1461	0.61
37	22.301	γ-Gurjunene	1479	1473	1.11
38	22.496	Germacrene D	1485	1481	5.26
39	22.588	Aristolochene	1488	1487	1.31
40	22.645	β-Eudesmene	1489	1486	0.40
41	22.868	Valencene	1496	1492	1.49
42	22.965	Bicyclogermacrene	1499	1495	4.53

Table 1 ***cont***.

No.	RT (min)	Compounds	RI (cal.)	RI (lit.)	Content (%)
43	23.057	α-Muurolene	1502	1499	0.24
44	23.286	β-Bisabolene	1511	1509	2.05
45	23.480	γ-Cadinene	1518	1513	0.38
46	23.743	δ-Cadinene	1527	1524	0.86
47	24.894	*trans*-Nerolidol	1567	1564	1.29
48	25.334	Spathulenol	1581	1576	0.79
49	25.500	Caryophyllene oxide	1587	1581	0.50
50	27.405	β-Eudesmol	1655	1649	0.41
51	27.514	Pogostole	1659	1655	0.98
52	28.292	α-Bisabolol	1686	1684	0.75
53	28.567	α-*trans*-Bergamotenol	1696	1700	0.39
54	31.388	Ambrial	1802	1809	0.57
Total					94.05
Monoterpene hydrocarbons					57.69
Oxygenated monoterpenes					1.70
Sesquiterpene hydrocarbons					28.98
Oxygenated sesquiterpenes					5.68

RT: Retention time (min); RI (cal.): Retention Indices calculated in relation to a series of C_7 - C_{30} *n*-alkanes; RI (lit.): Retention Indices found in the literature

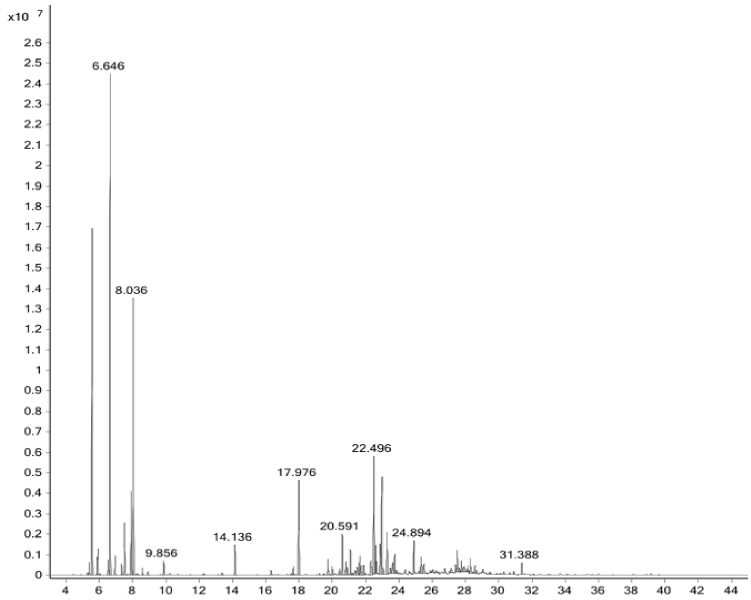

Figure 1. The GC chromatogram of the essential oil from *Z. plicatum* rhizomes

essential oil of *Z. densissimum* rhizomes was found to contain mainly β-pinene (38.36%), β-phellandrene (26.85%), and α-pinene (13.31%)[20]. The main constituents of *Z. monophyllum* rhizome and leaf oils were β-pinene (29.8 - 44.9%), borneol (3.1 - 5.2%), α-pinene (1.8 - 4.1%), and myrtenal (3.2 - 4.0%)[21]. The rhizome essential oil of *Z. eberhardtii* consists of linalool (34.9%) and 1,8-cineole (12.7%) as the principal components, while the compositions of

essential oil from the rhizomes of *Z. skornickovae* mainly comprise of β-pinene (25.0%), (E)-caryophyllene (8.8%), and α-pinene (7.9%)[22]. The most abundant compounds in the essential oils from *Z. atroporphyreus* rhizomes and leaves were β-pinene (40.6 - 48.0%), α-pinene (12.0 - 14.7%), β-elemene (11.7 - 4.4%), and sabinene (3.8 - 6.1%)[23]. Interestingly, α-pinene and β-pinene were identified as the dominant components in almost all the essential oils of *Zingiber* from Vietnam.

Antioxidant activities

In this study, the antioxidant activities of *Z. plicatum* rhizomes were evaluated through two different methods, DPPH-free radical scavenging and ferric reducing power assays. DPPH, the synthetic free radical, is commonly used to determine the stabilizing capabilities of pharmaceutical compounds, while the FRAP method measures the donating electron power of substances, reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions[13].

The essential oil showed relatively mild antioxidant activities, with an IC_{50} of 84.53 mg/mL for DPPH scavenging activity and an EC_{50} of 17.3 mg/mL for ferric reducing power. The antioxidant activities of essential oil were weaker than those of Trolox, with its IC_{50} of 0.038 mg/mL and EC_{50} of 0.1 mg/mL (Table 2).

Several previous studies showed the antioxidant activities of other *Zingiber* species' essential oils. The essential oil of the rhizomes of *Z. striolatum*, comprising β-phellandrene (24.0%), sabinene (17.3%), β-pinene (11.4%), geranyl linalool (8.6%), terpinen-4-ol (8.3%), α-pinene (5.6%) and crypton (4.5%), exhibited antioxidant power, with an IC_{50} of 3.42 mg/mL against DPPH free radicals[6]. The essential oil of *Z. officinale* roots from India, including main compounds of zingiberene (31.08%), ar-curcumene (15.35%), α-Sesquiphellandrene (14.02%), β-Bisabolene (13.81%), sabinene (8.28%), and camphene (5.14%), displayed DPPH radical scavenging activity, with an IC_{50} greater than 1.0 mg/mL. This essential oil also showed scavenging superoxide and hydroxyl radical activities and inhibition of lipid peroxidation[24], while the essential oil of *Z. officinale* roots from Ecuador, mainly containing citral (geranial 10.5% and neral 9.1%), α-zingiberene (17.4%), camphene (7.8%), α-farnesene (6.8%), and β-sesquiphellandrene (6.7%), showed greater antioxidant activity, with a DPPH scavenging activity IC_{50} of 0.675 µg/mL[9]. Even if the plant sample was collected in the same region, different altitudes could lead to variations in chemical composition and antioxidant activities. The essential oil of *Z. officinale* roots in higher altitudes was rich in geraniol (10.56%), geranial (9.92%), and eucalyptol (6.62%), whereas the oil from lower altitudes was rich in β-phellandrene (10.52%), α-funebrene (10.35%), and geranial (7.24%). Correspondingly, the ginger essential oil from the higher altitude showed greater antioxidant activity than the lower one[25].

The antioxidant activities of essential oil from *Z. plicatum* could be associated with major monoterpene compositions, such as α-pinene, β-pinene, and limonene. Monoterpenes, the major components of the essential oils of many plants, are well known for their natural antioxidants' potential[26]. According to the previous study, the increasing concentrations of α-pinene could lead to an elevation of antioxidant activities determined by DPPH and FRAP assays[27]. The IC_{50} values of α-pinene were 3116.3 µg/mL and 2245.0 µg/mL for DPPH and ABTS, respectively[28]. Limonene also demonstrates the ability to convert DPPH radicals to stable molecules and reduce ferric to ferrous ions[29]. Other essential oils contain α-pinene, β-pinene,

Table 2. Antioxidant activities of the essential oil of *Z. plicatum* rhizomes

Sample	DPPH scavenging activity IC_{50} (mg/mL)	Ferric reducing antioxidant power EC_{50} (mg/mL)
Essential oil	84.53 ± 0.60	17.3 ± 0.01
Trolox	0.038 ± 0.00	0.10 ± 0.00

and limonene as the main active compounds contributing to their antioxidant activities. The essential oil from the fresh peel of citrus plants primarily contained limonene, γ-terpinene, and β-pinene, exhibiting DPPH scavenging activity, with an IC_{50} of 1.17 mg/mL. The monoterpene hydrocarbon fraction also showed a positive correlation with DPPH and the β-carotene bleaching test[30]. In another study, essential oil from *Elaeoselinum asclepium*, comprising a high content of monoterpenes like α-pinene (43.9%), followed by sabinene (27.9%), β-pinene (16.0%), and limonene (2.0%), showed the IC_{50} of 48.26 mg/mL and EC_{50} of 0.513 mg/mL determined by DPPH and FRAP assays, respectively[31].

Besides, the components with lower concentrations, such as germacrene D, bicyclogermacrene, β-bisabolene, α-thujene, camphene, sabinene, γ-terpinene, p-cymenene, can also directly contribute to the essential oil's antioxidant activities or through the synergy among them[13,32-34].

Conclusion

The present study reports the essential oil composition of *Z. plicatum* rhizomes and its antioxidant activities for the first time. The GC-MS analysis identified fifty-four volatile components, with 28.98% of sesquiterpene and 57.69% of monoterpene hydrocarbons. Some major compounds were identified as β-pinene (26.85%), α-pinene (11.73%), limonene (10.03%), and germacrene D (5.26%). The essential oil exhibited mild antioxidant activities, with IC_{50} DPPH scavenging activity and EC_{50} ferric reducing power of 84.53 mg/mL and 17.3 mg/mL, respectively. These findings contribute more information to the literature on the *Zingiber* genus's chemical composition and bioactivity.

Competing interests

No potential conflict of interest was reported by the authors.

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