Journal of Essential Oil Bearing Plants

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/teop20

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To cite this article: Hieu Nguyen-Ngoc, Le Duc Giang, Hieu Tran-Trung, Tran Dinh Thang, Nguyen Hoang Tuan, Dang Khoa Nguyen, Nguyen Danh Duc, Nguyen Thanh To Nhi, Chi Toan Le & Nguyen Thi Giang An (2024) Chemical constituents and *in vitro* antimicrobial activity of rhizome essential oils of *Zingiber densissimum* S.Q.Tong & Y.M.Xia and *Kaempferia laotica* Gagnep. growing wild in Vietnam, Journal of Essential Oil Bearing Plants, 27:1, 73-81, DOI: 10.1080/0972060X.2024.2307905

To link to this article: https://doi.org/10.1080/0972060X.2024.2307905

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JOURNAL OF ESSENTIAL OIL BEARING PLANTS

Taylor & Francis
Taylor & Francis Group

DOI: 10.1080/0972060X.2024.2307905

Research Article

Chemical constituents and *in vitro* antimicrobial activity of rhizome essential oils of *Zingiber densissimum* S.Q.Tong & Y.M.Xia and *Kaempferia laotica* Gagnep. growing wild in Vietnam

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Received 29 September 2023
Revised 10 January 2024
Accepted 15 January 2024

Abstract

Zingiber densissimum S.Q.Tong & Y.M.Xia and *Kaempferia laotica* Gagnep., belong to the Zingiberaceae family, which were found in Vietnam and Laos in recent years. To date, there has been very limited information on their phytochemical profiles and pharmacological activities. The present study was conducted to give insights into the chemical composition of the essential oils isolated from two plants and their antimicrobial activities. Gas Chromatographic–Mass Spectral (GC-MS) analysis was utilized to identify the chemical composition of the rhizome essential oils of *Z. densissimum* and *K. laotica*. The antimicrobial activities were evaluated on three Gram-positive bacterial strains, three Gram-negative bacterial strains, and a pathogenic yeast. The GC-MS analysis showed that β-pinene (38.36%), β-phellandrene (26.85%), and α-pinene (13.31%) were the most dominant components in *Z. densissimum* rhizome essential oil, while *K. laotica* rhizome essential oil was found to contain mainly camphene (23.44%), α-pinene (10.69%), 3-carene (8.60%) and β-pinene (6.85%). As for the antimicrobial activity test results, the rhizome essential oil of *Z. densissimum* displayed potent activities against *Candida albicans* (MIC value = 2 µg/mL), *Enterococcus faecalis*, *Staphylococcus aureus*, and *Escherichia coli* (MIC values = 8 µg/mL). Meanwhile, the rhizome essential oil of *K. laotica* showed weaker activities against all investigated microbial strains (MIC values ranging from 32 to 256 µg/mL). The study results indicated the chemical constituents of the essential oils prepared from these plants. The two essential oil samples were also shown to exhibit potential antimicrobial activities against several pathogenic bacterial and fungal strains.

Keywords
Zingiber densissimum, *Kaempferia laotica*, GC-MS analysis, Essential oil, Antimicrobial activities

INTRODUCTION

The genus *Zingiber* is the third largest of the Zingiberaceae family and contains over 140 species. Most of the species are edible and medicinal plants that possess aromatic odors. Many species from the genus, for example, *Z. zerumbet*, *Z. officinale* (commonly known as ginger), *Z. corallinum*, and *Z. mioga*, have long been used in folklore medicine for the treatment

of nausea, vomiting, common cold, and for relieving symptoms from arthritis and abdominal pain[1]. Among recorded species, *Z. densissimum* was first discovered by Tong and Xia in southern Yunnan, China, in 1987, and then by Triboun in Thailand in 2014. Recently, *Z. densissimum* has been found in Lam Dong Province, Vietnam.

The genus *Kaempferia* is a medium-sized genus in the Zingiberaceae family with around 60 species distributed in Asia, mainly in India and Southeast Asia. In Vietnam, only seven species were discovered in natural habitat, including some plants widely used in traditional medicine, such as *K. galanga*. Vietnamese people have long used some *Kaempferia* species for the treatment of indigestion, and the alcoholic extract can be used to relieve symptoms of arthritis. In 2015, Tuan Nguyen-Hoang *et al*.[2] discovered the natural occurrence of *K. laotica* in Ba Ria-Vung Tau Province, Vietnam. Literature research showed that *K. laotica* is only distributed in Xiangkhuang Province (Laos), Ubon Ratchalthani and Buri Ram Provinces (Northeast Thailand)[2].

To date, there have been no studies on these two species of Zingiberaceae in terms of phytochemistry and bioactivity. In the present study, *Z. densissimum* rhizomes and *K. laotica* rhizomes (Fig. S1) were examined about the chemical composition of the essential oils (EOs) and their antimicrobial activities. These findings are part of research about the understudied ginger species growing wild in Vietnam, contributing to our literature about the natural product profiles and antimicrobial efficacy of these species.

Materials and Methods

Plant materials

In this study, *Z. densissimum* rhizomes were collected from Da Chais Commune, Lac Duong District, Lam Dong Province (GPS: 12°09'37.10"N 108°39'53.49"E), Vietnam in August 2022, while *K. laotica* rhizomes were gathered from Big Mountain, Vungtau City, Ba Ria Vung Tau Province (GPS: 10°22'47.72"N 107°03'29.81"E), Vietnam in July 2022. The identification and authentication of the two plants were done by Assoc. Prof. Dr. Nguyen Hoang Tuan. Voucher specimens (NHTuan 057 and NHTuan 058, respectively) were deposited at the herbarium of Hanoi National University.

Essential oil isolation

The fresh rhizomes (500 g) were cleaned, cut into small pieces, mixed with 1.5 L of distilled water, and then hydrodistilled using a Clevenger apparatus. The isolation process lasted until no further EO could be extracted (3.5 h). The collected EOs were treated with anhydrous Na_2SO_4 (Merck) to remove the traces of water and stored in sealed vials in the dark at 4°C until analysis. The experiment was done in triplicate. The EOs were obtained in yields of 0.20% (v/w, *Z. densissimum* rhizome EO) and 1.09% (v/w, *K. laotica* rhizome EO), calculated on a fresh weight basis.

Gas chromatographic-mass spectral (GC-MS) analysis and identification of the constituents

The GC-MS analysis was employed to identify the chemical composition of the EOs from *Z. densissimum* and *K. laotica* rhizomes, which was performed on an Agilent Technologies 7890B GC System with an HP-5MS UI column (size: 30 m × 0.25 mm, 0.25 µm film thickness), and coupled with an Agilent 5977B MSD model. Analysis conditions were set up as follows: Helium was the carrier gas (flow rate: 1.0 mL/min); injection volume: 1.0 µL with a split ratio of 25:1; oven temperature 0-2 min: 50°C; 2-22 min: 50-150°C (temperature rising rate: 5°C/min); 22-32 min: 150°C; 32-45 min: 150-280°C (temperature rising rate: 10 °C/min). The respective temperatures were also set up for the injector (300°C), MS Quad (150°C), transfer line temperatures (300°C), and MS source (230°C). The MS conditions were documented at 70 eV with a range of 50-550 amu at 2.0 scan/s. The individual chemical components were identified based on comparison of their retention indices (RI) and their mass spectral fragmentation patterns to those listed in the reference libraries (NIST17 and Adams book)[3-6]. The relative percentage of compounds was calculated from the peak area percentage in the GC chromatogram[7,8].

Antimicrobial activity test

The antimicrobial activities of the EO samples were evaluated on Gram-positive bacterial strains (including *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579), Gram-negative bacterial strains (including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076), as well as a pathogenic yeast (*Candida albicans* ATCC 10231), which were provided by National Institute for Food Control, Vietnam. The antimicrobial activities were assessed by microdilution broth susceptibility assay method, which was referenced from the standards issued by the Clinical and Laboratory Standards Institute (CLSI supplement M100, 2020)[9]. Luria Bertani Agar (LBA) and Sabouraud Dextrose Agar (SDA) were used in bacteria and yeast cultures on 96-well plates, respectively. The positive controls used in the study were streptomycin (for bacterial testing) and cycloheximide (for yeast testing), which were provided by the Institute of Drug Quality Control in Ho Chi Minh City, Vietnam.

The experiment was performed according to the following steps: (1) The EO samples were diluted in 10% DMSO to prepare eight different concentrations (2-256 µg/mL); (2) The bacteria and fungi (yeast) were standardized with a concentration of 2×10^5 CFU/mL; (3) the mixtures of microbial and testing samples were incubated at 37°C (18-24 h) for bacteria and at 35-37°C (36-48 h) for yeast. The minimum inhibitory concentration (MIC) value was determined as the lowest concentration that completely prevented the visible *in vitro* microbial growth after 24 h of culture. This is accomplished by spreading cultures out on an agar plate. All experiments were done three times, in duplicates.

Results and Discussion

Chemical composition analysis of the EOs

The GC-MS analysis detected 37 compounds in the rhizome EO of *Z. densissimum* (Fig. S2), of which monoterpene hydrocarbon was the largest chemical group, with 85.40% of the total content, followed by oxygenated sesquiterpenes, sesquiterpene hydrocarbons, diterpenes, and oxygenated monoterpenes based on peak area in the GC chromatogram. β-Pinene was found to be the most dominant component, accounting for 38.36% of the total content, followed by β-phellandrene (26.85%) and α-pinene (13.31%). Noticeably, these three compounds belong to the monoterpene hydrocarbon group (Table 1).

Similarly, the GC-MS analytical results revealed that a total of 52 components in the rhizome EO of *K. laotica* (Fig. S3), contained mainly monoterpene hydrocarbons (61.09%), followed by sesquiterpene hydrocarbons (15.18%), oxygenated monoterpenes (7.24%), oxygenated sesquiterpenes (4.62%) and diterpenes (0.47%). Camphene was found to be the most dominant volatile compound in the EO sample with a percentage of 23.44% based on peak area in the GC chromatogram. The EO from *K. laotica* rhizomes also contained a significant amount of α-pinene (10.69%) and β-pinene (6.85%) while a considerable amount of 3-carene was also detected (8.60%). Similar to the EO prepared from *Z. densissimum* rhizomes, all major components from the EO of *K. laotica* rhizomes belong to the monoterpene hydrocarbon group (Table 2).

The EOs of the most common ginger species, *Z. officinale*, contained mainly α-zingiberene, ar-curcumene, and geranial, which indicated the vast difference between the chemical composition of the EOs of *Z. densissimum* and *Z. officinale*[10]. When compared with other *Zingiber* species, the EO of *Z. densissimum* was shown to be very different in terms of chemical composition. Specifically, the EOs of *Z. montanum* and *Z. cassumunar* contained sabinene (37-56%), terpinen-4-ol (7-30%), and (*E*)-1-(3-4-dimethoxyphenyl) butadiene (11-16%)[11,12] while *Z. nimmonii* EO was found to contain myrcene, α-caryophyllene, β-caryophyllene, α-humulene, and α-cadinol[13].

As for the *Kaempferia* genus, the EO of *K. galanga* contained ethyl-p-methoxy-cinnamate, ethyl cinnamate, 1,8-cineole, borneol, camphene, linoleoyl, methyl-cinnamate, and pentadecane[14]. The EO of *K. angustifolia* collected in Thailand was found to contain camphene (27.98%), camphor (18.73%) and α-pinene (7.42%) while

Table 1. Chemical composition of the rhizome EO of *Z. densissimum*

No.	RT (min)	Compounds	RI (obsd.)	RI (lit.)	Percentage (%)
1	6.823	Tricyclene	926	925	0.08
2	6.966	α-Thujene	932	929	0.13
3	7.155	α-Pinene	939	937	13.31
4	7.550	Camphene	954	952	0.86
5	8.283	4(10)-Thujene (=sabinene)	978	974	0.78
6	8.397	β-Pinene	982	979	38.36
7	8.752	β-Myrcene	993	991	1.22
8	9.129	α-Phellandrene	1005	1005	1.82
9	9.301	3-Carene	1012	1011	0.15
10	9.496	α-Terpinene	1020	1017	0.09
11	9.730	p-Cymene	1028	1025	0.51
12	9.896	β-Phellandrene	1034	1031	26.85
13	10.119	(*Z*)-β-Ocimene	1042	1038	0.13
14	10.423	(*E*)- β-Ocimene	1053	1049	0.74
15	10.737	γ-Terpinene	1063	1060	0.14
16	11.607	Terpinolene	1090	1088	0.23
17	11.933	Linalool	1100	1099	0.53
18	12.345	*exo*-Fenchol	1116	1115	0.07
19	14.142	Isocamphopinone	1178	1173	0.05
20	14.771	Myrtenal	1198	1193	0.07
21	15.441	Fenchyl acetate	1224	1223	0.08
22	17.272	Bornyl acetate	1289	1285	0.56
23	18.674	δ-Elemene	1343	1338	0.24
24	19.698	α-Copaene	1381	1376	0.06
25	20.110	β-Elemene	1396	1391	0.07
26	20.837	Caryophyllene	1425	1419	0.41
27	21.071	β-Copaene	1435	1432	0.05
28	21.157	γ-Elemene	1438	1433	0.06
29	21.689	Humulene	1459	1454	0.19
30	22.387	Germacrene D	1486	1481	0.79
31	22.788	Valencene	1501	1492	0.22
32	23.028	α-Farnesene	1509	1508	0.11
33	23.160	β-Curcumene	1513	1514	0.08
34	23.525	Cadina-1(10),4-diene (=δ-Cadinene)	1525	1524	0.07
35	25.529	Caryophyllene oxide	1586	1581	0.08
36	33.557	Xanthorrhizol	1764	1753	3.11
37	39.376	Geranyl linallol	2035	2034	2.17
		Monoterpene hydrocarbons			85.40
		Oxygenated monoterpenes			1.36

Table 1 ***cont.***

	Compounds	Percentage (%)
	Sesquiterpene hydrocarbons	2.35
	Oxygenated sesquiterpenes	3.19
	Diterpens	2.17
	Total	94.47

RT: Retention time (min); RI (obsd.): Retention indices calculated in the investigation; RI (lit.): Retention indices from literature

Table 2. Chemical composition of the rhizome EO of *K. laotica*

No.	RT (min)	Compounds	RI (obsd.)	RI (lit.)	Percentage (%)
1	6.824	Tricyclene	926	925	2.43
2	6.967	α-Thujene	932	929	0.26
3	7.161	α-Pinene	939	937	10.69
4	7.602	Camphene	955	952	23.44
5	8.260	4(10)-Thujene (= Sabinene)	978	974	0.28
6	8.351	β-Pinene	981	979	6.85
7	8.752	β-Myrcene	993	991	2.29
8	9.130	α-Phellandrene	1005	1005	0.13
9	9.318	3-Carene	1013	1011	8.60
10	9.490	α-Terpinene	1019	1017	0.08
11	9.650	o-Cymene	1025	1022	0.07
12	9.725	p-Cymene	1028	1025	1.76
13	9.850	Limonene	1033	1030	3.61
14	9.925	Eucalyptol (= 1,8-cineole)	1035	1032	2.35
15	10.732	γ-Terpinene	1069	1060	0.34
16	11.607	Terpinolene	1090	1088	0.26
17	11.933	Linalool	1100	1099	0.07
18	13.255	d-2-Bornanone	1149	1143	0.97
19	13.885	endo-Borneol	1170	1167	3.37
20	14.211	Terpinen-4-ol	1181	1177	0.17
21	14.588	α-Terpineol	1193	1189	0.10
22	14.766	Myrtenal	1198	1193	0.06
23	17.272	Bornyl acetate	1289	1285	0.15
24	18.668	δ-Elemene	1342	1338	0.06
25	19.578	α-Ylangene	1377	1372	0.06
26	19.692	α-Copaene	1381	1376	0.07
27	20.104	β-Elemene	1396	1391	0.18
28	20.333	Cyperene	1404	1399	0.33
29	20.585	α-Gurjunene	1415	1409	2.95
30	20.831	Caryophyllene	1425	1419	0.57
31	21.071	β-Copaene	1435	1432	0.16

Table 2 ***cont.***

No.	RT (min)	Compounds	RI (obsd.)	RI (lit.)	Percentage (%)
32	21.163	γ-Elemene	1438	1433	4.26
33	21.695	Humulene	1459	1454	0.23
34	21.878	Alloaromadendrene	1466	1461	0.65
35	22.250	γ-Muurolene	1481	1477	0.46
36	22.382	Germacrene D	1486	1481	0.67
37	22.468	Aristolochene	1489	1487	0.29
38	22.748	Viridiflorene	1499	1493	0.47
39	22.828	Epizonarene	1502	1501	0.83
40	23.269	γ-Cadinene	1517	1513	0.76
41	23.526	Cadina-1(10),4-diene (= δ-Cadinene)	1525	1524	1.47
42	24.110	Selina-3,7(11)-diene	1543	1542	0.55
43	24.608	Germacrene B	1559	1557	0.16
44	24.974	Palustrol	1570	1568	0.48
45	25.306	Spathulenol	1580	1576	0.87
46	25.529	Caryophyllene oxide	1586	1581	0.58
47	26.719	Di-epi-1,10-cubenol	1616	1614	0.16
48	27.852	α-epi-Cadinol	1640	1640	0.78
49	28.058	δ-Cadinol	1645	1645	0.13
50	28.441	α-Cadinol	1653	1653	1.05
51	30.547	Eudesm-7(11)-en-4-ol	1695	1692	0.57
52	38.403	Sandaracopimaradiene	1966	1960	0.47
		Monoterpene hydrocarbons			61.09
		Oxygenated monoterpenes			7.24
		Sesquiterpene hydrocarbons			15.18
		Oxygenated sesquiterpenes			4.62
		Diterpenes			0.47
		Total			88.6

RT: Retention time (min); RI (obsd.): Retention indices calculated in the investigation; RI (lit.): Retention indices from literature

K. marginata contained δ-3-carene (33.84%), β-pinene (12.21%) and camphene (11.51%) in its EO. Therefore, it was observed that the chemical composition of *K. laotica* EO, to some extent, was similar to those of *K. angustifolia* and *K. marginata* with camphene, α-pinene, β-pinene, and 3-carene as the most dominant compounds[15]. Compared to other species, camphor was found to be the main component in *Artemisia herba-alba* and *Lavandula dentata*, borneol in *Origanum vulgare* and *Thymus vulgaris*, and 1,8-cineole in *Rosmarinus officinalis*[16].

Antimicrobial activity of the EOs

The EOs were evaluated for their antimicrobial activities against some pathogenic bacterial and fungal strains (Table 3). Streptomycin and cycloheximide were used as positive controls, respectively. The testing results indicated that the rhizome EO of *Z. densissimum* strongly inhibited the growth of *C. albicans,* with a MIC value of 2 µg/mL, which was considerably lower than that of cycloheximide. As for antibacterial activities, the *Z. densissimum* rhizome EO only showed moderate activities,

Table 3. Antimicrobial activity of the studied EOs

Microorganisms	Minimum Inhibitory Concentration (MIC, µg/mL)			
	Z. densissimum	***K. laotica***	**ST**	**CY**
E. faecalis ATCC299212	8	-	256	-
S. aureus ATCC25923	8	256	128	-
B. cereus ATCC14579	16	32	128	-
E. coli ATCC25922	8	128	32	-
P. aeruginosa ATCC27853	128	256	256	-
S. enterica ATCC13076	32	64	128	-
*C. albican*s ATCC10231	2	128	-	32

ST: Streptomycin; CY: Cycloheximide

with the MIC values ranging from 8 to 128 µg/mL. Specifically, *E. faecalis*, *S. aureus*, and *E. coli* were the most affected trains, with the MIC values of 8 µg/mL, while *B. cereus* and *S. enterica* were less sensitive to the EO, with MIC values of 16 and 32 µg/mL, respectively. Noticeably, the EO only showed weak effects against the growth of *P. aeruginosa* with MIC value of 128 µg/mL.

In contrast, the EO of *K. laotica* rhizomes showed generally weaker antimicrobial activities in comparison with that of *Z. densissimum* rhizomes. Specifically, the most susceptible microbial strain with *K. laotica* rhizome EO was *B. cereus* (MIC = 32 µg/mL), followed by *S. enterica* (MIC = 64 µg/mL), *E. coli* and *C. albicans* (MIC = 128 µg/mL), *S. aureus* and *P. aeruginosa* (MIC = 256 µg/mL). As for *E. faecalis* strain, the MIC value could not be detected due to the weak activity.

In comparison with other species in the Zingiberaceae family, the rhizome EO from *Z. densissimum* showed more pronounced activity, while that from *K. laotica* was in similar ranges. The EO from *Hedychium yunnanense* rhizomes was reported to display antimicrobial activities on the same microbial strains, with MIC values of 32-64 µg/mL[17]. In another study, the EO from *K. attapeuensis* rhizomes showed the MIC values around 32 µg/mL against all tested microbial strains except for *P. aeruginosa*[18].

The antimicrobial effects of the EOs are related to the major compounds and their synergy with other minor ones present in the EOs. The major monoterpene hydrocarbons from the two plants' EOs were shown to be effective against a variety of microbial strains[19-21], which explained the pharmacological results in the present study. β-Pinene was shown to display potent anti-fungal effects by interfering with the cell wall and reducing the *Candida* biofilm adhesion[22]. Camphene was effective against most dermatophytes, which are responsible for superficial skin infections, by changing the fatty acid composition of cell membrane, leading to inhibition of respiration and changes of cellular permeability[23]. 3-Carene, a main component of pine and pepper volatile oils, was found to possess strong bactericidal effects against *Brochothrix thermosphacta* and *Pseudomonas fluorescens* through cell wall permeability changes, DNA structure disruption, and interfering with the metabolic processes[24]. α-Pinene, a monoterpene abundantly found in pine and some rosemary[25], was studied for its synergistic effects with antibiotics in the combat against antibiotic resistance. The monoterpene was shown to prevent the antimicrobial efflux of *Campylobacter jejuni* (the main cause of gastroenteritis), which was displayed by reducing the MIC value of concurrent antibiotics, such as ciprofloxacin, erythromycin and triclosan, up to 512 times[26]. α-Pinene was also found to act via NorA efflux pump inhibition against *S. aureus* and significantly enhance the potential of norfloxacin[27].

Conclusion

The present study reveals the chemical composition of EOs from the two understudied ginger species, *Z. densissimum* and *K. laotica*, by the GC-MS analysis, in which β-pinene (38.36%), β-phellandrene (26.85%), and α-pinene (13.31%) were found the most abundant in *Z. densissimum* whereas the major compositions of *K. laotica* rhizome essential oil were α-pinene (10.69%), β-pinene (6.85%), and 3-carene (8.60%). The study results have shown that the EO of *Z. densissimum* rhizomes exhibited strong inhibitory activities against some microbial strains, specifically *E. faecalis*, *S. aureus*, *E. coli* (MIC value = 8 µg/mL), and *C. albican*s (MIC = 2 µg/mL) while *K. laotica*'s rhizome EO only showed weak antimicrobial effects against all investigated species (MIC values ranging from 32-256 µg/mL). The study findings should encourage further scientific work on these two gingers, to clarify their phytochemical profiles and other pharmacological activities.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Supplementary Data

Figures S1 to S3 are given in supplementary file.

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