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Research Article

Essential oils of two Zingiber plants *Zingiber eberhardtii* Gagnep. and *Zingiber skornickovae* N.S. Lý: Chemical profiles and antimicrobial effects

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Abstract

Zingiber is a genus of medicinal plants containing a rich resource of essential oils. The current study aims to describe chemical analysis and antimicrobial assay for two *Zingiber* species *Zingiber eberhardtii* Gagnep. and *Z. skornickovae* N.S. Lý. The rhizome oils, which were obtained by hydro-distillation using a Clevenger apparatus, were analyzed by GC-MS (gas chromatography-mass spectrometry). Extraction of *Z. eberhardtii* rhizome gave a yellow oil with 41 identified compounds (96.3%), in which linalool (34.9%) and 1,8-cineole (12.7%) were the principal compounds. The yellow oil of *Z. skornickovae* rhizome included 53 identified compounds (97.8%), with β -pinene (25.0%), (*E*)-caryophyllene (8.8%), and α -pinene (7.9%) as the major agents. By broth dilution method, both oils moderately controlled the growth of Gram (+) bacteria *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 with the same MIC (minimum inhibitory concentration) value of 128 μ g/mL. In particular, *Z. skornickovae* rhizome oil showed the best antimicrobial activity against yeast *Saccharomyces cerevisiae* ATCC 4098 with a MIC value of 64 μ g/mL. The obtained results are promising information for future research.

Keywords

Zingiber eberhardtii, *Zingiber skornickovae*, essential oil, chemical profile, antimicrobial activity

Introduction

Zingiber is a genus of flowering plants in the family Zingiberaceae. This genus includes about 144 species, which are widely distributed from Southeast Asia to China, Japan, and India¹⁻³. Due to their value in foods and medicines, the plants of this genus are cultivated around the world, in which *Z. officinale* is a well-known species⁴. It turns out that *Zingiber* plants are among potential Zingiberaceae species to provide a rich resource of essential oils. The extraction yield of *Z. officinale* rhizome oil might reach up to 3%, depending on the botanical source⁵. Terpene derivatives are common components in

Zingiber oils^{5,6}. These oils exhibited a wide range of biological actions, including antibacterial, larvicidal, antioxidative, anticancer, anti-obesity, anti-inflammatory, neuroprotective and cardiovascular protective properties⁵. They have been also used historically to treat a variety of ailments, such as respiratory disorders, stomach ulcers, neurodegenerative diseases, eye inflammation, and cardiovascular diseases⁷.

About 32 *Zingiber* plants are currently detected in Vietnam¹. There have been numerous phytochemical studies on Vietnamese *Zingiber* oils. *Z. magang* leaf oil, collected from Quangngai, was dominated by β -pinene (12.3%) and (*E*)-nerolidol (10.3%), while its rhizome oil was characterized by α -selinene (10.5%), caryophyllene oxide (9.7%), and *neo*-intermedeol (7.5%)⁸. By the GC-MS analysis, zerumbone (72.3%) was elucidated as a major compound of Quangninh, Vietnam, *Z. zerumbet* rhizome oil⁹. Another example is that *Z. nudicarpum* rhizome oil, from north-central Vietnam, showed remarkable antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus* with MIC values of less than 8 μ g/mL¹⁰.

Zingiber eberhardtii Gagnep. is an endemic species of Vietnam¹¹. It can be found in Ninhbinh, Kontum, and Lamdong provinces¹¹. In the meantime, *Zingiber skornickovae* N.S. Lý was elucidated as a new species, and found in Quangngai, Vietnam in 2016¹². The purpose of the current study was to describe the chemical analysis and antimicrobial activities for essential oils from these two plants.

Materials and methods

Plant materials

The fresh rhizomes of two studied plants, *Z. eberhardtii* and *Z. skornickovae*, have been gathered from Lamdong and Quangngai provinces, Vietnam in 03/2023, respectively. Their identifications were confirmed by co-author Ly Ngoc Sam. Two corresponding specimens ZER-956 (*Z. eberhardtii*) and ZSR-986 (*Z. skornickovae*), have been deposited at Institute of Tropical Biology.

Hydro-distillation of essential oils

The fresh rhizomes of each plant (1.0 kg)

were washed and then chopped into pieces. Essential oils were obtained by hydro-distillation approach for 3.0 h using a Clevenger apparatus of 2 L capacity as it the optimized period for complete extraction of the ginger essential oil^{2,13}. The obtained oils were dried over Na₂SO₄, and maintained at 5 °C for further analyses.

GC-MS analysis

The GC-MS analysis for *Z. eberhardtii* rhizome oil was performed using a Shimadzu Technologies GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) chromatograph equipped with a fused silica Equity-5 capillary column (30 m, 0.25 mm, film thickness 0.25 μ m, Supelco, USA)¹⁴. The analytical settings were as follows: 1.5 mL/min of carrier helium, the temperature of injector and interface at 280°C, and the ramp temperature from 60°C (2 min hold) to 240°C (10 min hold) at 3°C/min and to 280°C at 5°C/min for the column (10-min hold). A split ratio of 10:1 was used to inject the samples. The inlet pressure was 93.2 kPa and the injection volume was 1.0 μ L. Ionization voltage of 70 eV, detector voltage of 0.82 kV, and acquisition scan mass range of 40-500 amu at a sampling rate of 0.5 scan/s were the MS settings. By co-injecting the constituents and comparing the results to a homologous series of *n*-alkanes (C₇-C₄₀), the retention indices (RI) of chemical constituents were calculated. Chemical identification was carried out by comparison of their RI values with those in the literature¹⁵. The MS fragmentations were revised against those of other essential oils of known compositions using NIST 11 and WILEY 7 Libraries. Quantification of each volatile compound was performed on the basis of the relative area of the total ion chromatogram (TIC) peaks of volatile compounds^{14,16,17}.

A similar procedure was applied to *Z. skornickovae* rhizome oil. However, the ramp temperature was established from 60°C (2 min hold) to 180°C (15 min hold) at 5°C/min and to 280°C at 5°C/min for the column (20-min hold).

Antimicrobial assay

The pathogenic ATCC (American Type Culture Collection) strains, including three Gram (+) bacteria *Bacillus subtilis* ATCC 6633,

Staphylococcus aureus ATCC 25923 and *Clostridium sporogenes* NCTC 12935, two Gram (-) bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15442, three fungi *Aspergillus brasiliensis* ATCC 16404, *A. niger* ATCC 1015 and *Fusarium oxysporum* ATCC 46591, and two yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 4098, were used in this study. All strains were cultured on Muller Hilton Agar (MHA, Merck) plates for one day at 37°C.

The assay was described in previous reports¹⁸⁻²⁰. Briefly, the oil samples were dissolved in EtOH to reach concentrations of 4-256 µg/mL. A total of 180 µL of bacterial suspension with 10⁶ CFU mL⁻¹ in Muller Hilton Broth and 20 µL of the essential oil were placed in each well (MHB, Merck). The mixture was incubated at 37°C, and the OD (optical density) was determined at 600 nm using an Elisa reader (RNE-9002, USA). The lowest concentration that showed no growth was identified as the MIC. The assays were performed three times. The same procedures were used for the negative control, which contained MHB and Tween, and the positive controls, which contained MHB and bacterial suspension without the tested sample. Streptomycin and tetracyclin were used as reference compounds for respective Gram (+) and Gram (-) bacteria, whereas nystatin was used for fungi and yeasts.

Results and discussion

Chemical profile of essential oils

Z. eberhardtii rhizome oil was obtained with yellow color (0.15% v/w, yield). By the GC-MS analysis, a total of 41 compounds were identified in this oil, which accounted for 96.3% of the composition (Table 1). Oxygenated monoterpenoids were the major compounds with 76.7%, followed by monoterpene hydrocarbons (12.3%), non-terpenic compounds (5.1%), and oxygenated sesquiterpenoids (2.1%). In this oil sample, the principal compounds were linalool (34.9%), and 1,8-cineole (12.7%). Some compounds were detected with the percentages greater than 1.0%, including α-pinene, camphene, β-pinene, o-cymene, limonene, cis-linalool oxide (furanoid), trans-linalool oxide (furanoid),

trans-pinocarveol, borneol, cis-linalool oxide (pyranoid), trans-linalool oxide (pyranoid), (3Z)-hexenyl butanoate, myrtenol, 3,7-dimethyl-octa-1,7-dien-3,6-diol, bornyl acetate, butyl butyryl lactate, and elemol.

Z. skornickovae rhizome oil was obtained with yellow color (0.17% v/w, yield). Fifty-three compounds were identified in this oil, which represented 97.8% of the composition (Table 2). This oil was dominated by monoterpene hydrocarbons (49.7%), and sesquiterpene hydrocarbons (26.9%). The two studied oils showed very different chemical profiles; sesquiterpene hydrocarbons were not detected in *Z. eberhardtii* rhizome oil. *Z. skornickovae* rhizome oil also contained the other chemical classes, consisting of oxygenated sesquiterpenes (9.1%), non-terpenic compounds (7.1%), and oxygenated monoterpenes (5.0%). β-Pinene (25.0%), (E)-caryophyllene (8.8%), and α-pinene (7.9%) represented as the principal compounds of *Z. skornickovae* rhizome oil. Some other compounds were also found in noteworthy concentrations, such as benzyl benzoate (5.4%), limonene (5.0%), and β-elemene (3.9%). The major compound of *Z. eberhardtii* rhizome oil, linalool, was absent in *Z. skornickovae* rhizome oil, whereas the other major compound, 1,8-cineole, was observed to be drastically decreased to 1.2% in *Z. skornickovae* rhizome oil. In contrast, β-pinene of the *Z. skornickovae* rhizome oil was higher than that of *Z. eberhardtii* rhizome oil by 20.8%. The difference in results might be due to different species, geographic factors, time collection, extraction method, etc.

Zingiber plants are a potential resource of essential oils. Hung *et al.* summarised that phenylpropanoids, phenylbutanoids, and zerumbone were characteristics of *Z. niveum* roots and rhizomes; *Z. cassumunar* rhizomes and *Z. neesatum* rhizomes; and *Z. zerumbet* roots and *Z. ottensii* rhizomes, respectively¹. The leaves of *Z. rufopilosum*, *Z. gramineum*, and *Z. purpureum*, and the rhizomes of *Z. rubens* and *Z. collinsii*, and *Z. pellitum* were commonly accompanied by the predominance of monoterpene and sesquiterpene derivatives¹. Our current results can be seen as new evidence for further research.

Table 1. Chemical compositions in *Z. eberhardtii* rhizome oil

No	Compounds ^a	RT	RI ^b	RI ^c	Concentration (%)
1	α -Pinene	6.71	932	932	1.6
2	Camphene	7.18	947	946	4.0
3	Sabinene	8.0	972	969	0.2
4	β -Pinene	8.12	976	974	4.2
5	Dehydroxy- <i>cis</i> -linalool oxide	9.71	1020	1006	0.1
6	<i>o</i> -Cymene	9.86	1023	1022	1.2
7	Limonene	10.04	1028	1024	1.1
8	1,8-Cineole	10.14	1030	1026	12.7
9	Lavender lactone	10.44	1038	1034	0.1
10	<i>cis</i> -Linalool oxide (furanoid)	11.82	1072	1067	5.4
11	<i>trans</i> -Linalool oxide (furanoid)	12.49	1088	1084	5.4
12	Linalool	13.12	1104	1095	34.9
13	<i>trans</i> -Pinocarveol	14.66	1138	1135	1.2
14	Camphor	14.91	1144	1141	0.8
15	Isoborneol	15.46	1157	1155	0.2
16	Pinocarvone	15.71	1162	1160	0.6
17	Borneol	15.85	1165	1165	3.5
18	<i>cis</i> -Linalool oxide (pyranoid)	16.0	1169	1170	2.0
19	<i>trans</i> -Linalool oxide (pyranoid)	16.22	1174	1173	1.3
20	Terpinen-4-ol	16.36	1177	1174	0.3
21	Cryptone	16.77	1186	1183	0.5
22	(3 <i>Z</i>)-Hexenyl butanoate	16.95	1190	1184	3.9
23	Myrtenol	17.22	1196	1194	1.5
24	Verbenone	17.82	1210	1204	0.3
25	<i>trans</i> -Carveol	18.21	1219	1215	0.2
26	Cumin aldehyde	19.12	1240	1238	0.2
27	Carvone	19.31	1244	1239	0.2
28	3,7-Dimethyl-octa-1,7-dien-3,6-diol	20.65	1274	1274	1.2
29	Bornyl acetate	21.19	1286	1284	2.7
30	Perilla alcohol	21.73	1298	1294	0.1
31	<i>iso</i> -Verbanol acetate	22.09	1307	1308	0.3
32	<i>p</i> -Vinylguaiaicol	22.32	1312	1309	0.1
33	Myrtenyl acetate	22.8	1323	1324	0.2
34	Butyl butyryl lactate	24.37	1360	1353	1.1
35	<i>cis-p</i> -Mentha-8-thiol-3-one	24.5	1363	1357	0.3
36	<i>trans-p</i> -Menth-6-en-2,8-diol	25.16	1378	1371	0.3
37	δ -8-Hydroxycarvotanacetone	27.11	1425	1424	0.2
38	Elemol	32.18	1550	1548	1.2
39	(<i>E</i>)-Nerolidol	32.73	1564	1561	0.5

Table 1 cont.

No	Compounds ^a	RT	RI ^b	RI ^c	Concentration (%)
40	Caryophyllene oxide	33.49	1584	1582	0.1
41	β -Eudesmol	36.04	1651	1649	0.4
Total					96.3
Monoterpene hydrocarbons (Sr. no. 1-4, 6-7)					12.3
Oxygenated monoterpenes (Sr. no. 5, 8-21, 23-33, 35-37)					76.7
Oxygenated sesquiterpenes (Sr. no. 38-41)					2.2
Non-terpenic compounds (Sr. no. 22, 34)					5.1
^a Elution order on Equity-5 column; ^b Retention indices on Equity-5 column; ^c Literature retention indices (see references)					

Table 2. Chemical compositions in *Z. skornickovae* rhizome oil

No	Compounds ^a	RT	RI ^b	RI ^c	Concentration (%)
1	Tricyclene	5.89	923	921	0.2
2	α -Thujene	5.98	927	924	0.2
3	α -Pinene	6.17	932	932	7.9
4	Camphene	6.55	947	946	2.4
5	Sabinene	7.17	972	969	3.5
6	β -Pinene	7.28	976	974	25.0
7	β -Myrcene	7.59	991	988	1.8
8	α -Phellandrene	7.99	1006	1002	1.0
9	δ -3-Carene	8.15	1012	1008	1.3
10	<i>o</i> -Cymene	8.54	1023	1020	0.4
11	Limonene	8.66	1028	1024	5.0
12	1,8-Cineole	8.75	1030	1026	1.2
13	(<i>E</i>)- β -Ocimene	9.18	1048	1049	0.2
14	γ -Terpinene	9.51	1060	1060	0.4
15	Terpinolene	10.4	1090	1086	0.4
16	<i>n</i> -Nonanal	10.8	1105	1100	0.2
17	Borneol	12.6	1165	1165	0.9
18	Terpinen-4-ol	13.0	1177	1174	0.2
19	Myrtenal	13.5	1200	1195	0.3
20	<i>endo</i> -Fenchyl acetate	14.2	1223	1218	0.6
21	(<i>E</i>)-Cinnamaldehyde	15.6	1273	1267	0.2
22	Bornyl acetate	16.0	1289	1284	0.7
23	Dihydroedulan I	16.3	1299	1289	0.3
24	Methyl myrtenate	16.3	1300	1293	0.2
25	δ -Elemene	17.4	1342	1335	2.0
26	α -Copaene	18.5	1382	1374	0.1
27	β -Elemene	18.9	1397	1389	3.9
28	α -Cedrene	19.5	1420	1410	0.6

Table 2 cont.

No	Compounds ^a	RT	RI ^b	RI ^c	Concentration (%)
29	(E)-Caryophyllene	19.6	1427	1417	8.8
30	γ -Elemene	20.2	1439	1434	0.8
31	(E)-Cinnamyl acetate	20.1	1448	1443	0.3
32	cis-Muurolo-3,5-diene	20.2	1450	1448	0.8
33	Neryl propanoate	20.3	1455	1452	0.6
34	α -Humulene	20.5	1462	1452	1.2
35	α -Amorphene	21.0	1483	1483	0.6
36	Germacrene D	21.2	1489	1480	0.5
37	β -Selinene	21.3	1494	1489	0.8
38	Valencene	21.5	1501	1496	1.9
39	α -Muurolole	21.5	1507	1500	1.9
40	(E,E)- α -Farnesene	21.8	1510	1505	0.7
41	(Z)- α -Bisabolene	21.8	1514	1506	0.7
42	7- <i>epi</i> - α -Selinene	22.1	1527	1520	0.4
43	δ -Cadinene	22.2	1530	1522	0.5
44	Germacrene B	23.1	1567	1559	0.7
45	Caryophyllene oxide	23.7	1584	1582	0.9
46	Cedrol	24.1	1613	1600	3.6
47	Isospathulenol	24.7	1637	1631	0.7
48	α -Cadinol	25.3	1664	1652	0.7
49	Neocurdione	26.1	1698	MSref	0.4
50	Pentadecanal	26.5	1714	1715	1.0
51	Benzyl benzoate	27.9	1768	1759	5.4
52	Hexahydrofarnesyl acetone	30.2	1842	1845	1.9
53	(5E,9E)-Farnesyl acetone	33.1	1917	1913	0.9
Total					97.8
Monoterpene hydrocarbons (Sr. no. 1-9, 11, 13-15)					49.7
Oxygenated monoterpenes (Sr. no. 12, 17-20, 22-24)					5.0
Sesquiterpene hydrocarbons (Sr. no. 25-30, 32-44)					26.9
Oxygenated sesquiterpenes (Sr. no. 45-49, 52-53)					9.1
Non-terpeneic compounds (Sr. no. 10, 16, 21, 31, 50, 51)					7.1
^a Elution order on Equity-5 column; ^b Retention indices on Equity-5 column; ^c Literature retention indices (see references)					

Antimicrobial activity

The two essential oil samples were further submitted for antimicrobial screening, and the results are summarized in table 3. Both oils showed moderate activity against *B. subtilis* and *S. aureus* with the same MIC value of 128 $\mu\text{g/mL}$. *Z. eberhardtii* rhizome oil exhibited weak activity

against *C. sporogenes* with the MIC value of 256 $\mu\text{g/mL}$, but *Z. skornickovae* was inactive (MIC > 256 $\mu\text{g/mL}$). Regarding Gram (-) bacteria, only *Z. eberhardtii* rhizome oil suppressed the growth of *P. aeruginosa* with the MIC value of 128 $\mu\text{g/mL}$. The two oils were active against the fungus *A. niger* with MIC values of 128 and 256

Table 3. Antimicrobial activity of the studied essential oils

Microbial strains	Minimum Inhibitory concentration (MIC: µg/mL)				
	<i>Z. eberhardtii</i>	<i>Z. skornickovae</i>	Streptomycin	Tetracycline	Nystatin
Gram (+)	<i>B. subtilis</i>	128	128	4	
	<i>S. aureus</i>	128	128	8	
	<i>C. sporogenes</i>	256	> 256	8	
Gram (-)	<i>E. coli</i>	> 256	> 256		4
	<i>P. aeruginosa</i>	128	> 256		4
	<i>A. brasiliensis</i>	> 256	> 256		8
Fungi	<i>A. niger</i>	128	256		8
	<i>F. oxysporum</i>	> 256	> 256		8
Yeasts	<i>C. albicans</i>	> 256	> 256		4
	<i>S. cerevisiae</i>	128	64		8

µg/mL, respectively, but they failed to control *A. brasiliensis* and *F. oxysporum*. *Z. eberhardtii* rhizome oil (MIC 128 µg/mL) moderately inhibited the growth of the yeast *S. cerevisiae*. *Z. skornickovae* rhizome oil, in particular, showed the best antimicrobial activity to inhibit the proliferation of *S. cerevisiae* with the MIC value of 64 µg/mL, in comparison with that of the positive control nystatin (MIC 8 µg/mL).

Our current result matches well with previous reports since Vietnamese *Zingiber* oils themselves were the potential agents in antimicrobial treatments. For instance, *Z. castaneum* stem oil established the MIC values of 12.5, 50, and 50 mg/mL against *P. aeruginosa*, *A. niger*, and *F. oxysporum*, respectively²¹. *Z. magang* rhizome oil caused the inhibition to *Enterococcus faecalis* and *S. aureus* with the MIC value of less than 9.99 µg/mL, while *Z. tamii* leaf oil was the most active against *E. coli* (MIC 44.38 µg/mL) and *C. albicans* (MIC 45.62 µg/mL)⁸.

Conclusions

For the first time, chemical analyses and antimicrobial assays for the rhizome oils of *Z. eberhardtii* and *Z. skornickovae* were carried out. Two main compounds linalool (34.9%) and 1,8-cineole (12.7%) represented *Z. eberhardtii* essential oil. *Z. skornickovae* rhizome oil was marked with the appearance of β-pinene (25.0%),

(*E*)-caryophyllene (8.8%), and α-pinene (7.9%). Two tested oil samples showed antimicrobial effect against pathogenic strains *B. subtilis*, *S. aureus*, and *S. cerevisiae*. The current result gives new evidence and a basic foundation for further research.

Competing interests

No potential conflict of interest was reported by the authors.

Supplementary data

GC chromatograms of studied essential oils are given as supplementary information.

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