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Chemical Compositions and Antimicrobial Activity of Essential Oils From the Leaves of 4 Vietnamese Zingiberaceae Species

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Abstract

Essential oils of the leaves of 4 Vietnamese Zingiberaceae species were first obtained by hydro-distilled, and their chemical compositions were identified by gas chromatography-flame ionization detection/mass spectrometry . β-Pinene (21.7%), sabinene (12.8%), and α-pinene (8.0%) were the main compounds in *Wurfbainia tenella* leaf oil. The essential oil of *Hedydium villosum* var. *tenuifolium* leaf was dominated by β-pinene (23.7%), β-caryophyllene (21.6%), and 1,8-cineole (14.0%). β-Pinene (27.4%), α-pinene (21.2%), limonene (12.1%), and myrcene (8.6%) were the characteristic components of *Meistera sudae* leaf oil, and β-pinene (32.8%) and (*E*)-methyl cinnamate (15.8%) of *Alpinia hongiaoensis* leaf oil. The 4 oil samples exhibited antimicrobial activity against *Bacillus subtilis* (American Type Culture Collection [ATCC] 27212), *Staphylococcus aureus* (ATCC 12222), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 25923), *Aspergillus niger* (ATCC 9763), *Fusarium oxysporum* (ATCC 48112), *Candida albicans* (ATCC 10231), and *Saccharomyces cerevisiae* (ATCC 2601) at different levels. Especially, the leaf oil of *M sudae* showed strong activity against *B subtilis*, *S aureus*, and *S cerevisiae* with a minimum inhibitory concentration (MIC) value of 25.0 μg/mL.

Keywords

Zingiberaceae, essential oil, monoterpene, β-pinene, antimicrobial activity

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Introduction

Zingiberaceae is the largest family of the eight in the order Zingiberales with about 50 genera and 1600 species.[1] They are found across the tropical world, but Southeast Asia has the highest diversity.[1] Many ginger species are valuable economically because they provide food, fragrances, sauces, dyes, and textiles.[2,3] They are also widely used in folk medicine, and play a key role in pharmacological drug developments.[2,3] As a typical instance, *Curcuma longa* (turmeric) has long been used as a spice, but is also renowned for disease treatments such as against infection, depression, stress, and cancer.[4,5]

The ginger family consists of 21 genera and 100 species in Vietnam,[6] and they are always recognized to be an interesting subject for phytochemical investigations to identify chemical compositions in their essential oils. The seed oil of *Amomum tsao-ko*, collected from Laocai-Vietnam, was dominated by 1,8-cineole (30.6%), 2-decenal (17.3%), and geranial (10.6%),[7] whereas the antimicrobial activity of the leaf oil of another Vietnamese *Amomum*, *Amomum cinnamomeum*, was primarily dependent on the role of the main constituents β-pinene (35.8%), (*E*)-cinnamaldehyde (11.5%), and caryophyllene oxide (5.9%).[8] β-Pinene (20%-31.8%) was among the major compounds present in the leaf oils of *Hedychium stenopetalum,* and *Hedychium coronarium*, gathered from Sonla, Vietnam.[9] Similarly, β-pinene (12.1%-33.5%) was the principal compound

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in the leaf oils of another 2 Vietnamese species, *Alpinia globosa* and *Alpinia tonkinensis*.[10]

In the current study, we report the chemical compositions of the essential oils of 4 other Vietnamese Zingiberaceae species, *Wurfbainiaes tenella* (Lamxay & M.F.Newman) Skornick. & A.D. Poulsen, *Hedychium villosum* var. *tenuiflorum* Wall. ex Baker, *Meistera sudae* Šída f. & Škorničk, and *Alpinia hongiaoensis* Tagane, as well as their antimicrobial activity.

Results and Discussion

The leaf oil of *W tenella* (0.20% yield, w/w) was obtained with a yellow color. By gas chromatography-flame ionization detection/mass spectrometry (GC-FID/MS) analysis, 37 compounds were identified, which accounted for 91.5% of the total oil (Table 1). Monoterpenes and their oxygenated derivatives reached the highest amounts of 56.1% and 23.0%, respectively. Sesquiterpenes (1.4%) and their oxygenated derivatives (9.1%) were also found in this oil, whereas the remaining percentage of 1.9% was assigned to non-terpenic compounds. Monoterpenes β-pinene (21.7%), sabinene (12.8%), and α-pinene (8.0%), and the sesquiterpenoid caryophyllene oxide (5.7%) could be seen as the major compounds in this sample. Additionally, *p*-cymene (4.3%), limonene (4.1%), terpinen-4-ol and myrtenyl acetate (3.2%), cryptone (3.0%), linalool (2.8%), *trans*-sabinol (2.6%), myrtenol and myrtenal (1.5%), 1,8-cineole (1.1%), and pinocarvone (1.0%) were detected in amounts greater than 1.0%.

The hydro-distilled extract of *H villosum* var. *tenuiflorum* leaf gave a yellow oil in 0.15% yield, w/w. Thirty-one compounds were identified, which represented 97.5% of the total. This leaf oil included monoterpenes (56.0%), and sesquiterpenes (28.0%), while their oxygenated derivatives ranged from 3.5% to 4.3%. The essential oil of *H villosum* var. *tenuiflorum* leaf was dominated by the monoterpenes β-pinene (23.7%), 1,8-cineole (14.0%), and α-pinene (5.1%), and the sesquiterpene β-caryophyllene (21.6%). Coronarin E (5.5%) was the only diterpenoid detected, as well as non-terpenic compounds, which appeared in only trace amount of 0.2%. The oil of this plant was also associated with the presence of other significant compounds, such as sabinene (4.1%), camphene and caryophyllene oxide (3.1%), and α-humulene (2.8%).

The third yellow oil (0.17% yield, w/w) was extracted from the leaf of *M sudae*, in which 39 compounds (99.5%) were identified (Table 1). This oil was dominated by the appearance of monoterpenes (74.8%), followed by sesquiterpenoids (12.6%), monoterpenoids (7.3%), sesquiterpenes (4.4%), and non-terpenic compounds (0.4%). The principal compounds were the monoterpenes β-pinene (27.4%), α-pinene (21.2%), limonene (12.1%), and myrcene (8.6%). Apparently, the percentages of these 4 compounds in this oil were superior to those in the two-first oils. However, no monoterpenoid, sesquiterpene, or sesquiterpenoid attained an appreciable percentage.

A yellow oil (0.25% yield, w/w) was collected from *A hongiaoensis* leaves by hydrodistillation in which 36 compounds (89.8%) were identified (Table 1) including monoterpenes (40.1%), sesquiterpenes (16.9%), monoterpenoids (8.1%), and sesquiterpenoids (7.9%). In contrast to the first 3 oils, non-terpenic compounds in *A. hongiaoensis* oil reached a remarkable 16.8%. The percentage of the major compound, β-pinene (32.8%), in this sample was higher than in the 3 above samples, which contained 11.1%, 9.1%, and 5.4%, respectively. (*E*)-Methyl cinnamate, a non-terpenic compound, formed 15.8% of the oil, but was absent from the 3 previous oils. Various compounds were observed in amounts greater than 1.0%, including *cis*-eudesma-6,11-diene (4.5%), α-pinene (3.7%), 10-*epi*-junenol (3.0%), aristolochene (2.9%), linalool (2.5%), *trans*-muurola-4(14),5-diene (2.4%), *cis*-β-elemene (1.8%), *trans*-sabinol and γ-cadinene (1.6%), pinocarvone (1.4%), myrtenol and humulene epoxide II (1.2%), (*E*)-nerolidol and caryophyllene oxide (1.1%), and 1,10-*di-epi*-cubenol (1.0%). However, the percentages of 1,8-cineole and β-caryophyllene, the major components of the leaf oil of *H villosum* var *tenuiflorum*, and those of myrcene and limonene, the principle constituents of the leaf oil of *M sudae*, were either absent or present in insignificant amounts in the leaf oil of *A. hongiaoensis*.

It should be noted that the 2 genera *Wurfbainia* and *Meistera* have previously been classed as synonyms of *Amomum*.[13-15] Thereby, there are a lot of essential oil and pharmacological studies focusing on the 2 big genera *Amomum* and *Alpinia*. The most striking feature is that β-pinene is likely to be the main compound present in Vietnamese ginger essential oils. This result also matches well with previous studies. As mentioned above, β-pinene accounted for 35.8% of the leaf oil of *A cinnamomeum*,[8] from 20% to 31.8% of the leaf oils of 2 other Vietnamese species, *H stenopetalum* and *H coronarium*,[9] and 33.5% of the leaf oil of *A tonkinensis*.[10] β-Pinene was also recorded in high amounts of 85.8%-71.3% in the rhizome oil of *A purpurata* and 31.4% in the leaf oil of *H coronarium*, collected from Fiji.[16] Therefore, it is expected that Vietnamese ginger plants are a good resource of essential oils containing a high level of β-pinene.

The 4 essential oils were subjected to antimicrobial assay, and the results are provided in Table 2. The leaf oil of *W tenella* established a minimum inhibitory concentration (MIC) value of 256 μg/mL against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative bacterium *Escherichia coli*, as well as inhibited the growth of the yeast *Saccharomyces cerevisiae*, with a MIC value of 128 μg/mL. However, this oil failed to control the Gram-negative bacterium *Pseudomonas aeruginosa*, and fungi *Aspergillus niger* and *Fusarium oxysporum* (MIC > 400 μg/mL). The leaf oil of *H villosum* var *tenuiflorum* showed antimicrobial activity against *A niger* with a MIC value of 256 μg/mL, but did not affect the remaining microorganisms. As compared to the positive control, the leaf oil of *M sudae* exhibited strong activity, with the same MIC value of 25 μg/mL against the 2 Gram-positive bacteria, *B subtilis* and *S aureus*, and the yeast *S cerevisiae*. This good result may be due to the role of the major components α-pinene, β-pinene, and limonene. Leit et al[17] demonstrated that α-pinene and

Table 1. The Identified Compounds (%) in the Leaf Oils of 4 Zingiberaceae Plants.

Rt	RI_E	RI_L	Constituents	*Wurfbainia tenella*	*Hedydium villosum* var *tenuiflorum*	*Meistera sudae*	*Alpinia hongiaoensis*
10.03	931	924	α-Thujene	0.4	0.5	0.2	
10.30	940	932	α-Pinene	**8.0**	**5.1**	**21.2**	3.7
10.72	953	945	α-Fenchene			0.1	
10.80	956	946	Camphene	2.3	3.1	0.8	
11.50	979	969	Sabinene	**12.8**	4.4	0.3	0.7
11.69	985	974	β-Pinene	**21.7**	**23.7**	**27.4**	**32.8**
11.90	992	988	Myrcene	0.5	0.6	**8.6**	0.2
12.48	1010	1002	α-Phellandrene	0.3			
12.70	1017	1008	δ-3-Carene				0.5
12.90	1022	1014	α-Terpinene		0.4		
13.15	1030	1022	*p*-Cymene	4.3	1.0	3.8	0.6
13.31	1034	1024	Limonene	4.1	1.4	**12.1**	0.9
13.36	1036	1025	β-Phellandrene		0.2		
13.44	1038	1026	1,8-Cineole	1.1	**14.0**	0.1	0.7
14.32	1064	1054	γ-Terpinene	0.6	1.3		
15.38	1095	1086	Terpinolene		0.3	0.2	
15.62	1102	1095	Linalool	2.8	0.2	1.8	2.5
16.33	1122	1114	*endo*-Fenchol			0.4	
17.29	1149	1137	*trans*-Sabinol	2.6		0.4	1.6
17.51	1155	1141	Camphor	0.7	1.5		
17.65	1159	1145	Camphene hydrate			0.2	
17.90	1166	1154	Sabina ketone	0.4			
18.12	1173	1160	Pinocarvone	1.0		0.1	1.4
18.22	1175	1165	Borneol	0.8	0.2	0.4	
18.59	1186	1174	Terpinen-4-ol	3.2	1.4	1.7	0.3
18.94	1196	1183	Cryptone	3.0			
19.02	1198	1186	α-Terpineol		0.2	1.4	
19.25	1205	1194	Myrtenol	1.5		0.4	1.2
19.32	1207	1195	Myrtenal	1.5		0.3	
19.77	1220	1204	Verbenone	0.3			
20.79	1249	1238	Cumin aldehyde	0.6			
20.89	1252	1239	Carvone	0.2			
22.35	1295	1284	Bornyl acetate	0.4		0.2	0.3
22.42	1297	1289	Thymol	0.8			
22.57	1301	1290	Dihydroedulan II			0.4	
22.79	1308	1298	Sabinyl acetate				0.5
22.91	1311	1299	(*Z*)-Methyl cinnamate				0.5
23.43	1327	1319	4-Hydroxy-cryptone	0.8			
23.65	1334	1324	Myrtenyl acetate	3.2			0.3
23.99	1344	1330	3-*oxo*-*p*-Menth-1-en-7-al	0.6			
25.51	1390	1374	α-Copaene			0.6	0.9
25.57	1392	1376	(*E*)-Methyl cinnamate				**15.8**
25.86	1400	1387	β-Bourbonene			1.0	
25.96	1404	1389	*cis*-β-Elemene	0.6			1.8
26.09	1408	1403	Methyleugenol		0.2		
27.07	1439	1417	β-Caryophyllene		**21.6**	1.7	
27.77	1461	1440	(*Z*)-β-Farnesene		1.8		
28.11	1472	1452	α-Humulene		2.8	0.3	
28.34	1479	1464	9-*epi*-(*E*)-Caryophyllene	0.3	0.5		0.7
28.69	1491	1478	γ-Muurolene			0.3	0.6
28.80	1494	1484	*cis*-Eudesma-6,11-diene				4.5
28.94	1498	1484	Germacrene D		0.5		
29.04	1502	1487	Aristolochene		0.2		2.9
29.05	1502	1489	(*E*)-Methyl isoeugenol	0.5			
29.14	1505	1491	β-Selinene	0.5		0.2	0.6
29.29	1510	1491	γ-Amorphene				0.4
29.38	1513	1493	*trans*-Muurola-4(14),5-diene				2.4
29.42	1514	1500	Bicyclogermacrene		0.5		

(Continued)

Table 1. Continued.

Rt	RI_E	RI_L	Constituents	*Wurfbainia tenella*	*Hedydium villosum* var *tenuiflorum*	*Meistera sudae*	*Alpinia hongiaoensis*
29.89	1530	1513	γ-Cadinene			0.3	1.6
30.10	1537	1522	δ-Cadinene		0.1		0.5
30.88	1563	1548	Elemol	0.3		0.5	
31.07	1570	1561	(*E*)-Nerolidol			0.3	1.1
31.90	1597	1577	Spathulenol	2.3		1.4	
32.13	1605	1582	Caryophyllene oxide	**5.7**	3.1	2.6	1.1
32.47	1617	1590	10-*epi*-Junenol				3.0
32.88	1632	1608	Humulene epoxide II	0.8	0.2	0.3	1.2
32.94	1634	1618	1,10-*di*-*epi*-Cubenol				1.0
33.43	1651	1625	γ-Eudesmol			1.8	0.2
33.60	1657	1627	Caryophylla-3(15),7(14)-dien-6-ol		1.0		
33.66	1658	1638	*epi*-α-Cadinol			0.2	0.3
34.06	1673	1649	β-Eudesmol			3.6	
34.12	1675	1652	α-Eudesmol			1.9	
46.09	2155	2135	Coronarin E		**5.5**		
			Total	**91.5**	**97.5**	**99.5**	**89.8**
			Monoterpenes	56.1	56.0	74.8	40.1
			Monoterpenoids	23.0	3.5	7.3	8.1
			Sesquiterpenes	1.4	28.0	4.4	16.9
			Sesquiterpenoids	9.1	4.3	12.6	7.9
			Diterpenoids		5.5		
			Non-terpenic compounds	1.9	0.2	0.4	16.8

Abbreviations: Rt, retention time; RI_E, retention indices relative to *n*-alkanes (C_7-C_{30}) on HP-5 MS column; RI_L, retention indices from Adams[11] and the NIST standard database.[12] Bold font signifies compounds present in amounts greater than 5%.

Table 2. Antimicrobial Activity of Zingiberaceae Leaf Oils.

		Minimum inhibitory concentration (MIC: μg/mL)						
Microbial strains		*Wurfbainia tenella*	*Hedydium villosum* var *tenuiflorum*	*Meistera sudae*	*Alpinia hongiaoensis*	Streptomycin	Tetracyclin	Nystatin
Gram (+)	*Bacillus subtilis*	256	> 400	25	128	6.25		
Staphylococcus aureus		256	> 400	25	128	12.5		
Gram (-)	*Escherichia coli*	256	> 400	> 400	> 400		6.25	
	Pseudomonas aeruginosa	> 400	> 400	> 400	> 400		12.5	
Fungi	*Aspergillus niger*	> 400	256	> 400	256			25.0
	Fusarium oxysporum	> 400	> 400	> 400	> 400			12.5
Yeasts	*Candida albicans*	> 400	> 400	> 400	256			12.5
	Saccharomyces cerevisiae	128	> 400	25	128			6.25

β-pinene had strong antimicrobial activity against *S aureus*, *S epidermidis*, *S. pyogenes*, and *S. pneumoniae* with MIC values of 5 to 40 μg/mL. Another example is that limonene could inhibit *S aureus* with a MIC value of 20 mL/L.[18] The limonene-rich oil extracted from *Schinus areira* leaves and fruits also successfully controlled the growth of *S aureus*.[19] In the final case, with a MIC of 128 μg/mL, the leaf oil of *A. hongiaoensis* was moderately active against 2 Gram-positive bacteria, *B subtilis* and *S aureus*, and the yeast *S cerevisiae*, and successfully controlled the fungus *A niger* and the yeast *Candida albicans*. However, neither of the leaf oils of *M sudae* and *A hongiaoensis* inhibited the growth of the 2 tested Gram-negative bacteria, *E coli* and *P aeruginosa*, and the fungus *F oxysporum*.

The leaf oil of Vietnamese *A cinnamomeum* possessed a MIC value of 64.0 μg/mL against *C albicans*, as compared with that of its rhizome oil (MIC = 32.0 μg/mL).[7] *Amomum rubidium* leaf oil demonstrated antimicrobial activity against *E coli* and *F oxysporum* with the same MIC value of 50.0 μg/mL.[20] The leaf oils of Vietnamese *Alpinia* species *A globosa* and *A tonkinensis* were responsible for antimicrobial activity against *E coli*, *S aureus*, and *F oxysporum* with the same MIC value of 50.0 μg/mL.[10] Our evidence, once again, confirmed the useful

applications of Vietnamese ginger essential oils in antimicrobial treatments.

Materials and Methods

Plant Materials

The fresh leaves of *W tenella*, *A. hongiaoensis* leaves, and *M sudae* were collected from Lamdong, Vietnam in 03/2022, whereas the fresh leaves of *H villosum* var *tenuiflorum* were collected from Nghean, Vietnam in 04/2022. The plants were identified by the co-authors Ly Ngoc Sam and Le Thi Huong. The voucher specimens, including Ly-1617 (*W tenella* leaves), Huong-962 (*H villosum* var *tenuiflorum*), Ly-1622 (*A. hongiaoensis* leaves), and Ly-1623 (*M sudae* leaves), have been deposited in the VNM Herbarium, Institute of Tropical Biology. The obtained samples (2.5 kg each) were immediately cut into pieces, and hydro-distilled using a Clevenger apparatus for 2.5 h to give the yellow essential oils. The yields of extraction, which were calculated from the dried materials, reached a range of 0.15% to 0.25%.

GC-FID/MS Analysis

The GC-FID analysis was carried out following the conditions[21-23]: Agilent Technologies HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 m), helium carrier gas (1.1 mL/min), injector temperature 260 °C, detector temperature 270 °C, column temperature program: 65 °C (3 min hold), increase to 230 °C (4 °C/min), 230 °C (10 min hold), inlet pressure of 6.0 kPa, split mode injection (split ratio, 10:1), 1.1 µL injection volume.

GC-MS analysis was performed in the same manner: Agilent Technologies HP 7890A Plus Chromatograph (Santa Clara, CA, USA), HP-5 MS (30 m × 0.25 mm, film thickness 0.25 µm) column, HP 5973 MSD mass detector, helium carrier gas (1.1 mL/min), MS ionization voltage of 70 eV, emission current of 40 mA, acquisitions range of 40-400 amu, sampling rate of 1.0 scan/s. The gas chromatography (GC) was operated under the same conditions as GC-FID. The retention indices were based on a series of *n*-alkanes, co-injection with either pure compounds (Sigma-Aldrich, St. Louis, MO, USA) or identified essential oil components, MS library search (NIST 17 and Wiley Version 10), and comparison with the literature MS fragmentation used to identify the chemical components of the essential oils.[21-23] Based solely on the GC peak area (flame ionization detection response) and without the use of correction factors, the relative concentrations (%) of the constituents were computed. The measurements were made 3 times.

Antimicrobial Assay

Antimicrobial activity of the essential oils was evaluated against eight strains,[21-23] including Gram-positive (*B subtilis* and *S aureus*), Gram-negative (*E coli* and *P aeruginosa*), filamentous fungi (*A niger* and *F. oxysporum*), and yeasts (*C albicans* and *S cerevisiae*). All strains were acquired from American Type Culture Collection. Each strain was sub-cultured for 24 h on either Tryptic soil agar at 37 °C (bacteria) or potato dextrose agar at 35 °C (yeasts). The assays were performed in Mueller-Hinton broth (bacteria) and RPMI 1640 culture medium (yeasts). The inoculum was adjusted to 5×10^5 CFU/mL for bacteria and 2.5×10^3 CFU/mL for yeasts.

The tested oil samples were dissolved in ethanol and diluted in a culture medium to achieve concentrations from 400 to 4 µg/mL. Inoculated wells with and without antimicrobial agents were assayed to control the adequacy of the broth for microorganism growth and medium sterility, respectively. The final concentration of ethanol (5%) was also evaluated. The microplates were incubated at either 37 °C (bacteria) or 35 °C (yeasts) for 24 h. After that, resazurin (aqueous solution 0.02%) was added to the microplates to indicate the microorganism viability. Before that, aliquots were aseptically removed from each well, plated onto an adequate culture medium, and incubated as previously described. The lowest concentration that allowed no discernible growth of the tested microorganism was identified as the MIC. Streptomycin and tetracycline served as the standards for Gram-positive and -negative bacteria, respectively, while nystatin was used as the standard for fungi and yeasts. dimethyl sulfoxide (DMSO) at 5% was used as a negative control. Each experiment was performed 3 times.

Conclusions

For the first time, the chemical compositions of the essential oils of 4 Zingiberaceae species were reported. Monoterpenes were the main chemical class, including *W tenella* leaf oil (56.1%), *H villosum* var. *tenuiflorum* leaf oil (56.0%), *M sudae* leaf oil (74.8%), and *A. hongiaoensis* leaf oil (40.0%). The monoterpene β-pinene was the main compound in the 4 oil samples, and its percentage ranged from 21.7% to 32.8%. A few other compounds were also present in significant amounts, such as sabinene (12.8%) and α-pinene (8.0%) in *W tenella* leaf oil, β-caryophyllene (21.6%) and 1,8-cineole (14.0%) in *H villosum* var. *tenuiflorum* leaf oil, α-pinene (21.2%) and limonene (12.1%) in *M sudae* leaf oil, and (*E*)-methyl cinnamate (15.8%) in *A. hongiaoensis* leaf oil. The 4 oil samples showed different levels of antimicrobial activity. Especially, the leaf oil of *M sudae* showed strong activity against 2 of the tested Gram-positive bacteria, *B subtilis*, and *S aureus*, and the yeast *S cerevisiae* with the same MIC value of 25.0 µg/mL.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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