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Article

Essential Oils of Two Ginger Plants *Newmania orthostachys* N.S. Lý & Škorničk. and *N. serpens* N.S. Lý & Škorničk.: Chemical Compositions and Antimicrobial Activity

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Abstract: The current study describes chemical compositions and antimicrobial activity of essential oils from the rhizome of two Vietnamese ginger plants *Newmania orthostachys* N.S. Lý & Škorničk. and *Newmania serpens* N.S. Lý & Škorničk.. 49 compounds (98.4%) were identified in *N. orthostachys* rhizome oil, whereas 54 compounds (93.4%) were identified in *N. serpens* rhizome oil. *N. orthostachys* rhizome essential oil was dominated by monoterpene hydrocarbons (74.2%), in which β-pinene (35.7%), α-pinene (13.4%), sabinene (8.0%), camphene (6.7%), and limonene (5.1%) were characteristic compounds. Monoterpene hydrocarbons (33.5%) and sesquiterpene hydrocarbons (44.1%) represented *N. serpens* rhizome essential oil, as well as β-pinene (18.5%), bicyclogermacrene (12.4%), β-selinene (8.2%) were the principal compounds. In an antimicrobial assay against three tested Gram-positive bacteria *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579, both two essential oils showed the minimum inhibitory concentration (MIC) values of 16-64 µg/mL lower than those of positive control Streptomycin (MIC 128-256 µg/ml). These two essential oils with the MIC values of 8-16 µg/mL are also better than the positive control cycloheximide (MIC 32 µg/mL) against the yeast *Candida albicans* ATCC 10231.

Keywords: *Newmania*, essential oil, chemical composition, antimicrobial activity.

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Introduction

Zingiberaceae (the ginger family) is a large family of flowering plants, consisting of 53 genera and more than 1600 species [1]. Members of this family are spread over tropical and subtropical regions [2,3]. The ginger species are small to large herbaceous plants with tuberous rhizomes and distichous leaves [4]. As can be seen, Zingiberaceae plants are important ornamental, condiment, or medicinal plants [5,6]. it is also recognized that the ginger plants are a rich resource of essential oils. A great number of chemical compositions were documented previously in Zingiberaceae essential oils including ketones, alcohols, especially terpene derivatives [7].

About 100 Zingiberaceae species assigned to 21 genera were found in Vietnam [8]. Essential oils of Vietnamese gingers were associated with the predominance of terpene derivatives, as well as they make appropriate for drug development due to their pharmacological activities [9,10]. For instance, hydro-distilled extraction of *Zingiber officinale* root, collected from northern Vietnam, gave a yellow essential oil containing monoterpene hydrocarbons (28%), oxygenated monoterpenes (37%), and sesquiterpene hydrocarbons (25%) [9].

The rhizome essential oil of *Elettariopsis triloba*, gathered from Vu Quang National Park, Vietnam, was pharmacologically active against microbacterial strains *E. faecalis*, *S. aureus*, and *B. cereus* [10].

Newmania N.S. Lý & Škornič**k**. is a new endemic ginger genus, which was discovered and described and illustrated from central Vietnam in 2011 by Ngoc Sam Lý (co-author) and Skorničkova [11]. Currently, six species were recorded available in central Vietnam, encompassing *N. orthostachys*, *N. serpens, N. sessilanthera, N. cristata, N. gracilis* and *N. sontraensis* [11,12]. Among them, essential oil of the rhizomes of the last species *N. sontraensis* has been analyzed, by which its rhizome essential oil was dominated by β-pinene (22.41%), 1,8-cineole (8.32%), bicyclogermacrene (6.94%), α-terpinyl acetate (5.74 %), α-pinene (5.71 %), and camphene (5.58%) [13]. In the current study, we wish to report chemical compositions in essential oils of two other Vietnamese *Newmania* species *Newmania orthostachys* N.S. Lý & Škorni**čk**. and *N. serpens* N.S. Lý & Škorni**č**k., as well as their antimicrobial activity.

Materials and methods

Reagents

All chemicals and materials in the experiment were pure products from Sigma-Aldrich (USA) distributor. They include Mueller-Hinton Agar, Dimethylsulfoxide, Streptomycin, and Cycloheximide.

The samples of N. orthostachys and N. serpens

The fresh rhizomes of two studied plants were collected from Dau mount, Nghiahanh district, Quangngai province, Vietnam in 07/2019. The Latin names were identified by the co-author Ly Ngoc Sam. The voucher specimens Lý 774-N (*N. orthostachys* rhizomes) and Lý 775-N (*N. serpens* rhizomes), were stored at the VNM herbarium Institute of Tropical Biology.

Hydrodistillation of the essential oils from the rhizomes of N. orthostachys and N. serpens

The obtained samples (2.0 kg each) were immediately cut into pieces, and hydro-distilled using a Clevenger apparatus for 2.5 h to give yellow essential oils. The yields of extraction, which were calculated following fresh materials, reached about 0.2% v/w.

Chemical analysis of the essential oils

Gas chromatography with flame ionization detection (GC-FID) was carried out following the conditions [14-16]: Agilent Technologies HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 µm), Helium carrier gas (1.1 mL/min), injector temperature of 260°C, detector temperature of 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.

Gas chromatography-mass spectrometry (GC-MS) was performed in the same manner: From 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 46-400 amu, a sampling rate of 1.0 scan/s. The GC was operated under the same circumstances as GC-FID. The retention indices (RI) based on a series of n-alkanes, co-injection with 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 were used to identify the chemical components of essential oils [14-16]. Based solely on the GC peak area (FID response) and without the use of correction factors, the relative concentrations (%) of the constituents were computed. The measurements were repeated three times.

Microorganisms

The antimicrobial effect of two rhizome oils was performed using the broth dilution method [15,16]. Six pathogenic bacterial strains and one yeast strain were used, including three Gram-positive bacterial strains *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579, three strains of Gram-negative bacterial strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* ATCC 13076, and one yeast strain *Candida albicans* ATCC 10231.

Screening of the essential oils for antimicrobial activity

The selection of investigated concentrations was based on our previous publication [15,16], in which the tested essential oil was active with the specific concentration ranges. Stock solution of essential oil was prepared by DMSO (1%). Dilution series (2-fold) were prepared from 16.384 to 2 μg/mL. They were then transferred to 96-well plates. Bacteria grown in double-strength Mueller-Hinton broth were standardized to 5×10^5 CFU/mL. The last row of well plates containing only antibiotics without essential oils was used as a positive control. DMSO (1%) served as a negative control (no antimicrobial agent). Streptomycin and Cycloheximide were used as standards for antibacterial and anti-yeast activities, respectively. Experiments were repeated in triplicate. The results were displayed by the MIC values (the lowest dose at which bacterial growth is totally inhibited).

Results and discussion

Chemical constituents of the essential oils

By the GC-FID/MS analysis, a number of 49 compounds were identified in *N. orthostachys* rhizome essential oil, which accounted for 98.4% (Table 1). With 74.2%, monoterpene hydrocarbons were the chemical class of the identified compounds, followed by sesquiterpene hydrocarbons (10.7%), oxygenated sesquiterpenes (10.2%), and oxygenated monoterpenes (3.3%). As can be seen from Table 1, monoterpene β-pinene reached the highest amount of 35.7%, followed by monoterpenes α-pinene (13.4%), sabinene (8.0%), camphene (6.7%), and limonene (5.1%). Some compounds were recorded with an amount of greater than 1.0%, including *cis*-sesquisabinene hydrate (3.9%), β-chamigrene (2.2%), bicyclogermacrene (1.8%), *neo*-intermedeol (1.7%), caryophyllene oxide and zerumbone (1.4%), bornyl acetate (1.2%), β-caryophyllene (1.1%), and 1,8-cineole (1.0%).

Regarding the second species, a total of 54 compounds were identified in its rhizome essential oil, which represented 92.3% (Table 1). Sesquiterpene hydrocarbons (44.1%) and monoterpene hydrocarbons (33.5%) were the major chemical classes. *N. serpens* rhizome essential oil was also characterized by the presence of oxygenated sesquiterpenes (12.9%), oxygenated monoterpenes (1.5%), and oxygenated diterpene (0.3%). As compared with *N. orthostachys* rhizome essential oil, the percentage of monoterpenes in this sample has drastically decreased, but the amount of sesquiterpene hydrocarbons has significantly increased. The principal compound β-pinene (18.5%) was less than that of *N. orthostachys* rhizome essential oil by 17.2%. In contrast, the percentage of the principal sesquiterpene hydrocarbons bicyclogermacrene and β-selinene were more than those of N.

Table 1. Chemical compositions in the rhizome essential oils of two *Newmania* plants

No.	Compounds[a]	RT	RI[b]	RI[c]	Concentration (%) *N. orthostachys*	*N. serpens*
1	Tricyclene	10.06	928	921	0.2	-
2	α-Thujene	10.12	930	924	0.2	-
3	α-Pinene	10.39	939	932	13.4	2.9
4	Camphene	10.88	955	946	6.7	0.9
5	Sabinene	11.58	979	969	8.0	0.7
6	β-Pinene	11.78	985	974	35.7	18.5
7	Myrcene	11.98	992	988	1.6	0.5
8	α-Phellandrene	12.56	1010	1002	0.3	1.9
9	δ-3-Carene	12.78	1016	1008	-	0.1
10	α-Terpinene	12.96	1022	1014	0.2	0.4
11	o-Cymene	13.22	1029	1022	0.7	2.7
12	Limonene	13.38	1034	1024	5.1	1.8
13	β-Phellandrene	13.43	1035	1025	0.3	0.5
14	1,8-Cineole	13.50	1037	1026	1.0	-
15	(Z)-β-Ocimene	13.50	1037	1032	-	0.3
16	(E)-β-Ocimene	13.88	1049	1044	0.1	0.2
17	γ-Terpinene	14.37	1063	1054	0.5	1.5
18	Terpinolene	15.43	1094	1086	0.2	0.6
19	Linalool	15.66	1101	1095	-	0.2
20	Pinocarvone	18.16	1172	1160	0.2	-
21	Borneol	18.25	1175	1165	0.6	-
22	Terpinen-4-ol	18.62	1185	1174	0.2	0.2
23	Myrtenal	19.35	1206	1195	0.3	0.1
24	Bornyl acetate	22.37	1294	1284	1.2	0.7
25	Sabinyl acetate	22.81	1307	1289	0.2	-
26	Myrtenyl acetate	23.66	1333	1324	0.6	0.3
27	δ-Elemene	24.17	1348	1335	-	0.3
28	α-Terpinyl acetate	24.43	1356	1346	0.1	-
29	α-Copaene	25.53	1389	1382	0.1	0.2
30	*cis*-β-Elemene	25.97	1403	1389	0.3	0.8
31	Sesquithujene	26.27	1412	1405	0.2	0.4
32	α-*cis*-Bergamotene	26.68	1425	1411	-	2.0
33	β-Caryophyllene	27.03	1437	1417	1.1	1.3
34	Cycloseychellene	27.32	1446	1417	-	0.3
35	α-*trans*-Bergamotene	27.51	1452	1432	-	0.6
36	Guaia-6,9-diene	27.66	1457	1442	-	0.4
37	(Z)-β-Farnesene	27.77	1460	1440	-	-
38	(E)-β-Farnesene	27.92	1465	1454	0.4	0.5
39	α-Humulene	28.11	1471	1452	0.3	0.4
40	9-epi-(E)-Caryophyllene	28.36	1479	1464	-	2.4
41	γ-Curcumene	28.66	1488	1481	0.5	0.3
42	α-Curcumene	28.75	1492	1482	-	4.4
43	Germacrene D	28.94	1498	1487	0.1	0.5

table 1. (continued).

No.	Compounds[a]	RT	RI[b]	RI[c]	Concentration (%) *N. orthostachys*	Concentration (%) *N. serpens*
44	β-Chamigrene	29.02	1500	1488	2.2	-
45	Aristolochene	29.07	1502	1490	-	3.2
46	β-Selinene	29.15	1504	1491	0.9	8.2
47	γ-Amorphene	29.31	1510	1495	0.6	0.5
48	Bicyclogermacrene	29.42	1513	1500	1.8	12.4
49	β-Bisabolene	29.55	1518	1505	-	0.6
50	β-Curcumene	29.62	1520	1514	0.9	2.2
51	γ-Cadinene	29.9	1530	1513	-	0.2
52	Eugenol acetate	29.95	1531	1521	0.2	0.2
53	δ-Cadinene	30.11	1537	1522	-	1.1
54	7-epi-α-Selinene	30.11	1537	1526	0.8	-
55	α-Calacorene	30.78	1559	1544	0.2	0.4
56	*cis*-Sesquisabinene hydrate	30.91	1564	1559	3.9	-
57	(E)-Nerolidol	31.07	1569	1561	-	0.2
58	β-Calacorene	31.39	1580	1564	-	0.3
59	α-Turmerol	31.65	1589	1565	-	0.6
60	4α-Hydroxygermacra-1(10),5-diene	31.79	1593	1575	-	0.3
61	Spathulenol	31.88	1596	1577	0.4	2.9
62	Caryophyllene oxide	32.11	1604	1582	1.4	1.8
63	Viridiflorol	32.35	1613	1592	-	0.7
64	Ledol	32.69	1625	1602	-	0.9
65	6-epi-Cubenol	32.81	1629	1602	0.4	0.5
66	Humulene Epoxide II	32.88	1632	1608	-	0.3
67	α-Acorenol	33.41	1650	1632	0.5	-
68	β-Himachalol	33.42	1650	1637	-	0.2
69	epi-α-Muurolol	33.66	1659	1640	-	0.9
70	Eudesma-4(15),7-dien-1â-ol	33.85	1666	1647	-	0.4
71	α-Cadinol	34.02	1672	1652	0.3	-
72	Intermedeol	34.13	1675	1665	0.2	-
73	*neo*-Intermedeol	34.29	1681	1668	1.7	-
74	*trans*-Calamenen-10-ol	34.38	1684	1669	-	0.3
75	Bulnesol	34.53	1690	1670	-	0.4
76	Zerumbone	36.36	1757	1732	1.4	1.7
77	γ-Bicyclohomofarnesal	38.2	1827	1809	-	0.6
78	6,10,14-Trimethylpentadecan-2-one	38.72	1848	1848	-	0.2
79	Phytol	45.2	2117	1942	-	0.3
	Total				98.4	92.3
	Monoterpene hydrocarbons (Sr. no. 1-18)				74.2	33.5
	Oxygenated monoterpenes (Sr. no. 19-26)				3.3	1.5
	Sesquiterpene hydrocarbons (Sr. no. 27-55, 58)				10.7	44.1
	Oxygenated sesquiterpenes ((Sr. no. 56, 57, 59-78)				10.2	12.9
	Oxygenated diterpene ((Sr. no. 79)				0	0.3

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5 column;
[c] Literature retention indices (see references)

orthostachys rhizome oil by 10.6 and 7.3%, respectively. Additionally, several compounds with more than 1.0% were also found in N. serpens rhizome oil, consisting of a-curcumene (4.4%), aristolochene (3.2%), α-pinene and spathulenol (2.9%), o-cymene (2.7%), 9-epi-(E)-caryophyllene (2.4%), β-curcumene (2.2%), α-phellandrene (1.9%), limonene and caryophyllene oxide (1.8%), zerumbone (1.7%), γ-terpinene (1.5%), β-caryophyllene (1.3%), and δ-cadinene (1.1%).

To date, there has been only one previous report on essential oil of Newmania plants, in which β-pinene (22.41%), 1,8-cineole (8.32%), bicyclogermacrene (6.94%), α-terpinyl acetate (5.74%), α-pinene (5.71%), and camphene (5.58%) were the major compounds in the rhizome oil of Vietnamese *Newmania* plant *N. sontraensis* [13]. Significantly, β-pinene is a characteristic compound for not only genus *Newmania* but also other ginger plants. For instance, the percentage of this compound in Fijian *Alpinia purpurata* rhizome oil accounted for 71.3% [17]. The rhizome oil of Vietnamese *Zingiber magang* comprised mainly *β*-pinene (55.4%) [18]. β-Pinene reached up to 95.6% in the rhizome essential oil of Malaysian *Curcuma manga* [19]. Therefore, it is expected that the ginger plants seem to be a good resource of essential oils containing a rich monoterpene hydrocarbon *β*-pinene.

Results of the antimicrobial activity of the essential oils

Two essential oil samples have been further subjected to antimicrobial assay against three Gram-positive bacteria *E. faecalis*, *S. aureus*, and *B. cereus*, three Gram-negative bacteria *E. coli*, *P. aeruginosa*, and *S. enterica*, and one yeast *C. albicans* From Table 2, *N. orthostachys* rhizome essential oil showed the same MIC value of 32 µg/mL against three tested Gram-positive bacteria. In the meantime, *N. serpens* rhizome essential oil controlled the growths of *E. faecalis*, and *B. cereus* with the same MIC value of 16 µg/mL, and the growth of *S. aureus* with the MIC value of 64 µg/mL. Both two essential oils are better than the positive control streptomycin against these Gram-positive bacteria. However, they failed to inhibit three tested Gram-negative bacteria. In general, Gram-negative bacteria are often less sensitive to essential oils than Gram-positive bacteria, and this is directly related to composition of the bacterial cell wall. In Gram negative bacteria, the cell wall is a complex envelope constituted by the cytoplasmic membrane, the periplasm and the outer membrane [20]. It is, once again, viewed that both two essential oils successfully suppressed the growth of yeast *C. albicans* with the MIC values ranging from 8 to 16 µg/mL, and were better than that of the positive control Cycloheximide (MIC 32 µg/mL). Collectively, *N. serpens* rhizome essential oil is generally better than *N. orthostachys* rhizome essential oil in antimicrobial activity. It may be due to the role of sesquiterpene hydrocarbons, especially bicyclogermacrene.

This is the first time that *Newmania* essential oils have been applied to antimicrobial assay. In another approach, the ginger essential oils, especially Vietnamese ginger essential oils, proved their value in antimicrobial treatments.

Table 2. Antimicrobial activity of *Newmania* rhizome essential oils

	Minimum inhibitory concentration (MIC, µg/mL)						
	Gram (+)			**Gram (-)**			**Yeast**
Samples	***E. faecalis***	***S. aureus***	***B. cereus***	***E. coli***	***P. aeruginosa***	***S. enterica***	***C. albicans***
N. orthostachys rhizome	32	32	32	-	-	-	16
N. serpens rhizome	16	64	16	-	-	-	8
Streptomycin	256	128	128	32	256	128	-
Cycloheximide	-	-	-	-	-	-	32

As an example, Vietnamese *Z. magang* rhizome oil has potential antimicrobial activity against *E. faecalis* and *S. aureus* with the MIC values of 9.99 and 9.67 μg/mL, respectively, whereas other Vietnamese *Z. tamii* leaf oil was the most active against *E. coli* (MIC 44.38 μg/mL) and *C. albican*s ATCC 10231 (MIC 45.62 μg/mL) [18]. Similarly, Vietnamese *Z. nudicarpum* rhizome essential oil exhibited remarkable antibacterial activity against *E. faecalis*, *S. aureus*, and *B. cereus*, with the MIC values of 2, 8, and 1 μg/mL, respectively [15]. Our current result further confirmed the useful applications of Vietnamese ginger essential oils in antimicrobial treatments.

Conclusions

For the first time, the current study describes chemical compositions of essential oils of two *Newmania* species by the GC-FID/MS analysis. Monoterpene hydrocarbons (74.2%) were the main chemical class of *N. orthostachys* rhizome essential oil, whereas monoterpene hydrocarbons (33.5%) and sesquiterpene hydrocarbons (44.1%) represented *N. serpens* rhizome essential oil. *β*-Pinene (18.5-35.7%) was likely a major component present in both two oils. These two oils showed the MIC values of 8-64 μg/mL against three tested Gram-positive bacteria *E. faecalis*, *S. aureus*, and *B. cereus*, and one tested yeast *C. albicans*, and were better than those of the positive controls Streptomycin and Cycloheximide. More reports to identify chemical compositions in *Newmania* essential oils, secondary metabolites in the extracts, and pharmacological evaluations are expected.

Competing interests

No potential conflict of interest was reported by the authors.

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