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Original Article

Chemical Examination and Antimicrobial Activity of Essential Oils from the Leaves and Rhizomes of *Meistera caudata* Šída f. & Škorničk. (Zingiberaceae)

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Abstract: *Meistera caudata* is a new endemic Vietnamese species that belong to the Zingiberaceae family. In the present work, the chemical compositions of essential oils (EOs) from *M. caudata* leaves and rhizomes and their antimicrobial activities were reported for the first time. The gas chromatography-mass spectrometry (GC/MS) analytical results revealed that α-pinene, β-pinene, and camphene were the most abundant components in both EO samples. Specifically, the leaves EO contained 27.61% of α-pinene, 21.29% of β-pinene, and 8.62% of camphene while the figures for the rhizomes EO were 10.37%, 9.69%, and 12.1%, respectively. The EOs were also shown to exhibit strong antimicrobial activities against several pathogenic bacterial and fungal strains with MIC values ranging from 8-128 µg/mL, with the most potent activity against *Enterococcus faecalis* (8 µg/mL).

Keywords: Antimicrobial activity, essential oil, *Meistera caudata*.

Introduction

Meistera Giseke is a genus of the ginger family (Zingiberaceae) with 44 accepted species worldwide [1]. The species of this genus were recorded in India, the Himalayas, Southern China, Southeast Asia, New Guinea, Queensland, and

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Sri Lanka. Previously, the genus *Meistera* was known as a synonym of the genus *Amomum* Roxb., however since 2018, *Meistera* has been considered a separate genus [2]. Several species of the genus *Meistera* have been used as flavor enhancers in food, pain relievers, and immune modulators [3]. Up to now, about 12 species of the Meistera genus have been found in Vietnam, namely *M. aculeata*, *M. caudata*, *M. celsa*, *M. chinensis*, *M. cristatissima*, *M. elephantorum*, *M. gagnepainii*, *M. koenigii*, *M. muricarpa*, *M. sudae*, *M. tomrey*, and *M. vespertilio* [1]. Therein, *M. caudata* is a new endemic species with the local name of "Sa nhân đuôi" that was first discovered in 2019 from Bidoup-Núi Bà National Park in Southern Vietnam [4]. The botanical morphology of *M. caudata* appeared similar to that of *M. chinensis*, except for smooth leaves sheaths, green ligules, long caudate and larger, broadly trilobed anther crest of leaves blade [4]. To the best of our knowledge, there have been no published reports on the chemical composition and biological activities of the leaves and rhizomes EOs of this species. Therefore, this is the first study on the chemical compositions of EOs prepared from *M. caudata* leaves and rhizomes and their corresponding antimicrobial activity.

Material and methods

Chemical and reagents

Sodium sulfate (Na_2SO_4), tetracycline, and cycloheximide were purchased from Sigma-Aldrich Co. (USA). Dimethyl sulfoxide (DMSO) and *n*-hexane were obtained from Merck KgaA (Darmstadt, Germany). All the chemicals and reagents used were of the analytical grade.

Plant materials

The leaves and rhizomes of *M. caudata* were collected in May 2022 from Bidoup-Núi Bà National Park in Southern Vietnam. The plant was identified by Dr. Van-Son Dang, Institute of Tropical Biology, Vietnam Academy of Science and Technology, and a voucher specimen (No. HC_005) was preserved in the Herbarium of Department of Chemistry, College of Education, Vinh University, Vietnam.

Isolation of the essential oils

The EOs of the fresh leaves and rhizomes of *M. caudata* were isolated using the Clevenger apparatus. The parts of plant materials (350 g for each) were introduced into a 3L flask and distilled water was added to 2L. The hydrodistillation was repeated three times for each sample for 4 hr, according to the Vietnamese Pharmacopoeia [5]. All the EOs were combined, then dried with anhydrous sodium sulfate, and stored in a dark-colored vial at 4°C until analysis [6].

GC-MS analysis of EOs

The chemical constituents of EOs of *M. caudata* leaves and rhizomes were analyzed using an Agilent Technologies 7890B GC system equipped with an HP-5MS Ultra-Inert column (30 m × 0.25 mm; film thickness 0.25 µm) and detected using a 5977B MSD working in EI mode. Helium was the carrier gas at a flow rate of 1.0 mL/min. A sample of 1.0 µL of EO (diluted with *n*-hexane, 1:100 v/v) was injected manually and in a split mode of 1:25. The oven temperature was initially set up at 60ºC, which was kept constant for 1 min, then gradually increased to 240°C at the rate of 2°C/min, and finally kept with an isothermal end (240ºC) for 4 min. The injector, MS Quad, and transfer line temperatures were set at 300°C, 150°C, and 300°C, respectively, while the MS source was fixed at 230°C. The mass spectra were recorded in 70 eV with a mass scan range from m/z 50 to 550 (2.0 scan/s). The collected data were analyzed using MassHunter Workstation Software (Version B.08.00). The identification of EO constituents was carried out by comparison of their mass spectra and retention indices of the components with published data (NIST 17, Adams book) [7]. The formula used to calculate of $RI_{obsd.}$ was:

$$RI_{obsd.} = 100\ x\left[n + (N-n)\ x\ \frac{(\log RT_{unknown} - \log RT_n)}{N/(\log RT_N - \log RT_n)}\right]$$

Where RT = retention time of the respective compound; N= no. of carbon atoms in the larger alkane; n= no. of carbon atoms in the smaller alkane [8].

Finally, EO components are reported as a relative percentage of each peak area per total area in the GC/MS chromatogram.

Antimicrobial screening

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076, *Enterococcus. faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, and *Candida albicans* ATCC 10231 were purchased from the National Institute for Food Control. The antibacterial and antifungal properties of the EOs were measured using Hadacek and Greger's method [9]. Stock solutions of the EOs were prepared in 1% DMSO. In brief, the bacterial strains and yeast were incubated to reach approximately 2×10^5 CFU/mL, and then a volume of 50 µL of broth was inoculated into Luria-Bertani (LB) medium contaning different concentrations (256 µg/mL, 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL, and 2 µg/mL) of the EO and EO-free solutions. The mixture was incubated at 37°C for 24 hr. The antimicrobial properties of the EO were reported as MIC value (Minimum inhibitory concentration) which presents the lowest concentration of the EO completely inhibiting the growth of microorganisms after 24 hr of incubation. Positive control experiments were bacteria cells and yeast cells to tetracycline and cycloheximide exposure, respectively. All experiments were conducted in triplicate.

Results and discussion

Chemical composition of the EOs

Both the EOs of *M. caudata* leaves and rhizomes were light-yellow liquids having lower densities than water (the densities of EOs isolated from *M. caudata* leaves and rhizomes were 0.86 and 0.90 g/mL, respectively). The average yield of *M. caudata* leaves and rhizomes EOs was 0.28 and 0.34% (w/w), respectively. The GC/MS analysis (Figure 1 and Figure 2) indicated that a total of fifty-one (representing 98.17% of the total EO content) and sixty-two (representing 97.24% of the total EO content) volatile components in the leaves and rhizomes of *M. caudata*, respectively (Table 1). In general, monoterpene hydrocarbons (46.09 – 61.33%) and sesquiterpene hydrocarbons (28.15 – 39.4%) were the main classes of compounds identified in the two EO samples analyzed in the present study. Among these, pinenes and camphene were the most abundant volatile components in the studied EOs. Notably, while α- and β-pinenes made up 27.61 and 21.29% of the leaves EO, they accounted for 10.37 and 9.69% of the rhizome EO, predominated over by camphene (12.1%). The other volatile constituents which were detected at high percentages include β-elemene, selinenes, and δ-3-carene. The fragrance of α-pinene is reminiscent of pine, while that of β-pinene is associated with turpentine, possessing a dry, woody, or resinous aroma. Camphene has a potent, sharp scent with a woody, earthy undertone, and β-elemene carries a woody fragrance with a subtle sweetness. Collectively, these major constituents may bestow a pleasant and refreshing woody scent to the essential oil, with slight undertones of earthiness and spice. The other chemical classes detected in the EO, including non-terpenoid and oxygenated forms of monoterpenes and sesquiterpenes, accounted for 0.09 – 7.58%. Only one non-terpenoid, namely 1-methylhexyl acetate, was detected at roughly equal percentages between the leaf and rhizome oils. The results also indicated that the oxygenated compound groups present in the rhizome oil (24 constituents) outnumbered those in the leaf oil (16 constituents). Additionally, these groups were also in percentages total found to be more abundant in the rhizome essential oil (11.66%) compared to the leaves essential oil (8.57%). However, of these, eucalyptol and linalool in the leaf oil were detected at percentages 2 – 3 times as high as those in the rhizome oil.

There have been few studies on volatile profiles of *Meistera* species in the literature. Very recently, one study reported volatile components of *M. sudae* collected in Vietnam [10]. To our knowledge,

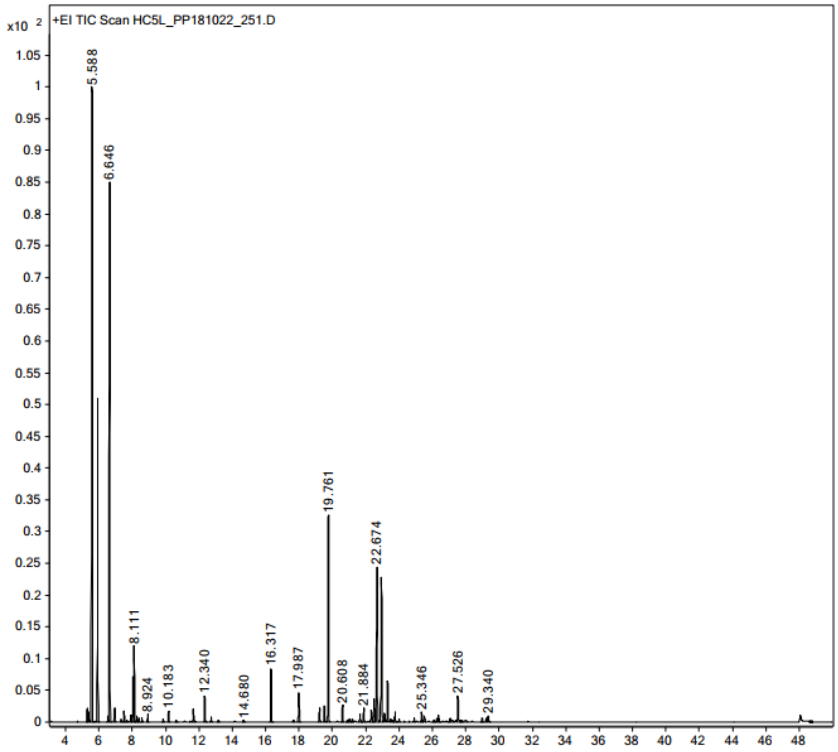

Figure 1. Gas chromatogram of *M. caudata* leaves essential oil on HP-5MS Ultra-Inert column (30 m × 0.25 mm; film thickness 0.25 µm)

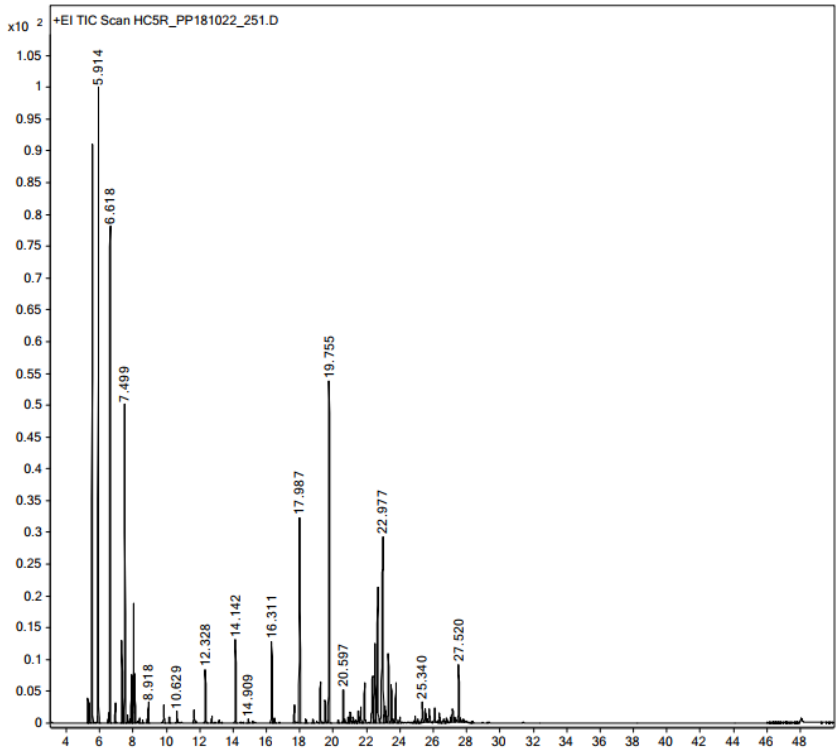

Figure 2. Gas chromatogram of *M. caudata* rhizome essential oil on HP-5MS Ultra-Inert column (30 m × 0.25 mm; film thickness 0.25 µm)

Table 1. Chemical constituents of *M. caudata* leaves and rhizomes EOs

No.	RT (min)	Compounds	$RI_{Cal.}$	$RI_{Lit.}$	Content (%)	
					Leaves	Rhizomes
1	5.29	Tricyclene	928	925	0.36	0.41
2	5.382	α-Thujene	932	929	0.25	0.34
3	5.588	α-Pinene	941	937	**27.61**	**10.37**
4	5.925	Camphene	955	952	**8.62**	**12.10**
5	6.526	Sabinene	978	974	0.18	0.18
6	6.646	β-Pinene	983	979	**21.29**	**9.69**
7	6.944	β-Myrcene	993	991	0.37	0.36
8	7.339	α-Phellandrene	1007	1005	0.07	1.58
9	7.505	δ-3-Carene	1014	1011	0.31	**6.22**
10	7.682	α-Terpinene	1020	1017	0.06	0.15
11	7.819	*m*-Cymene	1026	1023	-	0.07
12	7.905	*p*-Cymene	1029	1025	0.21	0.96
13	8.031	Limonene	1033	1030	1.38	2.76
14	8.111	1,8-Cineole (= Eucalyptol)	1036	1032	2.22	1.01
15	8.271	*cis*-β-Ocimene	1042	1038	0.17	-
16	8.392	1-Methylhexyl acetate	1046	1045	0.12	0.09
17	8.586	α-Ocimene	1052	1047	0.11	0.06
18	8.924	γ-Terpinene	1063	1060	0.24	0.42
19	9.845	Terpinolene	1091	1088	0.1	0.42
20	10.183	Linalool	1100	1099	0.35	0.14
21	10.640	Fenchol	1116	1113	0.06	0.26
22	11.659	Camphor	1149	1145	0.44	0.31
23	12.34	*endo*-Borneol	1170	1167	0.89	1.27
24	12.729	Terpinen-4-ol	1181	1177	0.18	0.17
25	13.164	α-Terpineol	1193	1189	0.07	0.06
26	14.142	Fenchyl acetate	1224	1223	-	1.94
27	14.909	Carvacrol methyl ether	1248	1244	-	0.09
28	16.317	Bornyl acetate	1289	1285	1.86	1.94
29	16.465	Thymol	1293	1291	-	0.12
30	17.987	δ-Elemene	1342	1338	1.04	4.97
31	18.359	α-Terpinyl acetate	1353	1350	-	0.09
32	18.783	*cis*-Carvyl acetate	1366	1362	-	0.09
33	19.229	β-Copaene	1380	1376	0.51	1.02
34	19.761	β-Elemene	1395	1391	**7.76**	**8.78**
35	20.299	α-Gurjunene	1413	1409	-	0.08
36	20.608	Caryophyllene	1423	1419	0.63	0.92
37	20.694	2,5-Dimethoxy-*p*-cymene	1426	1422	-	0.09
38	20.900	*cis*-β-Copaene	1433	1432	0.08	0.17
39	21.026	γ-Elemene	1437	1433	0.16	0.33
40	21.192	α-Guaiene	1443	1439	0.13	0.17
41	21.352	*iso*-Germacrene D	1448	1448	-	0.12
42	21.501	(-)-Aristolene	1453	1453	-	0.34
43	21.655	Humulene	1458	1454	0.32	0.41

table 1. (continued).

No.	RT (min)	Compounds	$RI_{Cal.}$	$RI_{Lit.}$	Content (%) Leaves	Rhizomes
44	21.884	Alloaromadendrene	1465	1461	0.55	1.09
45	22.330	γ-Muurolene	1480	1477	0.7	1.7
46	22.508	Germacrene D	1485	1481	0.89	2.12
47	22.674	β-Selinene	1490	1486	**6.14**	3.87
48	22.943	α-Selinene	1498	1494	**6.68**	**8.46**
49	23.126	*cis*-α-Bisabolene	1505	1504	0.42	0.59
50	23.309	β-Bisabolene	1511	1509	1.52	1.81
51	23.492	γ-Cadinene	1518	1513	0.14	1.13
52	23.595	7-*epi*-α-Selinene	1522	1517	-	0.1
53	23.755	Cadina-1(10),4-diene	1527	1524	0.38	1.05
54	24.001	*trans*-γ-Bisabolene	1536	1533	0.1	0.17
55	24.905	Nerolidol	1567	1564	0.15	0.15
56	25.346	Spathulenol	1582	1576	0.38	0.54
57	25.518	Caryophyllene oxide	1587	1581	0.21	0.36
58	26.090	Viridiflorol	1607	1591	0.09	0.42
59	26.370	Humulane-1,6-dien-3-ol	1617	1619	0.28	-
60	27.057	*iso*-Spathulenol	1642	1638	0.15	0.15
61	27.148	*tau.*-Cadinol	1646	1640	0.12	0.56
62	27.286	δ-Cadinol	1651	1645	-	0.17
63	27.526	*trans*-Guai-11-en-10-ol	1659	1655	1.12	1.61
64	27.635	*epi*-γ-Eudesmol	1663	1662	-	0.12
Monoterpene hydrocarbons (Sr. No. 1-13, 15, 17-19)					61.33	46.09
Oxygenated monoterpenes (Sr. No. 14, 20-29, 31, 32, 37)					6.07	7.58
Sesquiterpene hydrocarbons (Sr. No. 30, 33-36, 38-54)					28.15	39.4
Oxygenated sesquiterpenes (Sr. No. 55-64)					2.5	4.08
Non terpenoid (Sr. No. 16)					0.12	0.09

RT (min): Retention time;
$RI_{Cal.}$: Retention Indices obtained in column HP-5MS UI;
$RI_{Lit.}$: Retention Indices obtained from the literature (NIST 17 and Adams book)[7].

the present study is the first work focused on the investigation into volatile components of *M. caudata*. Interestingly, the combined proportion α- and β-pinenes (48.9%) constituted of the leaves EO from *M. caudata* was similar to that of *M. sudae* leaves EO (48.6%). Nevertheless, the leaves EOs from the two *Meistera* species showed significant differences in the percentage of the other volatile compounds. For example, β-elemene (not detected) and camphene (< 1%) in *M. sudae* leaves EO were identified as major volatile compounds in *M. caudata* leaves EO as stated earlier.

Evaluation of in vitro antimicrobial activity

The EO extracted from the leaves showed its strong antimicrobial properties against the examined bacteria and yeast with MIC ranging from 8 to 16 µg/mL for Gram (+), 16 to 128 µg/mL for Gram (-), and about 16 µg/mL for *C. albicans* (Table 2). Both the rhizome and leaves EOs exhibited the highest MIC value against *P. aeruginosa* among the investigated microorganisms, (64 µg/mL and 128 µg/mL, respectively). It could indicate that this bacterial strain is more resistant to the EOs than other bacteria. While the leaves EO displayed lower MIC values against *B. cereus* and *E. coli*

Table 2. Antimicrobial activity of EOs of *M. caudata* leaves and rhizomes

Microorganisms	MIC (µg/mL)			
	Leaves EO	Rhizomes EO	Tetracycline	Cycloheximide
E. faecalis ATCC 299212	8 ± 1.35	8 ± 0.12	4	NT
S. aureus ATCC 25923	16 ± 1.47	16 ± 1.98	16	NT
B. cereus ATCC 14579	16 ± 1.89	32 ± 2.12	64	NT
E. coli ATCC 25922	16 ± 1.56	32 ± 1.23	8	NT
P. aeruginosa ATCC 27853	128 ± 2.57	64 ± 1.35	256	NT
S. enterica ATCC 13076	16 ± 1.68	16 ± 1.12	64	NT
*C. albican*s ATCC 10231	16 ± 1.57	16 ± 1.21	NT	32

NT: Not tested; Mean ± SD, n = 3

compared to the rhizome EO. Interestingly, the EOs from *M. caudata* leaves and rhizomes presented higher antibacterial and anticandidal activities than those of the two drugs (tetracycline and cycloheximide), particularly against Gram (-) bacteria (*P. aeruginosa*, and *S. enterica*). As a result, the EOs exerted better inhibitory effects on bacterial growth of the investigated strains and *C. albicans*. The data suggest that these EOs are potential antibacterial and anticandidal agents for medical applications.

Pinene (α and β) and camphene were two dominant components of the EOs of the leaves (48.8% and 8.62%, respectively) and rhizome (20.06% and 12.1%, respectively). Besides, selinene occurred at a high concentration in the leaves EO (12.82%) and in rhizome EO (12.33%). High concentrations of α-pinene and β-pinene were shown to be associated with strong antibacterial properties against *B. subtilis* and *S. aureus* in previous studies [10,11]. Camphene is a secondary metabolite and a major component of EOs of many aromatic and medicinal plants. It has been shown to possess antibacterial and antifungal activities [12]. In addition to the two components, δ-3-carene was found to be more abundant in the rhizome EO (6.22%) compared to the leaves EO (0.32%). This compound was also identified as a major compound in the EOs of rhizomes of *Amomum agastyamalayanum* [13], and leaves, stems and roots of *A. muricarpum* [14]. Its presence is responsible for the antifungal activity of Eos [15], and antibacterial activity of ginger Eos [16]. As a result, the higher concentration of δ-3-carene may explain the lower MIC value against *P. aeruginosa* in the rhizome EO compared to the leaves EO.

Conclusions

In summary, the GC-MS analytical results showed that the EOs from leaves and rhizomes contained similar chemical compositions, including α-pinene (27.61% and 10.37%), β-pinene (21.29% and 9.69%), camphene (8.62% and 12.1%), β-elemene (7.76% and 8.78%), and α-selinene (6.68% and 8.46%). The *M. caudata* EOs also exhibited potential antimicrobial activity against *E. faecalis* ATCC 299212, *S. aureus* ATCC 25923, *B. cereus* ATCC 14579, *E. coli* ATCC 25922, *S. enterica* ATCC 13076, and *C. albicans* ATCC 10231 with MIC values ranging from 8-128 µg/mL. Of which, both EO samples showed the most potent anti-bacterial activities against *E. faecalis* (MIC= 8 µg/mL) while *P. aeruginosa* was shown to be less susceptible to the leaves and rhizome EOs (MIC values of 128 and 64 µg/mL). This is the first study to provide information on the chemical composition and antimicrobial activity of *M. caudata* leaves and rhizomes EOs. These findings provide important information on the pharmacological activities of the EOs and their potential to be employed as an antibacterial agent, as well as advantages to human health.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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