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CHEMICAL COMPOSITIONS AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS FROM THE LEAVES AND RHIZOMES OF *Meistera cristatissima* FROM VIETNAM

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Until now, there has been no information on the chemical constituents and biological activity of the essential oils and extracts of *Meistera cristatissima* (N.S.Ly & Skornick.) Skornick. (Zingiberaceae). As a part of the ongoing extensive research aimed at the characterization of the volatile constituents and antimicrobial activity of the poorly described species of Vietnamese flora [1–5], the chemical constituents and antimicrobial activity of essential oils hydrodistilled from the leaves and rhizomes of *M. cristatissima* from Vietnam are reported in this paper. *Meistera cristatissima* is regarded as a synonym of *Amomum cristatissimum* N.S. Ly & Skornick [6], is found in the Quang Ngai Province of central Vietnam and has echinate fruits and large yellow flowers [7].

The leaves and rhizomes of *M. cristatissima* were collected from Quang Ngai Province, Nghia Hanh District, Hanh Tin Dong Commune, Khanh Giang Village, Mount Dau (GPS 19°05′15″N, 104°38′09″ E), Vietnam. The collection was done in July, 2020. The plant was identified by Dr. L. N. Sam and a voucher specimen (VMN 891) was deposited in the plant specimen room, Vinh University, Vietnam. The leaves and rhizomes were separated from debris, stones and other substances via handpicking to obtain 2.0 kg of each sample, and were then subjected to separate hydrodistillation using a Clevenger-type apparatus as described previously [1–5, 8–10].

The chemical analysis of the essential oils was performed by using Gas chromatography (GC) on an Agilent Technologies HP 7890 Plus Gas chromatograph equipped with an FID and fitted with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were as described previously [1–5, 8–10]. An Agilent Technologies HP 7890N Plus Chromatograph fitted with a capillary HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with mass spectrometer HP 5973 MSD was used for this gas chromatography-mass spectrometry (GC-MS) experiment, under the same conditions as those used for gas chromatography analysis as described in the preceding. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35–350 amu at a sampling rate of 1.0 scan/s. The identification of constituents from the GC/MS spectra of *M. cristatissima* was performed based on retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_4–C_{40}), under identical experimental conditions. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition [11].

The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay [8–10]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO).

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TABLE 1. Chemical Constituents of Essential Oils from the Leaf and Rhizome of *M. cristatissima*

Compound [a]	RI[b]	Leaves	Rhizomes	Compound [a]	RI[b]	Leaves	Rhizomes
α-Pinene	939	19.8	–	β-Chamirene	1491	0.4	–
Camphene	955	3.4	–	γ-Muurolene	1492	0.4	–
Thuja-2,4(10)-diene	961	0.2	–	Aristolochene	1502	–	3.6
β-Pinene	984	3.9	–	β-Selinene	1505	3.2	2.4
o-Cymene	1029	2.7	–	γ-Amorphene	1512	0.6	0.8
Limonene	1034	1.5	–	α-Selinene	1515	1.1	–
1,8-Cineole	1037	1.9	–	β-Bisabolene	1519	1.8	1.1
Linalool	1101	1.6	0.2	γ-Cadinene	1531	0.3	0.3
endo-Fenchol	1122	0.3	–	7-*epi*-α-Selinene	1538	0.5	0.6
cis-Sabinol	1149	0.8	–	*trans*-Calamenene	1539	–	0.2
trans-Verbenol	1153	0.4	–	Elemol	1565	0.3	–
Camphor	1156	0.4	–	(*E*)-Nerolidol	1569	7.3	3.3
Pinocarvone	1173	0.4	–	4-*epi*-Maaliol	1588	–	1.8
Borneol	1176	0.5	0.9	Palustrol	1589	0.7	–
Terpinen-4-ol	1187	0.7	0.2	Spathulenol	1598	4.2	14.6
α-Terpineol	1198	0.8	–	Caryophyllene oxide	1605	9.8	7.1
p-Cymen-8-ol	1190	1.2	0.3	Cubeban-11-ol	1613	1.5	6.5
Myrtenol	1206	0.4	–	Rosifoliol	1620	–	0.6
Myrtenal	1208	0.4	–	Ledol	1628	0.4	1.6
Verbenone	1221	0.6	0.2	Humulene epoxide II	1632	1.1	5.3
Fenchyl acetate	1229	0.5	1.2	1-*epi*-Cubenol	1648	0.5	0.6
Bornyl acetate	1296	2.1	7.4	τ-Muurolol	1660	–	1.6
Dihydroedulane	1301	0.3	–	α-Muurolol	1663	–	1.0
Myrtenal acetate	1335	1.7	0.2	Desmethoxyenecalin	1668	0.3	–
trans-Carvyl acetate	1342	–	0.2	α-Cadinol	1674	1.1	2.6
α-Terpinyl acetate	1356	–	0.7	*neo*-Intermedeol	1679	1.7	2.1
cis-Carvyl acetate	1365	–	0.2	14-Hydroxyl-9-epi-(*E*)-caryophyllene	1688	–	0.3
endo-Isocamphanyl acetate	1380	–	0.5	Cadalene	1695	0.6	0.4
Geranyl acetate	1384	–	0.1	10-*nor*-Calamenen-10-one	1724	–	0.1
α-Copaene	1388	0.9	0.2	Cyclocolorenone	1774	0.5	–
cis-β-Elemene	1409	1.1	0.3	Phytol	2117	0.3	–
2,5-Dimethoxyl-*p*-cymene	1427	–	0.7	Total		92.0	71.2
β-Caryophyllene	1435	0.4	–	Monoterpene hydrocarbons		31.5	–
trans-α-Beramotene	1446	0.3	–	Oxygenated monoterpenes		15.7	12.3
Aromadendrene	1457	0.6	0.4	Sesquiterpene hydrocarbons		16.1	11.8
9-*epi*-(*E*)-Caryophyllene	1478	0.9	1.0	Oxygenated sesquiterpenes		29.4	49.1
Valencene	1490	–	0.5	Diterpenes		0.3	–

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5MS column; – Not identified.

Dilution series (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^{9}, 2^{7}, 2^{5}, 2^{3}, and 2^{1} μg/mL) were prepared in sterile distilled water inside the micro-test tubes from where they were transferred separately to 96-well microtiter plates. Bacteria grown in double-strength Mueller–Hinton broth or double-strength tryptic soy broth, and fungi sustained in double-strength Sabouraud dextrose broth, were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. DMSO was used as a negative control. Streptomycin was used as the antibacterial standard while nystatin and cycloheximide were used as antifungal standards. All experiments were performed in triplicate. After incubation at 37°C for 24 h, the MIC values were determined as the lowest concentration of essential oils of *M. cristatissima*, which completely inhibited the growth of the microorganisms. The IC_{50} values were determined by the inhibited growth of microorganisms based on the turbidity measurement data of EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and Rawdata computer software (Belgium).

The obtained essential oils were light-yellow coloured and the yields were 0.16% (leaf) and 0.25% (rhizome), which is consistent with values reported previously for the majority of the *Amomum* essential oils from Vietnam – for example, essential oils from the leaf and rhizome *A. cinnamomeum* were obtained in yields of 0.12 and 0.19%, respectively [1], while the leaves and stems of *A. rubidum* afforded essential oils in yields of 0.22 and 0.15%, respectively [2].

TABLE 2. The Antimicrobial Activity of the Leaf and Rhizome Essential Oils of *M. cristatissima*

Microorganism	MIC, μg/mL[a]		IC_{50}, μg/mL[a]	
	Leaves	Rhizomes	Leaves	Rhizomes
Enterococcus faecalis ATCC299212	64.0	128.0	20.45	39.45
Staphylococcus aureus ATCC25923	128.0	128.0	37.77	34.67
Bacillus cereus ATCC14579	64.0	256.0	29.45	98.67
Candida albicans ATCC 10231	64.0	256.0	18.45	102.56

[a] Means of three replicates; *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076 – no activity.

The main classes of compounds identified in the leaf essential oils of *M. cristatissima* were monoterpene hydrocarbons (31.5%), oxygenated monoterpenes (15.7%), sesquiterpene hydrocarbons (16.1%), and oxygenated sesquiterpenes (29.4%), as seen in Table 1. However, the rhizome oil consists mainly of oxygenated monoterpenes (12.3%), sesquiterpene hydrocarbons (11.8%), and oxygenated sesquiterpenes (49.1%). Monoterpene hydrocarbon compounds were not identified in the rhizome oil.

The main compounds of the leaf oil were α-pinene (19.8%), caryophyllene oxide (9.8%), and (*E*)-nerolidol (7.3%). The minor constituents consist of spathulenol (4.2%), β-pinene (3.9%), camphene (3.4%), β-selinene (3.2%), *o*-cymene (2.7%), and bornyl acetate (2.1%), whereas spathulenol (14.6%), bornyl acetate (7.4%), caryophyllene oxide (7.1%), cubeban-11-ol (6.5%), and humulene epoxide II (5.3%) were the main constituents identified in the rhizome essential oil. This is the first report on the essential oil constituents of *M. cristatissima*. It is well noted that terpene compounds predominate in the essential oils, and this has been previously reported for other *Amomum* essential oil samples grown in Vietnam [1, 2]. Typically, the chemical identities of the various terpene compounds present in the *Amomum* essential oils differed from one species to another, which is an indication of the chemical variability in their compositional pattern.

The essential oils from the leaves and rhizomes of *M. cristatissima* displayed moderate antimicrobial activity (Table 2). The essential oil from the leaves of *M. cristatissima* was the most active against *Enterococcus faecalis* ATCC299212, *Bacillus cereus* ATCC14579 and *Candida albicans* ATCC 10231, with minimum inhibitory concentration (MIC) values of 64.0 μg/mL. The obtained IC_{50} values were 20.45, 29.45, and 18.45 μg/mL, respectively. The rhizome essential oil displayed lower antimicrobial activity against the microorganism mentioned previously, with MIC values of 128.0, 256.0, and 256.0 μg/mL, respectively, and IC_{50} values of 39.45, 98.67, and 102.56 μg/mL, respectively. Both essential oils exhibited similar activity towards *Staphylococcus aureus* ATCC25923 (MIC = 128.0 μg/mL). In addition, both the leaf and rhizome oils exhibited no antimicrobial activity against Gram-negative microorganisms of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076. Overall, the studied essential oils exhibited moderate antimicrobial activity against the tested microorganisms. This shows that *Amomum* essential oils selectively inhibit the growth of different microorganisms. The chemical constituents and antimicrobial activity of *M. cristatissima* essential oils are being reported for the first time. It is believed that the constituents present in the studied essential oils might have influenced the observed antimicrobial activity of *M. cristatissima*. A section of the chemical compounds identified in the essential oils were reported previously, to possess antimicrobial activity, including α-pinene [12], β-pinene [12], bornyl acetate [12], camphene [13] caryophyllene oxide [14], (*E*)-nerolidol [14], and spathulenol [14].

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