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Research Article

Chemical composition and *in vitro* α-amylase inhibitory activity of *Meistera vespertilio* (Gagnep.) Skornick. & M.F. Newman essential oils collected in two different regions of Vietnam

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Abstract
Meistera, belongs to the Zingiberaceae family, is a genus of medicinal plants that contain a rich resource of essential oils (EOs) as well as have many valuable bioactivities. The aim of the present study was to investigate the EOs of *Meistera vespertilio*, which has not been reported before. The EOs were distilled by hydrodistillation method from *M. vespertilio* rhizomes collected in two different regions (Phu Tho and Nghe An Provinces) of Vietnam. The compositional analysis of two EO samples was carried out using Gas Chromatography-Mass Spectroscopy (GC-MS). GC-MS analysis showed that the two samples had a different number of chemical components. The Phu Tho EO sample consisted of more components (51 compounds) compared to the Nghe An EO sample (41 compounds). However, the two EOs were identified with some similar components including α-pinene, camphene, β-pinene, and limonene. Furthermore, both EO samples possessed good inhibitory effects on α-amylase, with average IC_{50} values ranging from 0.61 to 0.91 (mg/mL). Especially, the EO of *M. vespertilio* rhizomes from Nghe An Province showed intense α-amylase inhibitory activity (IC_{50} value of 0.61 ± 0.00 mg/mL) which was stronger than acarbose, an antidiabetic drug (IC_{50} value of 0.92 ± 0.01 mg/mL), while the figure for Phu Tho EO showed approximately equivalent activity. The results obtained in the present study showed the potential of *M. vespertilio* EOs as a valuable source of chemical compounds for the food and pharmaceutical industry.

Keywords
α-Amylase inhibition, Chemical composition, Essential oil, *Meistera vespertilio*

Introduction

Meistera is the most widespread genus of *Amomum* in the Zingiberaceae family, which includes 48 accepted species and is widely distributed from Sri Lanka and India to Indochina, Sundaland, Papua New Guinea, and Northern Australia[1]. Of these 12 species have been recorded in the Vietnamese flora[2]. To date, there has been quite little information on the chemical constituents and bioactivities of the genus *Meistera*. Previous research mainly

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focused on the study of chemical components of essential oils (EOs) from several species of this genus. For example, α-pinene (10.37-27.61%), β-pinene (9.69-21.29%), and camphene (8.62-12.1%) were identified as main compounds of *M. caudata* EOs[3]. The main constituents of *M. tomrey* EOs include 1,8-cineole (13.5-16.7%), β-pinene (6.6-14.2%), α-pinene (4.6-6.9%), and borneol (4.2-4.6%)[4]. The leaf EO of *M. sudae* contained β-pinene (27.4%), α-pinene (21.2%), limonene (12.1%), and myrcene (8.6%)[5]. Moreover, the EOs of the genus *Meistera* showed remarkable antimicrobial activity against several pathogenic bacterial and fungal strains[3,5].

Meistera vespertilio (Gagnep.) Škorničk. & M.F.Newman (local name: Sa nhân thầu dầu) is an herb 1.0-2.5 m tall, with red fruit similar to the fruit of the Castor oil plant (*Ricinus communis*), and is found in some regions of China, Laos, and Vietnam. To the best of our knowledge, there is no record of the chemical composition and biological activity of *M. vespertilio* EOs. The current study aims to investigate the chemical composition of and α-amylase inhibitory activity of the EOs of *M. vespertilio* rhizomes from two different regions of Vietnam.

Material and methods

Chemicals and reagents

Sodium sulfate (Na_2SO_4) and dichloromethane (CH_2Cl_2) were purchased from Merck KgaA (Darmstadt, Germany). α-Amylase from *Bacillus* sp. was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acarbose (94.1%) was purchased from the National Institute of Drug and Quality Control (Ha Noi, Vietnam). All other chemicals and reagents used were of the analytical grade.

Plant materials

M. vespertilio rhizomes used in the current study were collected in July 2022 from Xuan Dai, Tan Son District, Phu Tho Province, Vietnam (21°07'28.8"N; 104°58'21.2"E) and Dong Van, Que Phong District, Nghe An Province, Vietnam (19°45'32.5"N; 104°59'20.4"E). The identification of species was identified by Assoc. Prof. Dr. Nguyen Hoang Tuan (Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, Vietnam). Voucher specimens (HC.024.MVR-PT and HC.024.MVR-NA, respectively) were deposited at the Laboratory of the Department of Chemistry, Vinh University.

Hydrodistillation of the EOs

Fresh rhizomes of *M. vespertilio* (500 g) of each collection site were washed, cut into small pieces, and subjected to hydrodistillation for approximately 4 h using a Clevenger-type apparatus as previously described[6,7]. The hydrodistillation was repeated three times. Two EO samples were collected over water, dried with anhydrous sodium sulfate, and stored in the dark at 4°C ready for the experimental analysis.

Analysis and Identification of the EOs

Chemical components of both EO samples were identified in the analysis laboratory of Vinh University using Gas Chromatography-Mass Spectroscopy (GC-MS). GC-MS parameters used in this study are shown in Table S1.

Identification of the EOs components was carried out by comparing their mass spectra and retention indices (RI) with those contained in the literature (NIST 17 and Adams[8]). The formula for determination of the retention indices (RI) was employed as previously described[9,10]. Finally, quantification of the EO compounds was performed using the relative peak area percentage.

In vitro α-amylase inhibitory activity

The α-amylase inhibition of two EO samples was analysed as previously described[11]. The EOs were combined with a solution of α-amylase (0.14 U/mL) in phosphate buffer (pH 6.9). The resulting mixture was then subjected to incubation at 37°C for 15 min. Subsequently, the reaction was initiated with the addition of 15 mL of a 0.25% starch solution, followed by an additional 15-min incubation at 37°C. For the control sample, the same procedures were followed, excluding the addition of the enzyme. The reaction was halted by introducing 50 mL of 1 M HCl, succeeded by the addition of 100 mL of KI_3 solution. The absorbance at 595 nm was measured using a spectrophotometer. Acarbose was employed as a

positive control. The inhibition percentage was determined as follows:

Inhibition percentage = [1 – S/B] × 100

where, S and B stand for the absorbance of the sample and blank. IC_{50} (mg/mL) was used to predict the activity. Determination of this value was performed using the inhibition curve obtained for each extract by plotting the inhibition percentage (%) versus the extract concentration (mg/mL). The construction of the curve was conducted using Microsoft Excel.

Statistical analysis

Determination of α-amylase inhibitory activity was carried out in triplicate, and the data are shown as mean ± standard deviation. The Minitab 19 software package was employed for data analysis. Statistical comparisons were made through analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was defined as $p < 0.05$.

Results and discussion

Chemical composition of the EOs

Both EO samples of *M. vespertilio* rhizomes from Phu Tho and Nghe An Provinces were obtained with pale-yellow color with yields of 0.65 and 0.63% (w/w, fresh weight), respectively.

The chemical constituents of each EO sample were analyzed by GC-MS (Fig. S1 and Fig. S2, Supplemental material) showing that a total of 51 compounds (amounting to 97.06% of the content) and 41 compounds (amounting to 98.66% of the content) in the EOs of *M. vespertilio* rhizomes from Phu Tho and Nghe An Provinces, respectively (Table 1). The study results revealed it is quite similar chemical profiles of the EO samples of the two regions. Both EO samples were composed predominantly of monoterpene compounds including monoterpene hydrocarbons (83.00-82.35%) and oxygenated monoterpenes (6.91-13.33%). Moreover, the principal constituents in both EOs were α-pinene (54.46-54.05%), camphene (6.81-5.69%), β-pinene (5.96-7.47%), and limonene (7.72-6.40%). It is of interest to note that 1,8-cineole (5.52%) was identified as a main compound in the Nghe An EO sample, while it was found as a minor compound (1.04%) in the Phu Tho EO sample. In addition, some compounds have percentages of greater than 1.00% in both EOs, such as β-myrcene (2.97-2.92%), α-phellandrene (1.14-1.24%), p-cymene (1.13-2.24%), and fenchyl acetate (1.54-3.04%).

These present results were quite consistent with the chemical profiles of *Meistera* EOs as previously described. For example, α-pinene (10.37-27.61%), β-pinene (9.69-21.29%), and camphene (8.62-12.1%) were the main constituents in the EOs of *M. caudata*[3]. α-Pinene (4.6-6.9%), β-pinene (6.6-14.2%), and 1,8-cineole (13.5-16.7%) were the main constituents in the EOs of *M. tomrey*[4]. β-Pinene (27.4%), α-pinene (21.2%), limonene (12.1%), and myrcene (8.6%) were the main constituents of *M. sudae* leaf EO[5].

In vitro α-amylase inhibition of the EOs

The inhibition percentage of two EO samples (at various concentrations) on α-amylase is shown in Table 2. Increase in concentrations of the EO led to considerable inhibition of α-amylase in a dose-dependent manner. The EO from the *M. vespertilio* rhizome collected in Nghe An had a significantly lower IC_{50} (0.61 ± 0.00 mg/mL) compared to its counterpart from the rhizome in Phu Tho (IC_{50} = 0.91 ± 0.02 mg/mL), implying that it exhibited a higher inhibitory effect. Furthermore, it may have a stronger inhibitory activity than acarbose, an antidiabetic drug (IC_{50} = 0.92 ± 0.01 mg/mL). No data about anti-amylase activity of EOs from the genus *Meistera* has been found in the literature. However, a range of plant species belonging to the Zingiberaceae family have been reported to be assayed for this activity. For example, the EO of *Curcuma longa* fresh and dried rhizomes had a significantly lower IC_{50} compared to acarbose[12]. The EOs from *Aframomum melegueta* and *A. daniellii* seeds were reportedly able to inhibit α-amylase, albeit not as strong as acarbose[13].

Perhaps, the inhibitory effect of the *M. vespertilio* EOs on α-amylase was ascribed to the presence of monoterpenes and sesquiterpenes in their compositions. Previously, limonene and pinenes, which were identified as major constituents in the EOs, have been shown

Table 1. The EO components of *M. vespertilio* rhizomes from two different regions of Vietnam

No.	RT (min)	Compounds	RI (Exp.)	RI (Lit.)	Concentration (%)	
					Phu Tho EO	Nghe An EO
1	5.296	Tricyclene	928	925	0.20	0.15
2	5.382	α-Thujene	932	929	0.10	0.12
3	5.594	α-Pinene	941	937	**54.46**	**54.05**
4	5.914	Camphene	955	952	**6.81**	**5.69**
5	6.515	4(10)-thujene (= Sabinene)	978	974	0.10	0.12
6	6.618	β-Pinene	982	979	**5.96**	**7.47**
7	6.938	β-Myrcene	993	991	2.97	2.92
8	7.333	α-Phellandrene	1007	1005	1.14	1.24
9	7.676	α-Terpinene	1020	1017	0.20	0.29
10	7.905	p-Cymene	1029	1025	1.13	2.24
11	8.031	Limonene	1033	1030	**7.72**	**6.40**
12	8.111	1,8-cineole (= Eucalyptol)	1036	1032	1.04	**5.52**
13	8.266	(Z)-β-Ocimene	1042	1038	0.25	0.28
14	8.924	γ-Terpinene	1063	1060	0.86	1.56
15	9.839	Terpinolene	1091	1088	0.45	0.47
16	10.177	Linalool	1100	1099	0.45	0.88
17	10.635	Fenchol	1116	1113	0.05	0.11
18	11.653	Camphor	1149	1145	0.46	0.07
19	12.340	*endo*-Borneol	1170	1166	0.12	0.49
20	12.723	Terpinen-4-ol	1181	1177	0.16	0.41
21	13.158	α-Terpineol	1193	1189	0.15	0.41
22	13.353	Myrtenol (=α-pinene-10-ol)	1198	1195	0.06	0.06
23	14.142	Fenchyl acetate	1224	1223	1.54	3.04
24	14.400	Isobornyl formate	1232	1232	0.17	-
25	14.600	Methyl thymyl ether	1238	1235	-	0.11
26	14.812	(*Z*)-Citral	1245	1240	-	0.08
27	15.241	*trans*-Geraniol	1258	1255	0.15	0.18
28	15.790	(*E*)-Citral	1274	1270	0.05	0.10
29	16.311	Bornyl acetate	1289	1285	0.84	1.56
30	16.683	*trans*-Pinocarvyl acetate	1299	1297	0.07	-
31	16.769	Methyl myrtenate	1301	1301	0.07	-
32	17.593	Myrtenyl acetate	1329	1327	1.36	0.06
33	17.982	δ-Elemene	1341	1338	0.23	0.14
34	18.359	α-Terpinyl acetate	1353	1350	0.10	0.25
35	19.229	α-Copaene	1380	1376	0.07	-
36	19.441	Geranyl acetate	1386	1382	0.07	-
37	19.738	β-Elemene	1394	1391	0.20	-
38	20.603	α-Santalene	1423	1420	0.63	0.24

Table 1 cont.

No.	RT (min)	Compounds	RI (Exp.)	RI (Lit.)	Concentration (%)	
					Phu Tho EO	Nghe An EO
39	21.089	α-Bergamotene	1440	1435	0.18	0.09
40	21.335	Selina-5,11-diene	1448	1447	0.09	-
41	21.449	*epi*-β-Santalene	1451	1448	0.07	-
42	21.936	Alloaromadendrene	1467	1461	0.59	0.11
43	22.594	Aristolochene	1488	1487	0.32	0.10
44	22.868	Valencene	1496	1492	0.16	-
45	22.966	Bicyclogermacrene	1499	1495	0.28	0.15
46	23.292	β-Bisabolene	1511	1509	-	0.25
47	23.750	δ-Cadinene	1527	1524	0.11	0.08
48	24.505	Elemol	1553	1549	0.18	-
49	25.346	Spathulenol	1582	1576	0.33	0.52
50	25.529	Globulol	1588	1583	0.14	-
51	27.635	7-*epi*-γ-Eudesmol	1663	1662	0.14	0.44
52	28.505	Aristol-1(10)-en-9-ol	1694	1692	3.74	-
53	28.659	Germacrone	1699	1693	0.17	-
54	31.400	Ambrial	1803	1809	0.17	0.21
	Total				**97.06**	**98.66**
	Monoterpene hydrocarbons (No. 1-11, 13-15)				82.35	83.00
	Oxygenated monoterpenes (No. 12, 16-32, 34, 36)				6.91	13.33
	Sesquiterpene hydrocarbons (No. 33, 35, 37-47)				2.93	1.16
	Oxygenated sesquiterpenes (No. 48-54)				4.87	1.17

RT: Retention time (min); RI (Exp.): Retention Indices obtained in HP-5MS Ultra Inert column; RI (Lit.): Retention Indices obtained from the literature

Table 2. Inhibitory effect of the *M. vespertilio* rhizome EOs on α-amylase

Concentration (mg/mL)	Inhibition percentage (%)		
	Phu Tho EO	Nghe An EO	Acarbose
0.25	6.27 0.80	24.99 ± 1.12	-
0.50	24.82 ± 0.79	48.33 ± 0.20	-
1	58.16 ± 0.74	76.62 ± 0.14	-
2	84.83 ± 0.51	83.40 ± 0.67	-
4	91.39 ± 0.38	89.77 ± 0.94	-
IC_{50}	**0.91 ± 0.02^{a}**	**0.61 ± 0.00^{c}**	**0.92 ± 0.01^{b}**

Different letters (a, b, c) for the IC_{50} value stand for statistically significant differences among test samples ($p < 0.05$)

to possess potent inhibitory effects against α-amylase (IC_{50} = 0.435 ± 0.003 µL/mL)[14]. The anti-amylase activity observed in this species could also be due to synergistic or additive effects of the various constituents present in the EOs.

Conclusion

In conclusion, this study presents the first report on the chemical profile and *in vitro* α-amylase inhibitory activity of the EOs extracted from *M. vespertilio* rhizomes. GC-MS analysis results revealed that the EOs of *M. vespertilio* rhizomes from two different regions consisted mainly of monoterpenes, typically: α-pinene, camphene, β-pinene, and limonene. In addition, both EO samples showed potential α-amylase inhibitory activity with IC_{50} values of 0.61-0.91 mg/mL. The present study demonstrated that rhizome EOs of *M. vespertilio* have significant pharmaceutical potential although more study in the future will be required.

Competing interests

No potential conflict of interest was reported by the authors.

Supplementary data

Table S1 and Figs. S1-S2 are given in supplementary file available online at https://10.1080/0972060X.2023.2276724.

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