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Constituents of essential oils from the leaves and rhizomes of *Ophiopogon fruticosus* Aver., N. Tanaka & K.S. Nguyen from Vietnam

Pham Ngoc Khanh, Hoang Thi Ngoc Anh, Tran Thu Huong, Vu Thi Ha, Dinh Thi Thu Thuy, Rakesh Kumar Joshi and Nguyen Manh Cuong

Abstract

Ophiopogon fruticosus Aver., N.Tanaka & K.S. Nguyen (local name 'Địa Long', meaning "Earth Dragon") (Asparagaceae) was reported as a new *Ophiopogon* species in the flora of Laos and Vietnam. It is distributed in primary evergreen broad-leaved forests on limestone mountainous areas (500-1200 m above sea level) and locally abundant in Tuyen Quang province, in the North of Vietnam. From the fresh rhizomes of this plant, a pleasant smelling yellow essential oil was obtained in average of 0.99% yield (w/w) to the absolute dry mass of plant materials by steam distillation. It was soluble in alcohol and organic solvents and insoluble in water with a specific gravity of 0.875-0.975 g/mL (25 °C) and a refractive index of 1.466-1.566 (25 °C). Almost all components of the oil are terpenes including monoterpenes (hydrocarbon monoterpenes, 2 compounds, 1.06% of the total oil) and sesquiterpenes (hydrocarbon and oxygenated sesquiterpenes, 23 compounds, 62.83% and 14 compounds 28.72% of the total oil, respectively). The main constituents (>10 %) of the oil were sesquiterpene compounds with the highest relative amounts including cadina - 1(10), 7(11) diene (20.61%), ishwaraene (12.04%), longifolene (5.68%), α -selinene (4.13%), δ -cadinene (4.00%), and cis-calamenene (1.18%) and 1 sesquiterpene oxygenated as 1 β -H-presilphiperfolane-9a-ol (9.34%). The contents of the remaining components were below 5%, most of them (35 compounds) even below 2%.

Keywords *Ophiopogon fruticosus* Aver., N. Tanaka & K.S. Nguyen, new record, essential oil, Tuyen Quang

1. Introduction

Ophiopogon fruticosus Aver., N. Tanaka & K. S. Nguyen was reported as a new *Ophiopogon* species in the flora of Laos and Vietnam [1]. *O. fruticosus* (local name 'Địa Long', meaning "Earth Dragon") is a terrestrial and lithophytic undershrub species belonging to the Asparagaceae family. It is distributed in primary evergreen broad-leaved forests on limestone mountainous areas (500-1200 m above sea level) and locally abundant in Tuyen Quang province, in the North of Vietnam [1]. The stems, rhizomes and roots of this plant have been used in traditional folk medicine for treatment of skin diseases and limb pain. Till the time we write this paper, there are no reports on composition of the essential oil from this plant. Herein, we described for the first time the chemical composition of essential oil from the rhizomes of *O. fruticosus* collected in Vietnam.

2. Materials and methods**2.1 Plant material**

The whole plant *O. fruticosus* was collected at Na Hang district, Tuyen Quang province, Viet Nam, on 5th June 2019. A voucher specimen, C-623, was deposited at Department of bioactive compounds, Institute of natural products chemistry, Vietnam Academy of Science and Technology.

2.2 Methods**2.2.1 Essential oil extraction**

The fresh rhizomes of *O. fruticosus* were cleaned, sliced, chopped and subjected to steam-distillation in a Clevenger-type apparatus for 4 h at normal pressure, as described in Vietnamese Pharmacopoeia V [2]. The obtained essential oil was collected, dried over anhydrous sodium sulfate and stored in a sealed vial at 10 °C in the dark prior to analysis. The experiments were performed in triplicate.

2.2.2 Essential oil analysis

Essential oil composition of *O. fruticosus* rhizome was analyzed on an Agilent HP mode 7890A gas chromatograph coupled to an Agilent 5975C VL Triple-Axis mass spectrometer. A fused silica capillary Hewlett Packard HP5-MS (5% phenyl methyl siloxane) column (30 m x 0.25 mm i.d., 0.25 µm film thickness) was used for separation. Helium was used as carrier gas at a flow rate of 1.0 ml/min. Data acquisition and processing were performed using Agilent MSD productivity Chemstation Rev. E-02.02. Compounds also were identified by comparing their mass spectra with those contained in the NIST08 database. Data interpretation was performed using the Mass Finder 4.0 software.

2.2.3 GC-MS operation conditions

The mass spectrometer was operated in electron-impact (EI) mode, the ionization energy was 70 eV, the interface temperature was 250 °C, the ion source temperature was 250 °C, the MS quadrupole temperature was 150°C, and the scan range was 35-450 amu. A 0.1 µl of the oil sample was injected using split mode with a split ratio of 100:1. Initial temp of GC oven was set 60 °C, temperature increment 4°C/min to 240 °C.

2.2.4 Identification and quantification of essential oil constituents

Individual compounds in the oil were identified by comparison of their mass spectra and retention indices with those in GC-MS libraries (MS Wiley 8th and NIST 2008) and with those reported in literatures [3]. Retention indices of oil constituents were determined using standard C₈-C₂₈ straight chain hydrocarbons (Aldrich Chemical Company, USA) [3].

2.2.5 Quantitative analysis of rhizome essential oil

Essential oil content in percentages (volume / mass of absolute dry plant material) was calculated according to formula $X(\%) = \frac{V \cdot 10^4}{A \cdot (100 - B)}$, where X(%): Essential oil content (%); V : Essential oil volume obtained (ml), A: Mass of plant materials used for hydro-steam distillation (g) and B: Material moisture (%).

3. Results and discussion

3.1 Quantitative analysis of the rhizome's essential oil

The essential oil content of *O. fruticosus* determined as percent volume per dry medicinal material X (%) was shown in Tab. 1.

Table 1: Quantitative analysis of essential oil content in *O. fruticosus* rhizome after 3 times distillation

No.	Mass of plant materials used for hydro-steam distillation (g)	Material moisture (%)	Essential oil volume obtained (ml)	Essential oil content (%)
1 st	40.05	48.25	0.20	1.00
2 nd	48.63	48.25	0.25	0.99
3 rd	39.40	48.25	0.20	0.98

The essential oil of *O. fruticosus* rhizomes obtained in average was 0.99% yield (w/w) to the absolute dry mass of plant materials by steam distillation as a pleasant smelling yellow oil, soluble in alcohol and organic solvents, and insoluble in water with a specific gravity of 0.875-0.975 g/mL (25 °C) and a refractive index of 1.466 – 1.566 (25 °C).

3.2 Analysis of chemical composition of the rhizome's essential oil

A total ion chromatogram obtained from the GC-MS analysis is presented in Fig. 1. The composition of the essential oil is presented in Table 2, whereby all peaks with less than 0.1% area and unknown peaks with less than 0.5% area were not considered for analysis. The chemical structures of identified compounds were displayed in Fig. 2.

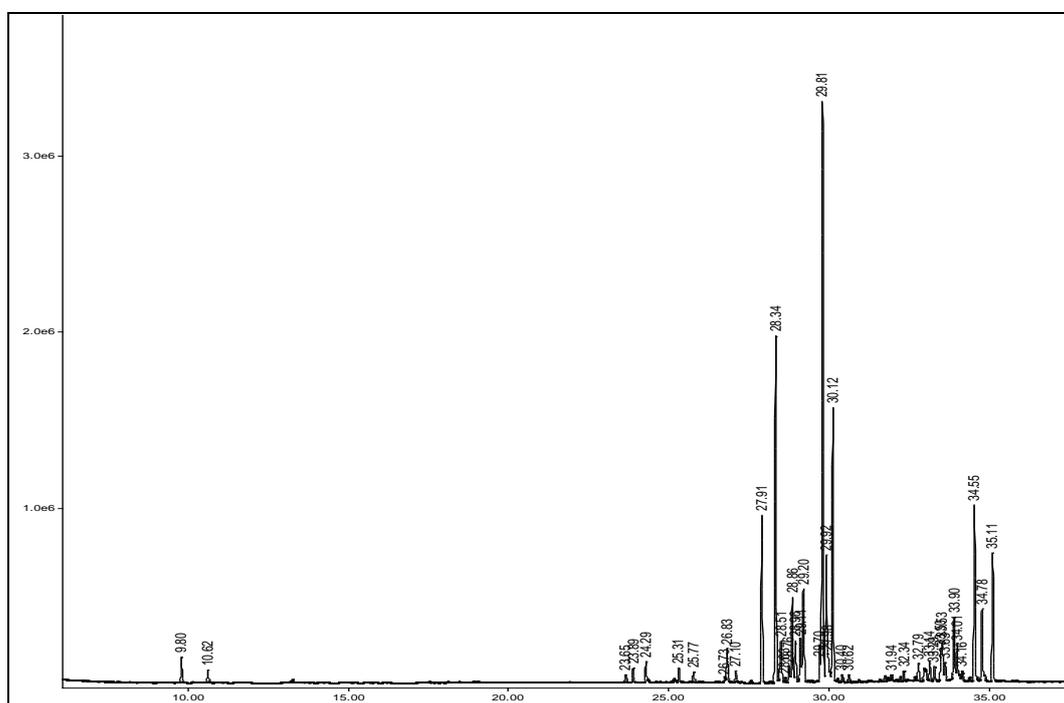
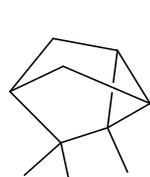


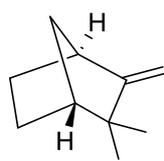
Fig 1: GC/MS total ion chromatogram of rhizome oil of *O. fruticosus*

Table 2: Chemical compositions of rhizome oil of *O. fruticosus*
 RI = Refractive index, RT = Retention time (min), MW = Molecular weight

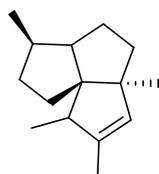
Peak No.	Retention time (min)	RI	Compounds	Molecular formula	MW	Relative amount (%)
1	9.80	928	Tricyclene	C ₁₀ H ₁₆	136.23	0.69
2	10.62	955	Camphene	C ₁₀ H ₁₆	136.23	0.37
3	23.65	1339	Silphiperfol-5-ene	C ₁₅ H ₂₄	204.36	0.24
4	23.89	1346	Presilphiperfol-7-ene	C ₁₅ H ₂₄	204.36	0.45
5	24.29	1358	Silphiperfol-6-ene	C ₁₅ H ₂₄	204.36	0.59
6	25.31	1389	α -Copaene	C ₁₅ H ₂₄	204.36	0.47
7	25.77	1403	cis- β -Elemene	C ₁₅ H ₂₄	204.36	0.33
8	26.74	1434	β -Copaene	C ₁₅ H ₂₄	204.36	0.17
9	26.83	1437	β -Caryophyllene	C ₁₅ H ₂₄	204.36	1.14
10	27.10	1445	β -Gurjunene (Calarene)	C ₁₅ H ₂₄	204.36	0.41
11	27.91	1471	Longifolene	C ₁₅ H ₂₄	204.36	5.68
12	28.34	1485	Ishwarane	C ₁₅ H ₂₄	204.36	12.04
13	28.51	1490	γ -Muuroolene	C ₁₅ H ₂₄	204.36	2.42
14	28.63	1494	α -Amorphene	C ₁₅ H ₂₄	204.36	0.20
15	28.76	1498	Germacrene D	C ₁₅ H ₂₄	204.36	0.59
16	28.86	1501	Aristolochene	C ₁₅ H ₂₄	204.36	2.98
17	28.96	1505	β -Selinene	C ₁₅ H ₂₄	204.36	1.55
18	29.11	1510	γ -Amorphene	C ₁₅ H ₂₄	204.36	1.81
19	29.20	1513	α -Selinene	C ₁₅ H ₂₄	204.36	4.13
20	29.70	1529	γ -Cadinene	C ₁₅ H ₂₄	204.36	1.21
21	29.81	1533	Cadina - 1(10), 7(11) diene	C ₁₅ H ₂₄	204.36	20.61
22	29.92	1537	δ -Cadinene	C ₁₅ H ₂₄	204.36	4.00
23	29.98	1539	cis - Calamenene	C ₁₅ H ₂₂	202.34	1.18
24	30.13	1544	1 β H-Presilphiperfolane-9a-ol	C ₁₅ H ₂₆ O	222.37	9.34
25	30.40	1553	α -Cadinene	C ₁₅ H ₂₄	204.36	0.35
26	30.62	1560	α -Calacorene	C ₁₅ H ₂₀	200.32	0.28
27	31.95	1605	Caryophyllene oxide	C ₁₅ H ₂₄ O	220.36	0.33
28	32.34	1619	Carotol	C ₁₅ H ₂₆ O	222.37	0.36
29	32.79	1635	1,10-di-epi-Cubenol	C ₁₅ H ₂₆ O	222.37	0.67
30	33.14	1647	cis-cadin-4-en-7-ol	C ₁₅ H ₂₆ O	222.37	0.73
31	33.29	1652	1-epi-Cubenol	C ₁₅ H ₂₆ O	222.37	0.63
32	33.50	1659	Tau-Cadinol (10-epi- α -Cadinol)	C ₁₅ H ₂₆ O	222.37	1.20
33	33.53	1661	T-Muurolol (epi- α -Muurolol)	C ₁₅ H ₂₆ O	222.37	1.22
34	33.63	1664	α -Muurolol (δ -Cadinol)	C ₁₅ H ₂₆ O	222.37	0.70
35	33.91	1674	α -Cadinol	C ₁₅ H ₂₆ O	222.37	2.70
36	34.01	1677	Intermedeol<neo->	C ₁₅ H ₂₆ O	222.37	1.38
37	34.16	1683	Intermedeol	C ₁₅ H ₂₆ O	222.37	0.48
38	34.55	1696	Amorph-4-en-10 β -ol	C ₁₅ H ₂₆ O	222.37	6.29
39	34.78	1705	Amorph-4-en-10 α -ol	C ₁₅ H ₂₆ O	222.37	2.69
40	35.11	1717	unknown (204, 222, RI 1717)			4.73
			Total			97.34
Total number of constituents						40
Number (%) of constituents identified						92.61%
Number (%) of monoterpene hydrocarbons						1.06%
Number (%) of oxygenated monoterpenes						0%
Number (%) of sesquiterpene hydrocarbons						62.83%
Number (%) of oxygenated sesquiterpenes						28.72%
Number (%) of unidentified compounds						4.73%



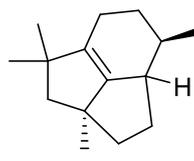
Tricyclene



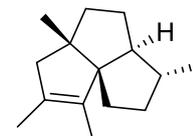
(+)-camphene



Silphiperfol-5-ene



Presilphiperfol-7-ene



Silphiperfol-6-ene

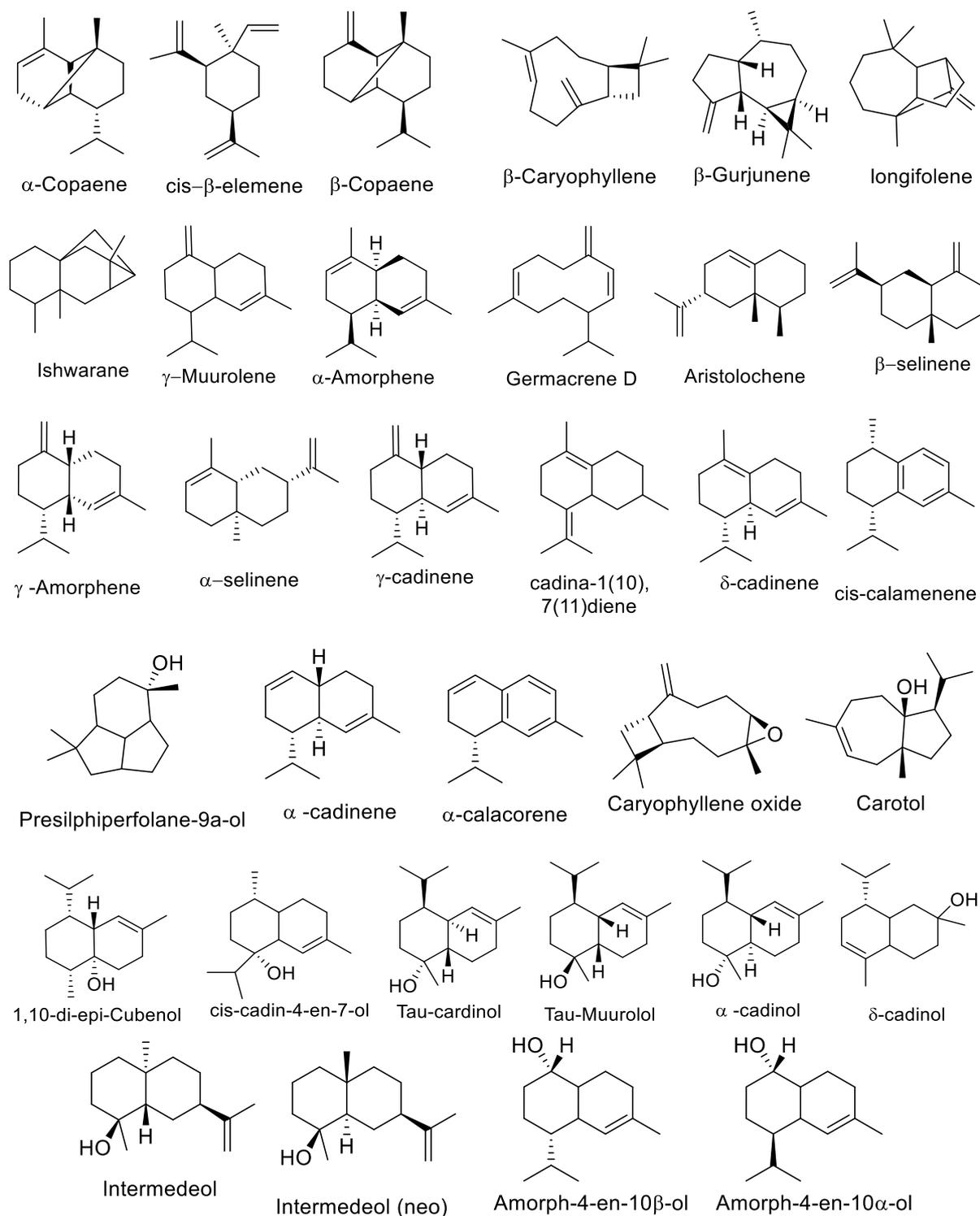


Fig 2: Chemical structures of identified compounds

As shown in Table 1 and Fig. 2, there are at least 40 compounds presented in the essential oil of *O. fruticosus* rhizome. The identification of these compounds was obtained through comparison of their mass spectra and retention indices with those in GC-MS libraries. Almost all components of the oil are terpenes including monoterpenes (hydrocarbon monoterpenes, 2 compounds, 1.06% of the total oil) and sesquiterpenes (hydrocarbon and oxygenated sesquiterpenes, 23 compounds, 62.83% and 14 compounds 28.72% of the total oil, respectively). The main constituent (>10%) of the oil was sesquiterpene compounds with the highest relative amounts including cadina-1(10), 7(11) diene (21, 20.61%), ishwarane (12, 12.04%), longifolene (11, 5.68%), α -selinene

(19, 4.13%), δ -cadinene (22, 4.00%), and cis-calamenene (23, 1.18%) and 1 sesquiterpene oxygenated as 1 β -H-presilphiperfolane-9a-ol (24, 9.34%). The contents of the remaining components were below 5%, most of them (35 compounds) even below 2%.

4. Conclusions

The chemical constituents of essential oil from the rhizome *O. fruticosus* was reported for the first time in the present study. The essential oil of *O. fruticosus* rhizomes obtained in average was 0.99% yield (w/w) to the absolute dry mass of plant materials by steam distillation as a pleasant smelling yellow oil, soluble in alcohol and organic solvents, and

insoluble in water with a specific gravity of 0.875-0.975 g/mL (25 °C) and a refractive index of 1.466 – 1.566 (25 °C). The main constituent (>10 %) of the oil was sesquiterpene compounds with the highest relative amounts including cadinene (10, 7.11%), diene (21, 20.61%), isohumulene (12, 12.04%), longifolene (11, 5.68%), α -selinene (19, 4.13%), δ -cadinene (22, 4.00%), and cis-calamenene (23, 1.18%) and 1 sesquiterpene oxygenated as 1 β -H-presilphiperfolane-9 α -ol (24, 9.34%). The contents of the remaining components were below 5%, most of them (35 compounds) even below 2%. However, the *O. fruticosus* essential oil should be selected for further studies regarding their bioactivity, especially anticancer features.

5. References

1. Averyanov LV, Tanaka N, Nguyen KS, Nguyen HT, Konstantinov EL. New species of *Ophiopogon* Ker Gawl., *Peliosanthes* Andrews and *Tupistra* Ker Gawl. (Asparagaceae) in the flora of Laos and Vietnam. *ADANSONIA* 2015;37(1):25-46.
2. Ministry of Health (MOH), Vietnam Pharmacopoeia V. Medical Publishing House, 2017.
3. Adams RPI ed. Identification of essential oil components by gas chromatography/Mass spectrometry, 4th Edition. Allured Publishing, Carol Stream 6, 2007.