Research Article

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Analysis and Biological Evaluation of Arisaema Amuremse Maxim Essential Oil

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Abstract: The chemical composition and antitumor activity of essential oil were compared for four parts of the Arisaema Amuremse Maxim. Chemical constituent of essential oil in every parts of Arisaema Amuremse Maxim was identified by GC-MS analysis systematically. Arisaema Amuremse Maxim had 114 kinds of essential oils. Tubers, petioles, leaves, and fruits were identified 53, 48, 5 and 21 species essential oil, respectively. The antiproliferative activity against Hep2, HCT-116, A549, SW480, HepG2 cell lines had been investigated by MTT assay. The essential oil of tubers, petioles, leaves and fruits all had strong antiproliferative activity. The IC₅₀ was 19.60 μg·mL⁻¹, 17.60 μg·mL⁻¹, 23.80 μg·mL⁻¹, and 30.23 μg·mL⁻¹, respectively. Among them, the essential oil of tubers and petioles had stronger antitumor activity. The essential oil of tubers, petioles, leaves and fruits had strong antiproliferative activity. This study was of great significance to increase the medicinal parts of Arisaema Amuremse Maxim.

Keywords: Arisaema; Amuremse; Maxim essential oil; MTT.

1 Introduction

Arisaema Amuremse Maxim, also called alias corn and snake corn, is traditional Chinese perennial herb medicine, mainly distributed in Heilongjiang, Jilin and Liaoning Provinces of China, as well as in Korea and Russia [1]. It is bitter in taste, pungent, warm, poisonous, and effects on fight swelling [2-3]. It can also be used to cure the dizziness, epilepsy, anti-tumor, coronary heart disease and inflammation [4-6]. Currently, there are not many reports on Arisaema Amuremse Maxim chemical components, mainly flavone glycosides, sterols, glycosides and brain esters, etc. As far as the essential oil of this plant was rarely studied, which chemical constituent is more complicated [7]. This essay concentrated on chemical constituent of essential oil. First, we used steam distillation to extract the tubers, petioles, leaves, and fruits of Arisaema Amuremse Maxim and obtained the essential oil. Then the essential oil was extracted by diethyl ether, dried with anhydrous sodium sulfate, and analyzed by GC - MS. Finally, the antineoplastic activities of the essential oils were tested by MTT assay [8-14]. The Arisaema Amuremse Maxim was adopted and identified by Professor Chendi of the School of Pharmacy of Jilin University in the Changbai Mountain area of Jilin Province in 2016.

2 Experimental section

2.1 Plant material

Arisaema Amuremse Maxim was collected from the Changbai Mountain in Jilin province and was identified by the School of pharmacy Chendi professor of Jilin University.

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2.2 Extraction and Identification

100 g dry tubers, petioles, leaves, fruits were put in the different essential oil extractor, Then, 1000 ml distilled water were added to extract for 10 hours. After extraction with diethyl ether and drying with anhydrous sodium sulfate, diethyl ether was evaporated to get essential oils which were all the pale yellow oily special aroma matters.

2.3 GC-MS chromatographic conditions

The GC-MS used for the gas chromatographic analysis consisted of a chromatographic column Agilent hp-5 (0.25mm×30m,0.25μm); the injection temperature was 260 °C and transmission line temperature was 280 °C, carrier gas was helium, flow rate was 1.0 mL/min, split ratio was 20:1, sample size was 1 μL. Temperature program: column temperature 80 °C for 3 min, in 10 °C / min up to 150 °C, not reserved, continued to heat in 5 °C / min to 240 °C, not reserved, then in 10 °C / min to 280 °C, keeping 10 min. Mass spectrometer conditions: EI ionization, electron bombardment energy 70 eV, ion source temperature 230 °C, scanning range from 20 to 800 amu; multiplier voltage 2141 V solvent delay 3 min, nist 2008 standard mass spectrometry gallery. Using gas chromatography data processing system, data processing by computer, identified the kinds of chemical composition by NIST 2008 standard mass spectrometry gallery. The relative content of each component in the sample was determined by peak area normalization method.

2.4 The antiproliferative activity against Hep2, HCT-116, A549, SW480, HepG2 cell lines has been investigated by MTT assay.

2.4.1 Cell culture:

DMEM complete medium containing 10% fetal bovine serum, 100 μg·mL⁻¹ penicillin and 100 μg·mL⁻¹ streptomycin. Under 37 °C, 5%CO₂, saturated humidity conditions. The medium was changed every other day and once every 3-4 days.

2.4.2 The tumor cells *in vitro* growth inhibition test (MTT)

Hep2 cells (throat cancer), HCT-116 cells (colon cancer), A 549(lung adenocarcinoma cells), SW480 (colon cancer)

and HepG-2 (HCC) cells were digested by 0.25% trypsin-0.01% EDTA solution, suspending in the culture medium and counting. Adjusting the cell density to 5 ×10⁴ (a ·mL⁻¹), added to 96 - well culture plate (100 μL per well). After 24h, the experimental group added 100 μl different concentration of each part of Arisaema Amuremse Maxim into each well. The concentration gradients of volatile oils in the four parts were 62.5 μg·mL⁻¹, 125 μg·mL⁻¹, 250 μg·mL⁻¹, 500 μg·mL⁻¹ and 1000 μg·mL⁻¹ and the positive control group added the same concentration gradient 5 - fluorouracil. The blank control group, cell control group, ethanol solvent control group were set up. Each group had three wells. They were put in the saturated humidity, 37 °C, 5% CO₂ incubator to continue culturing for 48 h. The cell morphology changes with the inverted microscope were observed

Before the end of the experiment 20 μL of 5mg·mL⁻¹ MTT were added to each well, removed the cell culture medium with a syringe. Then 150 μL DMSO were added into each well, and shaken on the shaking bed with low speed for 10 min to make crystals dissolve completely. The OD_{570nm} absorbance values were measured by an enzyme-linked immune detector.

The following formula was used to calculate the fruit growth inhibition rate (IR) of tumor cells: IR (%) = (1 - (dosing average OD value - blank group average OD value) / (control group average OD value - blank group average OD value) ×100%) and found the half inhibitory concentration (IC₅₀) by the Origin software.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Identification of essential oil

Aristides Amusement Maxim had 114 kinds of essential oil **as shown** in Table 1. The most was located in tubers, up to 53; the second was in petioles, totaling 48; the least was found in leaves, only 5. 2-pentadecanone, ethyl ester and ethyl oleate were more abundant in tubers and petioles. Phytone, hexadecanoic acid methyl ester, methyl oleate, heneicosane were common composition of tubers and fruits. Methyl palmitate and methyl oleate were similar in tubers, leaves, fruits, petioles and Leaves both had 6,10,14-trimethyl-2-pentadecane ketone. Nonadecane was common composition of petioles and fruits.

The systematic research of essential oil in Arisaema Amuremse Maxim in this paper was the foundation for

Table 1: Analysis on chemical constituent of essential oil in every parts of Arisaema Amurense Maxim by GC-MS.

Number	Chemical composition	Tubers	Petioles	Leaves	Fruits
		Relative Contents			
1	Hexanal	0.861			
2	trimethyl Oxazole	0.543			
3	2-Furanmethanol	5.501			
4	2,6-dimethyl Pyrazine	1.562			
5	2,4,5-trimethyl Thiazole	0.171			
6	2,3,5-trimethyl Pyrazine	1.988			
7	Benzeneacetaldehyde	1.235			
8	3-ethyl-2,5-dimethyl Pyrazine	1.317			
9	2,3-Dimethyl-5-ethylpyrazine	0.942			
10	Linalool(3,7-dimethyl-1,6-Octadien-3-ol)	0.516			
11	2-n-Butylfuran	0.491			
12	2-Isopropyl-4,5-dimethyl Thiazole	0.601			
13	2,3-diethyl-5-methyl Pyrazine	0.360			
14	2-ethyl-3-hydroxy-4H-Pyran-4-one	0.598			
15	2,5-dimethyl-3-(2-methylpropyl) Pyrazine	0.209			
16	5-ethyl-2-methyl-4-propyl Thiazole	0.382			
17	4,5-dimethyl-2-(2-methylpropyl) Thiazole	0.587			
18	2,5-dimethyl-3-(3-methylbutyl) Pyrazine	0.398			
19	(R)-1,5,5,9-tetramethyl-,Spiro[5.5]undeca-1,8-diene	0.264			
20	(+)-Epi-bicyclosesquiphellandrene	0.743			
21	2,6-Di(tert-butyl) benzo-1,4-quinone	0.853			
22	[1S-(1 π 4 π 5 π)]-1,8-dimethyl-4-(1-methylethenyl)- Spiro [4.5] dec-7-ene	0.296			
23	2-Tridecanone	1.708			
24	2,6-di-tert-butyl-p-cresol (Butylated Hydroxytoluene)	1.242			
25	2-Dodecanone	0.345			
26	1,3-bis(1,1-dimethylethyl)-2-methoxy-5-methyl Benzene	0.601			
27	(Z, Z)-6, 9-Pentadecadien-1-ol	0.705			
28	(Z, Z)-10,12-Hexadecadienal	0.387			
29	Cyclopentadecanone	2.076			
30	2-Pentadecanone	8.904	0.543		
31	4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)- 2H-Cyclopropa [g] benzofuran	0.621			
32	2-Nonanone	0.562			
33	Perhydrofarnesyl acetone 6,10,14-trimethyl-2-Pentadecanone	7.151			0.388
34	(Z)- 9,17-Octadecadienal	1.433			
35	5-heptenyl Benzene	1.064			

Table 1: Analysis on chemical constituent of essential oil in every parts of *Arisaema Amurense* Maxim by GC-MS.

Number	Chemical composition	Relative Contents			
		Tubers	Petioles	Leaves	Fruits
36	(E)-1-methoxy-9-Octadecene	0.822			
37	2-Heptadecanone	0.922			
38	(E,E)-6,10,14-trimethyl-5,9,13-Pentadecatrien-2-one	1.189			
39	Hexadecanoic acid methyl ester	1.333		38.622	53.452
40	Dibutyl phthalate	1.488			
41	Hexadecanoic acid ethyl ester	3.692			0.405
42	[1R-(1R*,3E,7E, 11R*,12R*)]-4,8,12,15,15-pentamethyl-Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol	1.034			
43	Aromadendrene oxide-(2)	0.598			
44	3-cyclohexyl-1-phenyl Propane	14.859			
45	(Z, Z)-9,12-Octadecadienoic acid methyl ester	1.475			5.402
46	(Z) 9-Octadecenoic acid methyl ester	0.866		12.276	0.117
47	Oxacycloheptadec-8-en-2-one	0.861			
48	9,12-Octadecadienoic acid, ethyl ester	5.619	3.586		
49	(Z, Z, Z)- 9,12,15-Octadecatrenoic acid ethyl ester	1.515			
50	Ethyl Oleate	1.839	0.527		
51	(Z, Z)- 9,12-Octadecadienoyl chloride	0.780			
52	Heneicosane	6.277			0.559
53	Pentacosane	2..601			
54	Tetradecane		0.098		
55	1-(2-nitropropyl)- Cyclohexanol		0.149		
56	1H-Cyclopropa[a]naphthalene		0.067		
57	2,6,10-trimethyl-Tetradecane		0.098		
58	Pentadecane		0.171		
59	Hexadecane		0.770		
60	Heptadecane		2.527		
61	5,6-bis(2,2-dimethylpropylidene)-(E,Z)- Decane		2.805		
62	1-chloro-Octadecane		1.043		
63	Octadecane		1.755		
64	1-Hexadecanol acetate		4.052		
65	6,10,14-trimethyl-2-Pentadecanone		0.997	46.276	
66	1,2-Benzenedicarboxylic-acid bis(2-methylpropyl) ester		1.091		
67	Nonadecane		1.032		0.279
68	14-Methylpentadecanoic acid methyl ester		7.699		
69	7,9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6,9-diene-2,8-dione		7.909		
70	tert-Hexadecanethiol		1.851		

Table 1: Analysis on chemical constituent of essential oil in every parts of Arisaema Amuremse Maxim by GC-MS.

Number	Chemical composition	Tubers	Petioles	Leaves	Fruits
		Relative Contents			
71	1,2-Benzenedicarboxylic acid butyl 8-methylnonyl ester		1.152		
72	Eicosane		1.012		
73	2,4,4,6,6,8,8-Heptamethyl-2-nonene		0.624		
74	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro [2.5] octan-4-one		0.599		
75	Methyl-11,14-eicosadienoate		0.570		
76	8-Octadecenoic acid methyl ester		0.755		
77	5-dodecyldihydro-2(3H)-Furanone		0.470		
78	Hexadecanoic acid butyl ester		2.189		
79	Docosane		0.587		
80	Behenic alcohol		5.025		
81	Heptacosane		4.005		
82	Butyl 9,12-octadecadienoate		0.555		
83	Tetracosane		6.873		
84	Octacosane		4.641		
85	1,2-Benzenedicarboxylic acid diisooctyl ester		0.516		
86	Hexacosane		0.463		
87	Sulfurous acid cyclohexylmethyl pentadecyl ester		0.462		
88	9-octyl-Tetracosane		0.576		
89	Hexatriacontane		6.734		
90	9-octyl-Hexacosane		0.486		
91	1-Hexacosene		0.538		
92	13-dodecyl-Hexacosane		5.129		
93	Nonacosane		0.773		
94	i-Propyl 24-methyl-pentacos-5,9-dienoate		5.109		
95	Triacantane		2.544		
96	Hentriacontane		3.285		
97	Dotriacontane		1.230		
98	Tritriacontane		1.701		
99	1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone			1.602	
100	3,7,11,15-Tetramethyl-2-hexadecen-1-ol			1.224	
101	Methyl-9-Tetradecenoate				0.115
102	Methyl tetradecanoate				1.539
103	Pentadecanoic acid methyl ester				0.095
104	12-methyl Tetradecanoic acid methyl ester				0.111
105	14-methyl-Pentadecanoic acid methyl ester				0.048

Table 1: Analysis on chemical constituent of essential oil in every parts of *Arisaema Amurensis* Maxim by GC-MS.

Number	Chemical composition	Tubers	Petioles	Leaves	Fruits
		Relative Contents			
106	(Z)- 9-Hexadecenoic acid methyl ester				0.915
107	Heptadecanoic acid, methyl ester				0.519
108	9,10,12-trimethoxy Octadecanoic acid methyl ester				1.305
109	9-Octadecenoic acid methyl ester				5.033
110	13-octadecenoate				7.818
111	Octadecanoic acid methyl ester				2.262
112	Ethyl 9-hexadecenoate				0.375
113	Methyl 12-hydroxystearate				3.005
114	Methyl 13-phenyl-tridecanoate				1.031

Table 2: The antiproliferative activity of essential oil.

Parts	IC ₅₀ (μg·mL ⁻¹)					Most Sensitive Cells
	Hep2	HCT-116	A-549	HepG-2	SW-480	
Fruits	50.47±5.26	59.26±8.38	105.00±18.26	40.14±6.12	30.23±4.99	SW-480
Tubers	26.71±3.84	19.60±0.95	27.44±1.23	19.83±1.56	35.46±2.37	HCT-116
Leaves	51.79±4.98	36.75±5.24	52.52±6.32	23.80±2.77	138.10±15.52	HepG-2
Petioles	23.60±1.94	19.52±1.36	20.80±7.64	17.60±1.96	27.72±7.11	HepG-2

further study, which was of great significance to increase the medicinal parts of *Arisaema Amurensis* Maxim.

3.2 The antiproliferative activity was investigated by MTT assay

The essential oil of tubers, petioles, leaves, and fruits was tested against cancer cell lines, including Hep2, HCT-116, A-549, SW-480, HepG-2 for proliferation and survival by MTT assay. In general, the essential oil of *Arisaema Amurensis* Maxim all activated on cancer cells effectively. The essential oil of tubers, petioles, leaves and fruits all had strong antiproliferative activity. Among them, the essential oil of tubers and petioles had stronger antitumor activity. The results were presented in Table 2.

4 Discussion

In the literature, reports were mainly on the study of essential oil of *Arisaema Amurensis* Maxim tubers. In this

paper, the antitumor activity of essential oil in various parts of *Arisaema Amurensis* Maxim (including tubers, petioles, leaves and fruits) was studied and compared for the first time.

Arisaema Amurensis Maxim had 114 kinds of essential oil. There were 24 kinds of essential oil of tubers with ingredients over 1%. The highest was three-cyclohexyl - 1-phenyl propane, occupied 14.859%. There were 25 kinds of essential oil of petiole with ingredients over 1%, the highest was 7,9-two tertiary butyl-1-oxygen screw [4.5]-decane-6, 9-diene-2,8-diketone (7.909%) and 14-methyl pentadecane acid methyl ester (7.699%). The highest in leaves was 6,10,14-trimethyl-2-pentadecane ketone (46.276%). The highest in fruits was methyl palmitate (53.452%). There were 5 kinds of essential oil for leaves with ingredients over 1%, the highest was 6, 10, 14-trimethyl-2-pentadecane ketone (46.276%). There were 9 kinds of essential oil of fruits with ingredients over 1%, the highest was methyl palmitate (53.452%).

The essential oil of tubers, petioles, leaves, and fruits was tested against cancer cell lines, including Hep2, HCT-116, A-549, SW-480, HepG-2 for proliferation and survival

Figure 1: GC-MS chromatograms showed the essential oil in every part of *Arisaema amuremse* Maxim: (a) Fruits, (b) Tubers, (c) leaves, (d) Petioles.

by MTT assay. Among them, the tubers were the most sensitive to HCT-116 cells, and the IC_{50} was 19.60 ± 0.95 .

Petioles was the most sensitive to HepG-2 cells, IC_{50} is 17.60 ± 1.96 ; Fruits were the most sensitive to SW-480 cells, IC_{50} was 30.23 ± 4.99 ; Leaves were most sensitive to HepG-2 cells, IC_{50} was 23.80 ± 2.77 . It can be seen from Table 2 that essential oil of tubers, petioles, leaves, and fruits of *Arisaema Amuremse* Maxim had a good in vitro inhibition over the above four experimental cells. With the increase of the dose, the inhibition of tumor cells gradually increased. Therefore, the four parts of *Arisaema Amuremse* Maxim, including petiole, leaf and fruit, could be used in medicine. The medicinal part of *Arisaema Amuremse* Maxim recorded by the first edition of the 2015 edition of the Chinese Pharmacopoeia was its dried tubers. In addition to the medicinal studies on the tuber parts of *Arisaema Amuremse* Maxim, the petiole, leaf and fruit parts of *Arisaema Amuremse* Maxim were also

studied in this paper. Therefore, this study was of great significance in increasing the effective medicinal parts of *Arisaema Amuremse* Maxim.

5 Conclusions

The essential oil components of the whole plant of *Arisaema Amuremse* Maxim were studied systematically in this paper, which would lay the foundation for further research on its chemical composition and increasing the effective medicinal parts of *Arisaema Amuremse* Maxim.

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