Research Article

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Analysis and Biological Evaluation of Arisaema Amuremse Maxim Essential Oil

https://doi.org/10.1515/chem-2019-0054
received June 11, 2018; accepted September 11, 2018.

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 19.60μg·mL⁻¹, 17.60μg·mL⁻¹, 23.80μg·mL⁻¹, and 30.23μg·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; Amuremese; Maxim essential oil; MTT.

1 Introduction

Arisaema Amuremese Maxim, also called aliased 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 Amuremese 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 Amuremese 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 Amuremese 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 Amuremese Maxim was collected from the Changbai Mountain in Jilin province and was identified by the School of pharmacy Chendi professor of Jilin University.
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℃ and transmission line temperature was 280℃, 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℃ for 3 min, in 10℃/ min up to 150℃, not reserved, continued to heat in 5℃/ min to 240℃, not reserved, then in 10℃/ min to 280℃, keeping 10 min. Mass spectrometer conditions: EI ionization, electron bombardment energy 70 eV, ion source temperature 230℃, scanning range from 20 to 800 aμm; 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℃, 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), A549 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 x10⁴ (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℃, 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 OD₅₇₀nm 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 Amuremese 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 |
|--------|--------------------------------------------------------------------------------------|--------|----------|--------|--------|
| 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-Pyan-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-(1π4π5π]-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-dimethyl)-2-methoxy-5-methyl Benzene                                     | 0.601  |          |        |        |
| 27     | (Z, Z)-6, 9-Pentadecadien-1-o1                                                      | 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  |          |        |        |
| Number | Chemical composition                                                                 | Tubers | Petioles | Leaves | Fruits |
|--------|---------------------------------------------------------------------------------------|--------|----------|--------|--------|
|        | Relative Contents                                                                     |        |          |        |        |
| 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-Octadecatrienoic 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-Cycloprop[a]napththalene                                                           | 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-dimethylpropyliidine)-(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-oxaspir (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 Amurense Maxim by GC-MS.

| Number | Chemical composition                                                                 | Tuber | Petiole | Leaf | Fruit |
|--------|---------------------------------------------------------------------------------------|-------|---------|------|-------|
| 71     | 1,2-Benzenedicarboxylic acid butyl 8-methylnonyl ester                               |       |         |      |       |
| 72     | Eicosane                                                                              |       |         |      |       |
| 73     | 2,4,4,6,6,8,8-Heptamethyl-2-nonene                                                     |       |         |      |       |
| 74     | 5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5] octan-4-one                        |       |         |      |       |
| 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 diisoctyl 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     | Triacontane                                                                           | 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 Pentadecanoic acid methyl est                                                |       |         | 0.111|       |
| 105    | 14-methyl-Pentadecanoic acid methyl est                                                |       |         | 0.048|       |
further study, which was of great significance to increase the medicinal parts of Arisaema Amuremse 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 Amuremse 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 Amuremse Maxim tubers. In this paper, the antitumor activity of essential oil in various parts of Arisaema Amuremse Maxim (including tubers, petioles, leaves and fruits) was studied and compared for the first time.

Arisaema Amuremse 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 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.
by MTT assay. Among them, the tubers were the most sensitive to HCT-116 cells, and the IC50 was 19.60±0.95℃. Petioles was the most sensitive to HepG-2 cells, IC50 is 17.60±1.96; Fruits were the most sensitive to SW-480 cells, IC50 was 30.23±4.99; Leaves were most sensitive to HepG-2 cells, IC50 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.

Acknowledgments: The authors expressed many thanks for the funding and support of Department of Pharmaceutical chemistry, College of Pharmacy, Jilin University, and also grateful for the assistance from Dr. Xiaohong Yang.
Conflict of interest: Authors declare no conflict of interest.

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