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Chemical Constituents Analysis of Petroleum Ether Extract from *Xylaria striata* by GC-MS

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To analyze the chemical constituents of petroleum ether extract of *Xylaria striata*.
Methodology: *Xylaria striata* was extracted by petroleum ether, and then the extract was analyzed by Gas Chromatography-Mass Spectrometer (GC-MS). Identification of compounds was achieved from their GC retention indices (RI) relative to *n*-alkanes and by computer search using libraries of NIST05, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature.
Results: Thirty-seven compounds were separated by gas chromatography. Based on the NIST spectral library and corresponding literature information, thirty compounds which covered 98.43% of the total peaks were identified. Most of them were fatty acids and their esters (77.58%), steroids (19.44%). The methyl linoleate covered 38.23% of the total peaks, while methyl palmitate was 19.12%, methyl linoleate (9-cis, 12-trans) was 5.15%, methyl oleate was 4.43% and methyl stearate was 3.3%.
Conclusion: This is the first report of chemical constituents of the petroleum ether extract of *Xylaria striata* by GC-MS. The result will provide ample information for further exploration and utilization of *Xylaria striata*.

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Keywords: Xylaria striata; petroleum ether extract; GC-MS; chemical constituents.

1. INTRODUCTION

Xylaria Hill ex Schrank, Baier. F1. 1:200(1789), the biggest genus of the family *Xylaria*ceae (Ascomycota), are widely distributed from the temperate zone to the tropic of the earth, and most of this genus are endophytes of vascular plant. There is an increasing number of novel secondary metabolites which possess biological activities relevant for drug discovery have been found in this genus in recent years.

The fungus *Xylaria striata* Pat. 1887 belongs to the family of *Xylaria Hill ex Schrank*. It is mainly growing on decayed barks and lived roots of broad-leaved woodland in summer. To our knowledge, this mushroom has been served as folk medicine and nutrient-dense food in China for long years [1]. Previous studies reported that the *Xylaria* species exhibited multifarious bioactivities [2-5], and a series of these bioactive components had been isolated [6-9]. Our group has been conducting systematic research on fungus *Xylaria striata* since 2013. We have completed and optimized the artificial cultivation methods of this mushroom [10-12]. In addition, the preliminary screening of biological activity has also been accomplished. We concluded that ethanol extract of *Xylaria striata* showed significant antimicrobial and antitumor activity [13-14]. According to the reports in the existing literature, there is no systematic report about chemical constituents of this fungus, so the present investigation was carried out to determine the possible chemical constituents of *Xylaria striata* by GC-MS, which could be a basis for further study of this fungus.

2. MATERIALS AND METHODS

2.1 Materials

Wild fruiting bodies (Fig. 1a) of *Xylaria striata* were collected from the trunk base and stumps of *Sophora japonica* in the town of Qingyi, Mianyang in January 2013. It was identified by one author of this article --- Prof. He, X.S., and the voucher specimen was kept in the Microbiology Laboratory of Southwest University of Science and Technology, Mianyang, Sichuan Province. *Xylaria striata* can easily form fruit bodies (Fig. 1b) directly in the agar culture medium. Using the optimized artificial culture methods [10], the quantities of material harvested in one growth expedition (50 days) of the fungus were approximately 65.91 g (fresh weight) or 20.33 g (dry weight) in each culture bottle (10 cm high, 8 cm diameter). In this article, we use 20 bottles of cultivation fruiting bodies as the experimental materials.

Fresh mushroom materials after removing external materials were dried at 50℃ to a constant weight. The dried materials were pulverized using a grinder and passed through a sieve (mesh size: 1 mm) for further use.

a. Wild fruiting bodies **b. Artificial cultivation fruiting bodies**

Fig. 1. The fruiting bodies of *Xylaria striata*

2.2 Preparation for Extract

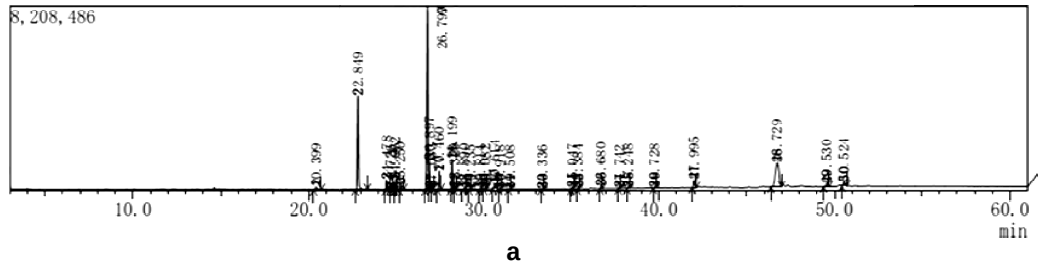
The dried powered *Xylaria striata* (450 g) was extracted with ethanol (5 times, 24 h/per time, and the solid-to-liquid ratio is 1:10). The extract obtained was filtered and concentrated using a rotary evaporator at 50℃. The extract (24.38 g) mixed with ultra-pure water, was successively extracted by petroleum ether, ethyl acetate, and *n*-butanol. The extracts were filtered and finally dried in vacuo evaporator respectively. The petroleum ether dry extract (2.7 g) was stored in a sealed glass bottle filled with N_2 at 4℃ for further use.

2.3 Instruments and Chromatographic Conditions

Before the GC-MS analysis, the petroleum extract were methylated with mixed solution composed of H_2SO_4 (95%): toluene: methanol =1:1:2 (v/v/v) for 12 h at 50℃. In order to obtain phase separation, 3 mL purified water was added and the upper phase was dehydrated by passing through a column filled with anhydrous sodium sulfate. After being filtered through a 0.22 μm filter, the sample was determined by an Aglient 7890A/5975C gas chromatography-mass spectrometry, equipped with a split-splitless injector and Agilent 19091S-433 column (30m×250μm ID, 0.25 μm film thickness). The sample was injected, and the split ratio was 10:1. The injector and detector temperatures were 200℃ and 220℃, respectively. The oven temperature programmed as follows: firstly, held for 2 min at 50℃, then 50～180℃ at 10℃/min, held for 5 min at 180℃, and then 180～280℃ at 5℃/min, held for 5 min, finally, 280～300℃ at 3℃/min for 10min. Helium (99.9%) was used as carrier gas at a flow rate of 1.0 mL/min. The mass detector was set to scan ions between 30-600 m/z using full scan mode and electron impact (EI, 70 eV). A hydrocarbon mixture of *n*-alkanes (C_8-C_{20}) was applied separately on GC-MS using the same chromatographic conditions as above. Identification of compounds was achieved from their GC retention indices (RI) relative to *n*-alkanes and by computer search using libraries of NIST05, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature.

3. RESULTS AND DISCUSSION

GC-MS chromatogram of the petroleum ether extract showed 37 peaks in *Xylaria striata* (Fig. 2), and 30 of them (98.43% of the total peaks) were identified. The fragmentation patterns for some of the peaks were compared with that of the library of compounds. The constituents along with their retention time, percentage area and similarity obtained from the GC-MS analyzer are tabulated in Table 1.



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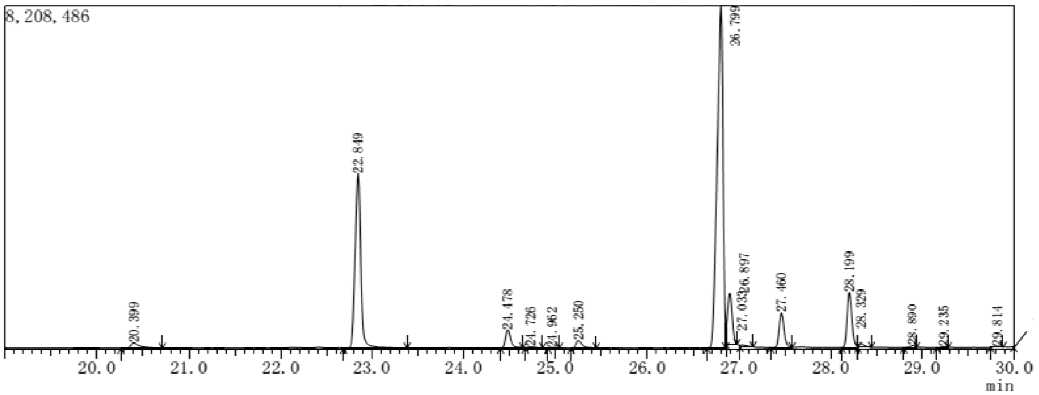

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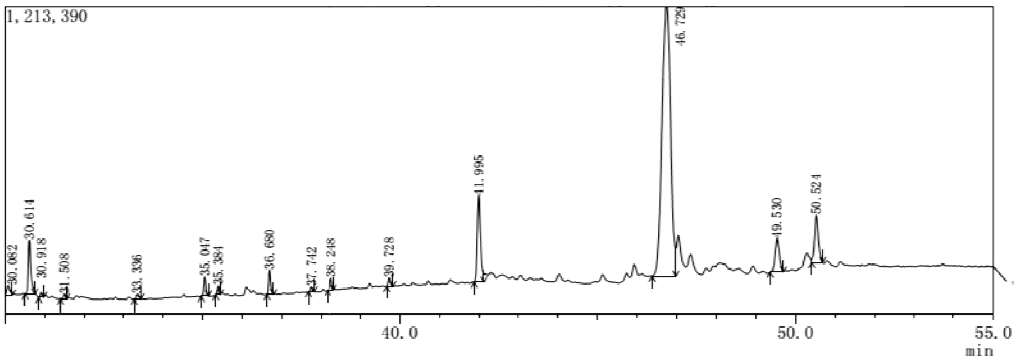

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Fig. 2. GC-MS ion flow chromatograms of petroleum ether extract (a: Total ion chromatogram, b: Enlarge figure of 19-30 min, c: Enlarge figure of 30-55 min)

Table 1. Components analysis of the petroleum ether extract

Number	Retention time (Min)	Area (%)	Molecular formula	Compound name	Similarity (%)
1	20.40	1.05	$C_{15}H_{30}O_2$	Methyl pentadecanoate	93
2	22.85	19.12	$C_{17}H_{34}O_2$	methyl palmitate	97
3	24.48	1.96	$C_{18}H_{36}O_2$	ethyl palmitate	93
4	24.73	0.16	$C_{19}H_{34}O_2$	Cis-methyl linoleate	84
5	24.96	0.05	$C_{18}H_{34}O_2$	2-hexyl-cyclopropane-methyl caprylate	78
6	25.25	0.98	$C_{19}H_{38}O_2$	Cymene-methyl margarate	93
7	26.80	38.23	$C_{19}H_{34}O_2$	Trans-methyl linoleate	95
8	26.90	4.43	$C_{19}H_{36}0_2$	Methyl oleate	93
9	27.03	0.23	$C_{23}H_{40}O_2$	32-carbon triolefinic acid-methyl ester	87
10	27.46	3.30	$C_{19}H38O_2$	Methyl stearate	95
11	28.20	5.15	$C_{19}H_{34}O_2$	(9-cis, 12-trans)-methyl linoleate	92
12	28.33	0.42	$C_{16}H_{30}O$	Cis-9-Hexadecenal	87
13	28.89	0.23	$C_{21}H_{42}O_2$	Ethyl nonadecanoate	86
14	29.24	0.13	$C_{20}H_{38}O_2$	cis-10-Nonadecenoic acid，methyl ester	86
15	29.81	0.17	$C_{19}H_{32}O_2$	(9cis，11trans，13trans)-γ-methyl linolenate	88
16	30.08	0.24	$C_{21}H_{36}O_2$	Cis-5，8，11-Eicosatrienoic acid methyl ester	86
17	30.61	1.03	$C_{19}H_{32}O_2$	(9cis，11cis，13cis)-γ- methyl linolenate	89
18	30.92	0.07	$C_{18}H_{36}0$	1，2-Epoxyoctadecane (EO-18)	81
19	31.51	0.09	$C_{27}H_{54}0_2$	Cerotic acid methyl ester	81
20	33.34	0.09	$C_{22}H_{44}O_2$	Methyl 18-methylicosanoate	82
21	35.05	0.31	$C_{27}H_{54}O_2$	Methyl-pentacosane acid-methyl ester	89
22	35.38	0.14	$C_{24}H_{38}O_4$	Phthalate(2-Ethylhexane)	89
23	36.68	0.39	$C_{22}H_{44}O_2$	Methyl tetracosanoate	86
24	37.74	0.08	$C_{30}H_{61}Br$	1-Bromotridecane	79
25	38.25	0.19	$C_{22}H_{44}O_2$	Tetradecanoic acid，5，9，13-trimethyl-，methyl ester	84
26	39.73	0.15	$C_{30}H_{50}O$	1，6，10，14，18，22-Tetracosahexaen-3-ol，2，6，10，15，19，23-hexamethyl，(all-E)	83
27	42.00	2.16	$C_{35}H_{46}O_2$	9,11-dehydro-ergosterol-benzoate	63
28	46.73	17.06	$C_{28}H_{44}O$	Ergosterin	89
29	49.53	1.07	$C_{30}H_{50}O$	Ergosterol	78
30	50.52	1.31	$C_{28}H_{40}O$	Ergot-sterone	80

Note: Similarity is a score based on mass spectra similarity matching between the compounds from the experimental materials and the NIST spectral library. It's automatically provided by the computer. The larger of the score is, the similarity of the structure is

The main constituents were fatty acids and their esters (77.58%), steroids (19.44%), including methyl linoleate (38.23%), methyl palmitate (19.12%), (9-cis, 12-trans)-methyl linoleate (5.15%), methyl oleate (4.43%), methyl stearate (3.3%), 9,11-dehydro-ergosterol-benzoate (2.16%), ethyl palmitate (1.96%), ergot-sterone (1.13%), lanosterol (1.07%), methyl pentadecanoate (1.05%), γ-methyl linolenate, etc.

3.1 Discussion

Yuan reported that ethanol extract of *Xylaria striata* had good inhibitory activity to plant pathogenic fungi and bacteria [13] as well as the liver cancer cells [14]. The GC-MS analysis found that the petroleum ether extract of *Xylaria striata* contains a high level of linoleic acid methyl ester and methyl palmitate which had strong antibacterial activity [15-16], especially to plant spider mites. Hence, we speculated the abilities of antimicrobial activity to plant pathogenic fungi and bacteria of *Xylaria striata* may come from these two compounds. In addition, many literatures reported that ergosterol and ergotsterone had potent antitumor activity [17-21]. Therefore, in some extent, these two compounds may be the material basis for the antitumor activity of *Xylaria striata*. Besides, Ergosterol is an important raw material in the fields of food and medical industries. For example, it is a major source of fat-soluble vitamin D_2, it also can be used in the production of "cortisone", "hormone progesterone", etc. [21].

Many other components found in GC-MS analysis also have many biological activities. For instance, lanosterol has the value of treatment of cataract [22], the linoleic acid methyl ester can be derivative into linoleic acid or conjugated linoleic acid, which has good antitumor activity and effective prevention to cardiovascular disease [23-27].

4. CONCLUSION

In conclusion, *Xylaria striata* has a good potential to be developed into a kind of bio-environmental protection pesticide and medicine. This study provides a material basis for further exploitation of functional components from *Xylaria striata*, and simultaneously provides a certain theory basis for its utilizations in the fields of food, agriculture, and medicine, etc.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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