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Chemical Constituents Analysis of Ethyl Acetate Extract from MSR-1707 by GC-MS

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Authors' contributions

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

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ABSTRACT

Aims: To analyze the chemical constituents of ethyl acetate extract from MSR-1707 to promote the rational utilization of the mushroom resources.
Methodology: MSR-1707 belongs to the genus *Nigrospora sp.* It was extracted by ethyl acetate, then the extract was analyzed by Gas Chromatography-Mass Spectrometer (GC-MS). Identification of compounds was achieved according to their GC retention indices (RI) and database search using the library of NIST05, as well as a comparison of the fragmentation pattern of the mass spectra with data published in the literature.
Results: Seventy-three compounds were separated by gas chromatography. Based on the NIST05 spectral library and corresponding literature information, fifty-three compounds were identified. Their relative percentage of contents accounted for 95.62% of the outflow peak. Some of the identified peaks are 9-Octadecenoic acid, methyl ester(E)(18.50%),9-Tricosene(Z)(8.30%), 13-Docosenamide (E)(5.26%) and Myristic acid glycidyl ester (3.11%).
Conclusion: This is the first report of chemical constituents of the ethyl acetate extract of *Nigrospora sp.* using GC-MS, which offer some theoretical basis for the further exploration and application of this mushroom.

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Keywords: Nigrospora sp.; ethyl acetate extracts; GC-MS; chemical constituents.

1. INTRODUCTION

In recent years, Fungi have been a research hotspot as they can produce a variety of active substances with potential medicinal and agricultural applications [1,2,3]. According to the statistical data, there are about 1.5 million species of fungi that exist in the world [4], and there is still a sea of species waiting to be researched and discovered [5].

Beginning in 2017, our team conducted a systematic study on mushrooms distributed in the southwest of China. As part of our research project, the various biological activity of large numbers of mushrooms and their endophytic fungi were evaluated and compared. After the preliminary screening, the mycelium's extract of one kind of fungus (Species Code: MSR-1707) which belongs to genus *Nigrospora sp.* exhibited good antioxidant activity.

Nigrospora sp. is a kind of plant endophytic fungus, belonging to the Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Trichosphaeriales, *Nigrospora.* The genus *Nigrospora sp*. has been extensively studied in the literature for the production of a variety of biologically active secondary metabolites [6]. like antiviral anthraquinones and azaphilones[7,8], and phytotoxic lactones [9]. However, the composition of the *Nigrospora sp*. with high antioxidant activity isolated from mushrooms in Sichuan province, west of China has not been reported. Hence, In the present study, the chemical composition of MSR-1707, one kind of genus *Nigrospora sp*. has been studied using gas chromatography-mass spectrometry technology (GC-MS) to analyze its possible chemical components. This research will provide a theoretical basis for the development and application of this fungus as an antioxidant candidate resource.

2. MATERIALS AND METHODS

2.1 Materials

The fungus was collected in Jiangyou, Sichuan Province, China. Its culture and isolation were completed in the Microbiology Laboratory of Southwest University of Science and Technology, Mianyang, Sichuan Province. The pure strain was deposited as MSR-1707 on June 18, 2017. Based on the microbial population identification sequencing (16S/18S/ITS) analysis, MSR-1707 was identified as *Nigrospora sp.* All other chemicals used in the experiment were of analytical grade and were purchased from Chengdu Kelon Chemical Reagent Factory.

2.2 Methods

2.2.1 Preparation for extract

MSR-1707 was inoculated on a PDA plate for activation. After 5～7 days, the activated strain was inoculated in a 500 mL triangle flask containing 300 mL PD medium and cultivated in a constant temperature oscillating incubator at 25℃ for 30 days. The fermentation state was observed and recorded as shown in Fig. 1. The culture was filtered and the filtered mycelium was extracted with ethyl acetate solution (ethyl acetate: H_2O = 5:1). The extraction and suction filtration was repeated several times until the extract solution was close to colorless. The extract solution obtained was filtered and concentrated using a rotary evaporator at 50℃, stored in a sealed glass bottle filled with N_2 untill further use.

2.2.2 Chemical analysis by GC-MS

60mg of the ethyl acetate extract and 2 mL of 5% sulfuric acid-methanol solution were added into a 10 mL test tube with a stopper and left for 60min at 70℃. constant shaking. Then 2 mL of *n*-hexane was added to the methyl ester solution and left to stand for 10 min with constant shaking. After being filtered through a 0.22 filter, the sample was determined by an Agilent 7890A/5975C GC-MS.

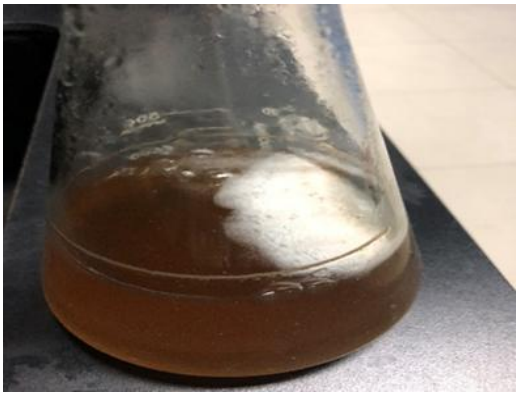
The chromatographic column was 19091S-433 (0.25μm, 30m × 250μm,), the carrier gas was helium at a flow rate of 3 mL/min, injection mode was splitless, injector and detector temperatures were 290℃ and 270℃, respectively. The GC oven temperature program was 40℃ for 1 min, 40~140℃ at 25℃/min, 140~240℃ at 20℃/min, and held 240℃~270℃ at 10℃/min for 10 min.

The mass detector was set to scan ions between 35~500 m/z using full scan mode and electron impact (EI, 70eV), the temperature of interface and ion source were 280℃ and 200℃, respectively.The individual compounds were identified by matching their mass fragmentation pattern with the National Institute of Standard Technology (NIST) Library.

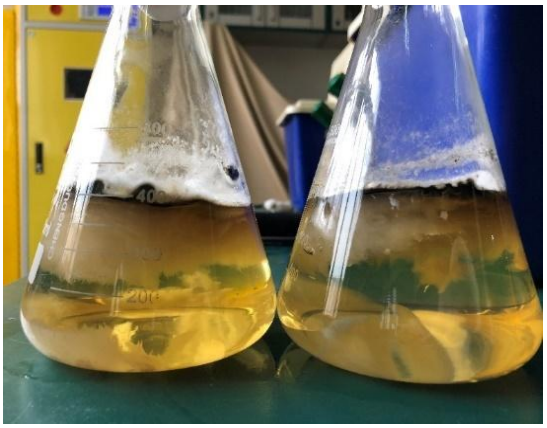
3. RESULTS AND DISCUSSION

In this experiment, GC-MS combined technology was used to analyze the components of the ethyl acetate extract of MSR-1707. The total chromatogram was shown in Fig. 2. The corresponding mass spectra of the 73 chromatographic peaks were automatically searched through the NIST spectral library, and related literature was consulted and compared with the standard spectra. The analysis results were shown in Table 1.

2 days

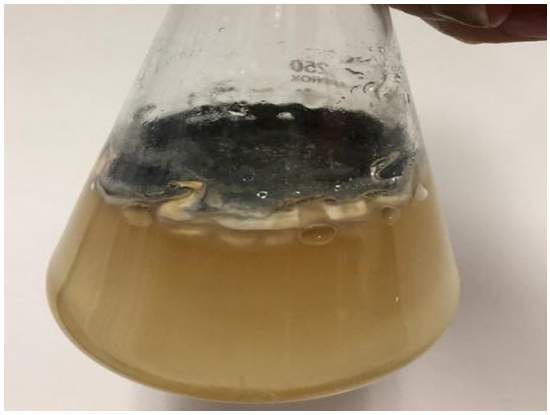


7 days



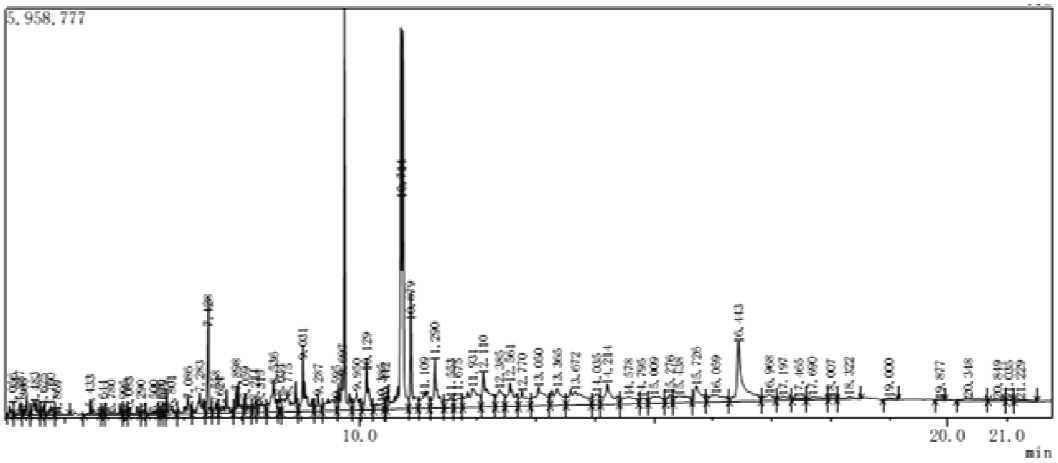
15 days

20 days

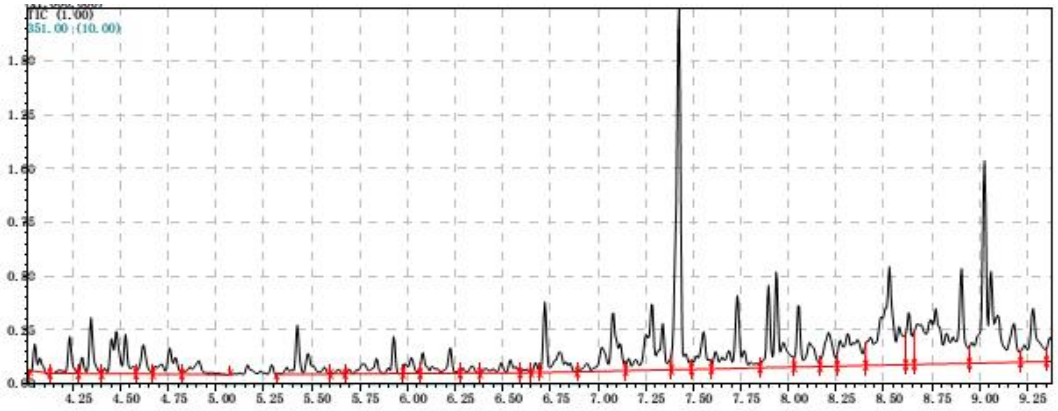


30 days

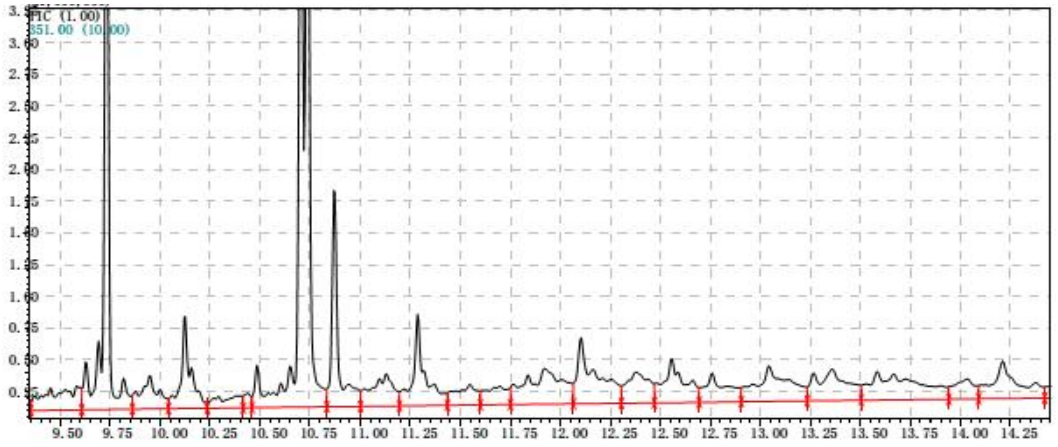
Fig. 1. Fermentation status of MSR-1707 under different culture time



a. Total ion chromatogram



b. Enlarge figure of 0～10 min



c. Enlarge figure of 10～14.5 min

Fig. 2. GC-MS ion flow chromatograms of ethyl acetate extract of MSR-1707

Table 1. Components analysis of the ethyl acetate extract from MSR-1707

Number	Retention time / min	Compound name	Molecular formula	Molecular weight	Area/%	Similarity/%	RI
1	4.05	Heptane, 2,2,4,6,6-pentamethyl	$C_{12}H_{26}$	170	0.18	95	981
2	4.235	Decane, 2,2-dimethyl	$C_{12}H_{26}$	170	0.23	94	1130
3	4.3	Heptane, 4-ethyl-2,2,6,6-tetramethyl	$C_{13}H_{28}$	184	0.39	88	1080
4	4.345	Nonane, 3-methyl-5-propyl	$C_{13}H_{28}$	184	0.52	90	1185
5	4.475	Hexane, 2,2,4-trimethyl	C_9H_{20}	128	0.71	91	767
6	4.62	Dodecane, 4-methyl	$C_{13}H_{28}$	184	0.24	89	1249
7	4.755	Dodecane, 4,6-dimethyl	$C_{14}H_{30}$	198	0.35	93	1285
8	4.785	*n*-Nonaldehyde	$C_9H_{18}O$	142	0.17	90	1104
9	4.91	Naphthalene, 1,2-dihydro	$C_{10}H_{10}$	130	0.2	83	1149
10	5.165	Silane, cyclonhexyldimethoxymethyl	$C_9H_{20}O_2Si$	188	0.08	93	1041
11	5.29	Benzeneacetic acid, methyl ester	$C_9H_{10}O_2$	150	0.67	95	1160
12	5.425	*n*-Dodecane	$C_{12}H_{26}$	170	0.5	96	1214
13	5.51	Undecane, 2,5-dimethyl	$C_{13}H_{28}$	184	1.2	85	1185
14	5.76	1-Decanol, 2-hexyl	$C_{16}H_{34}O$	242	0.42	87	1790
15	5.835	Undecane, 4,4-dimethyl	$C_{13}H_{28}$	184	1.46	90	1229
16	5.895	Hexadecane, 2,6,11,15-tetramethyl	$C_{20}H_{42}$	282	1.44	88	1753
17	5.99	*n*-Eicosane	$C_{20}H_{42}$	282	0.16	86	2009
18	6.02	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	0.25	73	1240
19	6.08	Pentadecane2,6,10-trimethyl	$C_{18}H_{38}$	254	0.34	91	1618
20	6.155	N,N-Dibutylcyanamide	$C_9H_{18}N_2$	154	0.78	74	1210
21	6.54	2-Bromo dodecane	$C_{12}H_{25}Br$	248	0.21	87	1446
22	6.67	1-Tetradecanol	$C_{14}H_{30}O$	214	0.05	93	1656
23	6.72	*n*-Tetradecane	$C_{14}H_{30}$	198	0.64	96	1413
24	7.08	Cycloheptasiloxane, tetradecamethy	$C_{14}H_{42}O_7Si_7$	518	0.88	82	1447
25	7.195	2,5-Cyclohexadiene-1,4-dione	$C_{14}H_{20}O_2$	220	1.29	83	1633
26	7.25	Silane, trichlorooctadecyl	$C_{18}H_{37}Cl_3Si$	386	1.14	79	2249
27	7.34	Heneicosane	$C_{21}H_{44}$	296	1.96	95	2109
28	7.425	ButylatedHydroxytoluene	$C_{15}H_{24}O$	220	2.32	95	1668
29	7.895	1-Hexadecanol	$C_{16}H_{34}O$	242	1.19	96	1854
30	8.05	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	0.62	89	1654
31	8.63	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	0.41	89	1680
32	8.905	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	2.36	82	1860

Number	Retention time / min	Compound name	Molecular formula	Molecular weight	Area/%	Similarity/%	RI
33	9.025	1-Nonadecene	$C_{19}H_{38}$	266	2.68	93	1900
34	9.18	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	0.69	94	1779
35	9.625	9-Hexadecenoic acid, methyl ester, (Z)	$C_{17}H_{32}O_2$	268	1.7	93	1886
36	9.73	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.04	96	1878
37	10.125	9-Tricosene, (Z)	$C_{23}H_{46}$	322	8.3	88	2315
38	10.155	*n*-Tetracosanol	$C_{24}H_{50}$	338	1.46	91	2407
39	10.605	1-Heptacosanol	$C_{27}H_{56}O$	396	1.21	88	2948
40	10.705	9,12-Octadecadienoic	$C_{19}H_{34}O_2$	294	2.86	93	2093
41	10.714	9-Octadecenoic acid	$C_{19}H_{36}O_2$	296	18.5	91	2085
42	10.87	Methyl stearate	$C_{19}H_{38}O_2$	298	3.06	95	2077
43	11.84	1-Tricosanol	$C_{23}H_{48}O$	340	2.99	89	2550
44	12.555	1-Hexacosanol	$C_{26}H_{54}O$	382	2.07	94	2848
45	12.665	2-Methylpentacosane	$C_{26}H_{54}$	366	1.55	80	2542
46	12.76	Phenol, 2,2'-methylenebis [6-(1,1dimethylethyl)-4-methyl	$C_{23}H_{32}O_2$	340	1.41	93	2788
47	13.045	Cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	666	2.59	83	1860
48	13.36	Myristic acid glycidyl ester	$C_{17}H_{32}O_3$	284	3.11	80	1969
49	13.585	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	0.81	90	2475
50	13.67	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	2.22	85	2704
51	14.035	Bumetrizole	$C_{17}H_{18}ClN_{30}$	315	0.94	88	2556
52	15.72	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	1.59	89	2674
53	16.435	13-Docosenamide, (Z)	$C_{22}H_{43}NO$	337	5.26	93	2625

As shown in Table 1, fifty-three compounds were identified by GC-MS, their relative percentage accounted for 95.62% of the outflow peak. Most of them were 9-Octadecenoic acid, methyl ester(E)(18.50%), 9-Tricosene (Z)(8.30%), 13-Docosenamide (Z)(5.26%) and Myristic acid glycidyl ester (3.11%), Methyl stearate (3.06%), 1-Tricosanol (2.99%), 9-Octadecenoic (2.86%) and 1-Nonadecene (2.68%).

More and more therapeutic drugs have been isolated from fungi, showing a good therapeutic effect in antitumor [10], antioxidant [11], and anti-inflammatory [12] field. The fungus isolated from soursop leaf has the potential to be used as a source of anticancer agents [13]. Besides, the antimicrobial potential of fungi isolated from geranium roots was also reported [14]. In the present study, the above identified compounds mostly showed a variety of biological activities. 9-Octadecenoic acid, 9-Tricosene, 13-Docosenamide, and 9-Octadecenoic have good antibacterial activity [15-17]. Besides, Myristic acid glycidyl ester and 1-Nonadecene, etc., have the antioxidant capacity [18,19]. Other compounds also show anti-inflammatory [20], nociception [21], immunomodulatory activity [22], and other abilities. The presence of these active compounds was of great significance for the development of this fungus.

4. CONCLUSION

In conclusion, the bioactive compounds of MSR-1707 .appeared to have potential as a useful drug source, due to the presence of various compounds that are essential for health. This experiment uses GC-MS technology, which has the advantages of simple operation, less solvent, fast analysis, and good effect, etc. This study provided the basis for further developing the functional components of genus *Nigrospora sp.*, and also provides a theoretical basis for the application of this fungus in agriculture, medicine, and other fields.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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