**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-Docosanamide (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.
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°C 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: H2O = 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°C, stored in a sealed glass bottle filled with N2 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°C. 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°C and 270°C, respectively. The GC oven temperature program was 40°C for 1 min, 40~140°C at 25°C/min, 140~240°C at 20°C/min, and held 240°C~270°C at 10°C/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°C and 200°C, respectively. The individual compounds were identified by matching their mass fragmentation pattern with the National Institute of Standard Technology (NIST) Library.

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

Fig. 1. Fermentation status of MSR-1707 under different culture time
Fig. 2. GC-MS ion flow chromatograms of ethyl acetate extract of MSR-1707

a. Total ion chromatogram

b. Enlarge figure of 0~10 min

c. Enlarge figure of 10~14.5 min
| 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_{9}H_{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_{9}H_{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, cyclohexylidimethoxymethyl | C_{9}H_{20}O_{2}Si | 188 | 0.08  | 93          | 1041 |
| 11     | 5.29                | Benzeneacetic acid, methyl ester | C_{9}H_{10}O | 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_{30}O_{6}Si_{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_{9}H_{18}N_{2} | 154 | 0.78  | 74          | 1210 |
| 21     | 6.54                | 2-Bromo dodecane | C_{12}H_{26}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_{40}O_{7}Si_{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, trichloroacetaldehyde | C_{18}H_{27}Cl_{3}Si | 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_{20}O | 220              | 2.32  | 95          | 1668 |
| 29     | 7.895               | 1-Hexadecanol | C_{16}H_{32}O | 242              | 1.19  | 96          | 1854 |
| 30     | 8.05                | Cyclooctasiloxane, hexadecamethyl | C_{16}H_{40}O_{8}Si_{8} | 592 | 0.62  | 89          | 1654 |
| 31     | 8.63                | Methyl tetradecanoate | C_{15}H_{20}O_{2} | 242 | 0.41  | 89          | 1680 |
| 32     | 8.905               | Cyclononasiloxane, octadecamethyl | C_{18}H_{30}O_{3}Si_{9} | 666 | 2.36  | 82          | 1860 |

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   |
|--------|---------------------|----------------------------------------------------|-------------------|-----------------|-------|--------------|------|
| 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_{32}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_{50}O     | 396             | 1.21  | 88           | 2948 |
| 40     | 10.705              | 9,12-Octadecadienoic acid                         | C_{19}H_{34}O_{2} | 294             | 2.86  | 93           | 2093 |
| 41     | 10.714              | 9-Octadecenoic acid                               | C_{19}H_{34}O_{2} | 296             | 18.5  | 91           | 2085 |
| 42     | 10.87               | Methyl stearate                                    | C_{19}H_{36}O_{2} | 298             | 3.06  | 95           | 2077 |
| 43     | 11.84               | 1-Tricosanol                                       | C_{23}H_{46}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_{30}O_{2} | 340             | 1.41  | 93           | 2788 |
| 47     | 13.045              | Cyclononasiloxane                                  | C_{18}H_{32}O_{9}Si_{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_{30}O_{4} | 390             | 2.22  | 85           | 2704 |
| 51     | 14.035              | Bumetrizole                                        | C_{17}H_{19}ClN_{30} | 315             | 0.94  | 88           | 2556 |
| 52     | 15.72               | Tetracosanoic acid, methyl ester                   | C_{26}H_{50}O_{2} | 382             | 1.59  | 89           | 2674 |
| 53     | 16.435              | 13-Docosenamide, (Z)                              | C_{22}H_{46}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 therapeutical effect in anti-tumor [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.

ACKNOWLEDGEMENTS

The work was supported by the Doctor Foundation of Southwest University of Science and Technology(16xz7161), Innovative Training Program for National College students (201710619023), Undergraduate Students Innovation Training Program of Sichuan Province (20xcy078). Student Innovation Fund Program of Southwest University of Science and Technology (jz19-062).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Parthasarathy R, Chandrika M, Yashavantha Rao HC et al. Molecular profiling of marine endophytic fungi from green algae: Assessment of antibacterial and anticancer activities. Process Biochemistry. 2020;96:11-20.
2. Huang L-Q, Niu Y-C, Su Let al. The potential of endophytic fungi isolated from cucurbit plants for biocontrol of soilborne fungal diseases of cucumber. Microbiological Research. 2020;231:126369.
3. Fouda AH, Hassan SED, Eid AM, EwaisEED. Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss.). Annals of Agricultural Sciences, 2015;60(1):95-104.
4. Hawksworth DL. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological research.2001;105(12):1422-1432.
5. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews. 2003;67:491-17.
6. Metwaly AM, Kadry HA, El-Hela AAet al. Nigrosphaerin A a new isochromene derivative from the endophytic fungus Nigrospora sphaerica. Phytochemistry Letters. 2014;7:1-5.
7. Zhang S-P, Huang R, Li F-F et al. Antiviral anthraquinones and azaphilones produced by an endophytic fungus Nigrospora sp. from Aconitum carmichaeli. Fitoterapia. 2016;112:85-89.
8. He J-W, Chen G-D, Gao Het al. Heptaketides with antiviral activity from three endolichenic fungal strains Nigrospora sp., Alternaria sp. and Phialophora sp. Fitoterapia. 2012;83:1087-1091.
9. Fukushima T, Tanaka M, Gohbara M, Fujimori T. Phytotoxicity of three lactones from Nigrospora sacchari. Phytochemistry. 1998;48:625-630.
10. Jong S-C, Donovick R. Antitumor and Antiviral Substances from Fungi. In: Advances in Applied Microbiology. Edited by: Neidleman SL. Academic Press. 1989;183-262.
11. Yang J, Huang Y, Xu H et al. Optimization of fungi co-fermentation for improving anthraquinone contents and antioxidant activity using artificial neural networks. Food Chemistry. 2020;313:126138.

12. Cheng J-J, Chao C-H, Chang P-C, Lu M-K. Studies on anti-inflammatory activity of sulfated polysaccharides from cultivated fungi Antrodia cinnamomea. Food Hydrocolloids. 2016;53:37-45.

13. Minarni, Artika IM, Julistiono Het al. Anticancer activity test of ethyl acetate extract of endophytic fungi isolated from sour sop leaf (Annona muricata L.). Asian Pacific Journal of Tropical Medicine. 2017;10:566-571.

14. Abobaker Z, Viljoen A, Chen W et al. Endophytic fungi isolated from Pelargonium sidoides DC: Antimicrobial interaction and isolation of a bioactive compound. South African Journal of Botany. 2019;122:535-542.

15. Sohn H-R, Baek K-Y, Hou CT, Kim H-R. Antibacterial activity of 7,10-dihydroxy-8(E)-octadecenoic acid against food-borne pathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2013;2:85-87.

16. Masui H, Kondo T, Kojima M. An antifungal compound, 9,12, 13-trihydroxy-(E)-10-octadecenoic acid, from Colocasia antiquorum inoculated with Ceratocystis fimbriata. Phytochemistry. 1989;28:2613-2615.

17. Garg M, Priyanka, Chatterjee M. Isolation, characterization and antibacterial effect of biosurfactant from Candida parapsilosis. Biotechnology Reports. 2018;18:e00251.

18. Prasath KG, Sethupathy S, Pandian SK. Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in Candida albicans. Journal of Proteomics. 2019;208:103503.

19. Yi F, Sun J, Bao X et al. Influence of molecular distillation on antioxidant and antimicrobial activities of rose essential oils. LWT. 2019;102:310-316.

20. Mužáková V, Meloun M, Jindrová A, Čegan A. The effect of fatty acids in red blood cell membranes on the dynamics of inflammatory markers following the coronary stent implantation. Journal of Pharmaceutical and Biomedical Analysis. 2019;166:310-325.

21. Ferdous A, Janta RA, Arpa RN et al. The leaves of Bougainvillea spectabilis suppressed inflammation and nociception in vivo through the modulation of glutamatergic, cGMP, and ATP-sensitive K+ channel pathways. Journal of Ethnopharmacology. 2020;261:113148.

22. Bergamo P, Luongo D, Miyamoto J et al. Immunomodulatory activity of a gut microbial metabolite of dietary linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, associated with improved antioxidant/detoxifying defences. Journal of Functional Foods. 2014;11:192-202.