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Research Article

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Characterization of volatile constituents from an endophytic *Aspergillus fumigatus* strain

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ABSTRACT

An endophytic isolate of Aspergillus fumigatus was obtained from the healthy stem of Cynodon dactylon. The fungus was identified on the basis of its morphology and aspects of its molecular biology. Volatile constituents were characterized from this fungus and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). As a result, a total of twenty-two compounds represented all of the ligarine extract were characterized. Higher amounts of oil-based straight-chained alkyl ethyl esters and fatty acids were found to compose major volatile chemotype accounted for 42.44% and 35.05% of this organism, respectively. The main components was demonstrated to be 9,12-octadecadienoic acid, ethyl ester (16.96%); linoleic acid (16.48%); ethyl oleate (15.14%); n-hexadecanoic acid (9.13%); hexadecanoic acid, ethyl ester (7.80%). Some pharmaceutical components were discovered such as linoleic acid, linoleic acid ethyl ester, α- and γ-tocopherol, which could potentially be used in the field of medicine. Antioxidant activity of the extract was assessed by the free radical scavenging (DPPH).

Keywords: *Aspergillus fumigatus*; volatile constituents; GC-MS analysis; chemical composition

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INTRODUCTION

Plant-associated endophytes represent a largely untapped source of small-molecule natural products, some with chemical structures that have been optimized by coevolution for biological and ecological relevance [1]. According to Hawksworth el al., more than 1.5 million endophytic fungi are now thought to live within 270000 species of vascular plants, however, the number of species described is only in the range of 70000–100000, the prospects for additional discoveries of interesting fungal metabolites are bright [2,3]. Many fungal species are known to emit volatile bioactive substances utilized for the healthcare purpose of humankind, especially ones that have distinctive odours, and this has prompted appropriate chemical analyses of the fungal volatile constituents [4].

The genus *Aspergillus* of the family *Moniliaceae* is a diverse genus with about 180 recognized species, has been proven to be a rich source of bioactive metabolites. The species *A. fumigatus* is one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency [5]. Several studies has already been conducted and it was reported that 226 compounds have been isolated from the fungus during the last two decade [6]. A number of indolic alkaloids with antimitotic properties were discovered [7]. The compounds of interest have been of a class known as tryprostatins, with spirotryprostatin B being of special interest as an anticancer drug. *A. fumigatus*

grown on certain building materials can produce genotoxic and cytotoxic mycotoxins, such as gliotoxin [8].

Previous chemical investigations of *A. fumigatus* in our laboratory led to the discovery of several new natural products such as aperfumoid, aperfumin, deacetylfumigaclavine C and 9-deacetoxyfumigaclavine C [9, 10]. To the best of our knowledge, only nonvolatile constituents have been previously identified from this fungus. The characteristic odor principle has not yet been investigated. In the current paper, we report the first time the results of a study aimed to define the volatile constituents from hexane extract from *Cynodon dactylon* endophytic *A. fumigatus*. Antioxidant activity of the extract was assessed by the free radical scavenging (DPPH). The findings from this work may add to the overall value of the airborne saprophytic fungi *A. fumigatus*.

EXPERIMENTAL SECTION

Isolation and identification of the fungus

The title strain of *A. fumigatus* (strain no. CY018) was isolated from the healthy stem of *C. dactylon* collected in November 2001 from Yancheng Biosphere Reserve, Jiangsu Province. The collected plants were authenticated by Prof. L. X. Zhang (Nanjing University), with a voucher specimen preserved in the herbarium of Nanjing University. The strain was identified by Dr. Y. C. Song. A voucher specimen is deposited in our laboratory at -80 ° C. The working strain was preserved on potato dextrose agar slants containing 10% NaCl and stored at 4 ° C.

Fermentation and Extraction

The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 5 days. The agar plugs were inoculated into Erlenmeyer flasks (1000 mL) each containing 300 mL of PDA medium. After 4 days, the seed culture was transferred into 1 L flasks, each preloaded with the Czapack medium containing sucrose (30.0 g/L), $NaNO_3$ (3.0 g/L), KCl (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $FeSO_4$ (0.01 g/L), K_2HPO_4 (1.0 g/L), and yeast extract (1.0 g/L). The culture was grown for 14 days at 28 ° C. The harvested culture (100 L) was extracted at room temperature with EtOAc (3 × 100 L). The mycelia and PDA medium were extracted with ethylacetate. The extract was further extracted with ligarine to get the volatile metabolites and yield 1.2 g residue.

Gas chromatography (GC) analysis

Ligarine extracts obtained from *A. fumigatus* was analyzed using Hewlett Packard 6890 GC equipped with a FID detector and HP-FFAP ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm). GC oven temperature was kept at 60 °C for 3 min initially, and then raised at the rate of 3 °C/min to 250 °C. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted samples 1/1000 in n-pentane, v/v) of 1.0 μL were injected manually and in the splitless mode. Peaks area percents were used for obtaining quantitative data.

Gas chromatography/mass spectrometry (GC/MS) analysis

The analysis of the ligarine extract was performed under the same conditions with GC, using a Hewlett Packard 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass selective detector in the electron impact mode (70 eV). Identification of the components was based on comparisons of their relative retention times and mass spectra with those obtained from standards and/or the NIST98 and Wiley275 library data.

Free radical-scavenging activity

The radical scavenging ability (RSA) of the hexane extract of *Pestalotiopsis* JCM2A4 was estimated by using 2,2'-diphenyl-b-picrylhydrazyl (DPPH) method described previously [11-15]. Thus, an aliquot of EO solution 1 mL was added to 3 mL of ethanolic DPPH (60 μM). The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark and absorbance was measured at 517 nm. The free radical scavenging activity was calculated as follows:

$$\%RSA = [(A_{blank} - A_{sample} / A_{blank}] \times 100\%$$

where A_{blank} was the absorbance of the control reaction(containing all reagents except the test compound), and A_{sample} was the absorbance of the test compound.

RESULTS AND DISCUSSION

The analysis of the ligarine extract from *C. dactylon* endophytic *A. fumigatus* was simultaneously performed using gas chromatography–mass spectrometry (GC–MS). The detected volatile components of the fungus and its relative percentages according to their relative retention indices (RI) are given in Table 1. Twenty-two components were identified representing all of the extract. 9,12-Octadecadienoic acid, ethyl ester (16.96%); linoleic acid (16.48%); ethyl oleate (15.14%); *n*-hexadecanoic acid (9.13%); hexadecanoic acid, ethyl ester (7.80%) were the main constituents and totally comprising 65.51% of the extract. In addition, high amounts of oil-based straight-chained alkyl ethyl esters and fatty acids were found to compose a major chemotype of the extract, such as tetradecanoic acid, ethyl ester; hexadecanoic acid, ethyl ester; tetradecanoic acid, ethyl ester; ethyl 9-hexadecenoate; ethyl oleate; 9,12-octadecadienoic acid, ethyl ester; 9,12,15-octadecatrienoic acid, ethyl ester; and linoleic acid ethyl ester, accounted for 42.44% of this organism; *n*-hexadecanoic acid; hexadecenoic acid, Z-11-; heptadecanoic acid; 14-pentadecenoic acid; octadecanoic acid; and linoleic acid accounted for 35.05% of this organism, respectively. Simultaneously, some pharmaceutical components were discovered. It is noteworthy to point out that unsaturated fatty acids are available to release the oxidative stress, especially of the blood vessels and nerves and to keep the skin and other tissues youthful and supple trough their lubricating quality [13]. Linoleic acid and its ethyl ester are being sold as a panacea that has the capability of reducing or eliminating cancer, preventing heart disease, improving immune function, and altering body composition to treat obesity or build lean body mass [14, 15]. α-Tocopherol and its analogue γ-tocopherol are different forms of methylated phenol vitamin E, both featuring a chromanol ring, with a hydroxyl group that can donate a hydrogen atom to reduce free radicals and a hydrophobic side chain which allows for penetration into biological membranes. Antioxidant function is reported to be its most important biological function [16]. Other functions include enzymatic activities, gene expression, and neurological function are also reported by many researchers [17, 18]. The above finding prompted us to investigate the antioxidant activity of our volatile constituents against DPPH radical. However, the extract proved to be devoid of significant activity at 400μg/mL in the bioassays used. Further in-depth studies are needed on evaluation of the nonvolatile chemical composition of the fungal extracts with the aim of separation and structure elucidation of their active components separately.

Table 1. Volatile constituents identified from *A. fumigatus* by GC-MS

No.	R.I. [a]	MF [b]	Components	Composition (%)
1	10.91	$C_{16}H_{32}O_2$	Tetradecanoic acid, ethyl ester	0.14
2	11.53	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	1.84
3	12.01	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester	7.80
4	12.60	$C_{18}H_{36}O_2$	Heptadecanoic acid, methyl ester	0.16
5	12.98	$C_{16}H_{32}O_2$	Tetradecanoic acid, ethyl ester	1.02
6	13.12	$C_{18}H_{34}O_2$	Ethyl 9-hexadecenoate	0.40
7	13.74	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester	3.47
8	14.21	$C_{20}H_{38}O_2$	Ethyl Oleate	15.14
9	14.75	$C_{20}H_{36}O_2$	9,12-Octadecadienoic acid, ethyl ester	16.96
10	15.08	$C_{20}H_{34}O_2$	9,12,15-Octadecatrienoic acid, ethyl ester	0.78
11	15.91	$C_{16}H_{22}O_4$	Dibutyl phthalate	1.79
12	16.47	$C_{20}H_{36}O_2$	Linoleic acid ethyl ester	0.20
13	17.78	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	9.13
14	17.99	$C_{16}H_{30}O_2$	Hexadecenoic acid, *Z*-11-	1.23
15	18.61	$C_{18}H_{36}O_2$	Heptadecanoic acid	1.22
16	18.91	$C_{15}H_{28}O_2$	14-Pentadecenoic acid	1.10
17	19.58	$C_{18}H_{36}O_2$	Octadecanoic acid	4.67
18	19.79	$C_{57}H_{104}O_6$	9-Octadecenoic acid, (*E*)-	12.11
19	20.30	$C_{18}H_{32}O_2$	Linoleic acid	16.48
20	20.80	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid	1.57
21	21.78	$C_{29}H_{50}O_2$	α-tocopherol	1.45
22	23.14	$C_{30}H_{50}O_3$	γ-tocopherol	1.35

[a] R.I. = Relative retention indices on HP-5 ms column in reference to n-alkanes.
[b] MF = Molecular formula of the Component

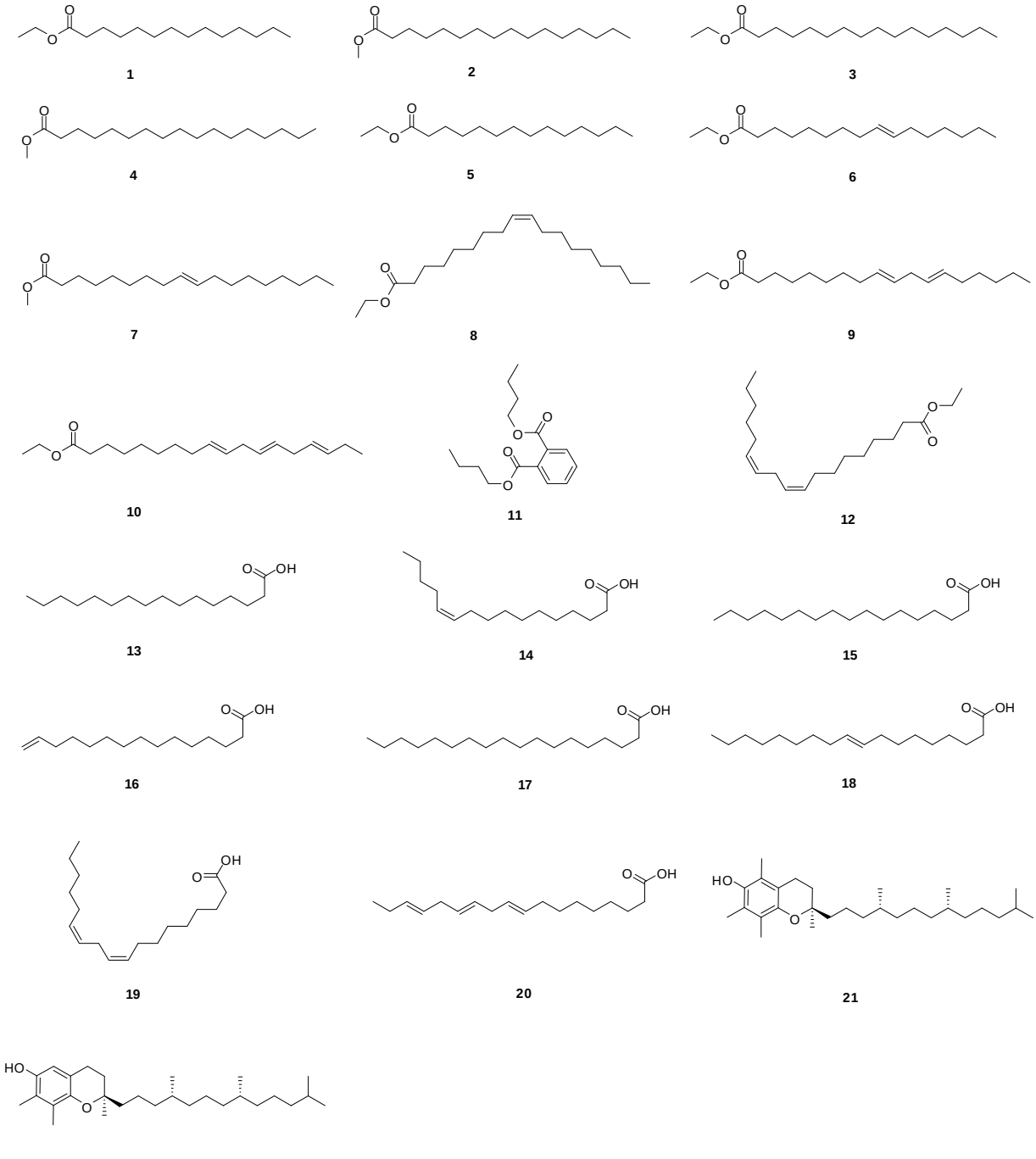

Figure 1. GC–MS profile of volatile constituents of ligarine extract of *A. fumigatus*

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REFERENCES

[1] PA Paranagama; EMK Wijeratne; AAL Gunatilaka, *J. Nat. Prod.*, **2007**, 70, 1939–1945.

[2] DC Hawksworth; AY Rossman, *Phytopathology*, **1987**, 87, 888–891.
[3] S. F. Brady, M. M. Wagenaar, M. P. Singh, J. E. Janso, J. Clardy. *Org. Lett*., **2000**, 2, 4043–4046.
[4] G. A. Strobel, E. Dirkse, J. Sears and C. Markworth. *Microbiology*, **2001**, 147, 2943–2950.
[5] J. P. Latgé. Clin. *Microbiol. Rev.*, **1999**, 12, 310–350.
[6] C. F. Jens, R. Christian, F. N. Kristian, O. L. Thomas, *Med. Mycol.*, **2008**, S1–S19.
[7] C. B. Cui, H. Kakeya, H. Osada. *J. Antibiot.*, **1996**, 49, 832–835.
[8] S. M. Nieminen, R. Kärki, S. Auriola, M. Toivola, H. Laatsch, R. Laatikainen, A. Hyvärinen and A. Wright. *Appl. Environ. Microbiol.*, **2002**, 68, 4871–4875.
[9] J. Y. Liu, Y. C. Song, Z. Zhang, L. Wang, Z. J. Guo, W. X. Zou, R. X. Tan, *J. Biotechnol*., **2004**, 114, 279–287.
[10] H. M. Ge, Z. G. Yu, J. Zhang, J. H. Wu, R. X. Tan. J. Nat. Prod., **2009**, 72, 753–755.
[11] D. H. Li, Z. Y. Liang, M. F. Guo, J. Zhou, X. B. Yang, J. Xu. *Afri. J. Biotechnol.*, **2012**, 11, 4513–4517.
[12] M Chatatikun1; A Chiabchalard, *J. Chem. Pharm. Res*., **2013**, 5(4), 97-102.
[13] TB Shyma; AG Deviprasad; MP Raghavendra, *J. Chem. Pharm. Res*., **2012**, 4(10), 4501-4505
[14] SG. Alsabri; HM El-Basir; NB Rmeli; SB Mohamed, AA Allafi, AA Zetrini, AA Salem, SS Mohamed, AGbaj; MM El-Baseir, *J. Chem. Pharm. Res*., **2013**, 5(1):32-36.
[15] A Malik; A Kushnoor; V Saini; S Singhal; S Kumar; YC Yadav, *J. Chem. Pharm. Res.*, **2011**, 3(3):659-665
[16] S. Kalmijn, E. J. M. Feskens, L. J. Launer, *Am. J. Epidemiol.*, **1997**, 145, 33–41.
[17] L. D. Whigham, M. E. Cook and R. L. *Atkinson: Pharmacol. Res.*, **2000**, 42, 503-510.
[18] T. Sudha, S. Chidambarampillai and V. R. Mohan. *J. Curr. Chem. Pharm. Sc.*, **2013**, 3, 113-122.
[19] E. F. Bell. *Am. J. Clin. Nutr*., **1987**, 46, 183–186.
[20] Azzi. *Free Radical Bio. Med.*, **2007**, 43, 16–21.
[21] Zingg; Azzi, A. *Curr. Med. Chem.*, **2004**, 11, 1113–1133.