NPC Natural Product Communications

2014
Vol. 9
No. 6
863 - 864

Chemical Composition and Antimicrobial Activity of the Essential Oil from *Allium hookeri* Consumed in Xishuangbanna, Southwest China

Ren Li[a, b], Yuan-Fei Wang[c], Qian Sun[c] and Hua-Bin Hu[a, *]

[a]*Key Laboratory of Tropical Plant Resource and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, PR China*

[b]*University of Chinese Academy of Sciences, Beijing 100049, PR China*

[c]*Yunnan Traditional Chinese Medical College, Kunming 650500, PR China*

huhb@xtbg.ac.cn (H.-B. Hu)

Received: February 10th, 2014; Accepted: March 24th, 2014

The essential oil from the rhizomes of *Allium hookeri* Thwaites, obtained by simultaneous distillation extraction, was analyzed by GC-MS. Di-2-propenyl trisulfide (31.8%), diallyl disulfide (28.4%), and dipropyl trisulfide (8.4%) were the main constituents among the thirteen identified components. The essential oil showed strong activity against *Aspergillus fumigatus* and *Candida albicans* with MIC (the minimal inhibitory concentration) and MBC (minimal bactericidal concentration) values ranging from 32−64 μg/mL. These results suggest that the essential oil from the rhizomes of *A. hookeri* could be used as a potential antimicrobial ingredient in the food industry.

Keywords: *Allium hookeri*, Essential oil, Chemical composition, Antimicrobial, Xishuangbanna.

Ethnobotanical studies showed that the rhizome of wide chive (*Allium hookeri* Thwaites), as well as the leaf of Chinese chive (*A. tuberosum* Rottler) has been consumed as a daily vegetable and spice by the local people in Xishuangbanna, Southwest China. One difference between the two chives is that the leaf breadth of wide chive (5-28 mm) is greater than that of Chinese chive (1.5-8 mm) [1a]. Use of wide chive is more popular for the reason that its fleshy rhizome is longer and thicker than that of Chinese chive. *Allium* species have been used in traditional medicine for the prevention of infection, coronary thrombosis and atherosclerosis [1b, 1c]. The essential oils of *Allium* species, such as Chinese chive and garlic, have been reported to have antimicrobial activities [2a, 2b] and to be rich in disulfide and trisulfide compounds [3a, 3b]. However, to the best of our knowledge, there has been no study on the chemical composition and bioactivities of the essential oil from the rhizome of *A. hookeri* (EOA). We identified the main components of EOA by GC/MS, and investigated its antimicrobial activity by the disk diffusion method. In addition, this study also provides scientific information for further exploration and applications of this plant.

The chemical components of the EOA are listed in Table 1, in order of retention time. The sample yielded 0.1%, w/w, of a smelly yellow oil. The identified compounds made up 83.1% of the oil. The major constituents, which were also found in previous studies of the chemical composition of the essential oils from *Allium* species [4a, 4b], were 2-propenyl trisulfide (31.8%), diallyl disulfide (28.4%), and dipropyl trisulfide (8.4%).

As shown in Table 2, the EOA had an effect on seven out of the eight tested microbial strains. Compared with other *Allium* species, for example onion, the EOA exhibited ether better or similar antimicrobial activities [2b]. For instance, the EOA was extremely active against the tested fungi, such as *Aspergillus fumigatus* and *Candida albicans*, with MICs and MBCs ranging from 32−64 μg/mL. EOA was found to have activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are common food borne pathogens and could cause some stomach diseases, with MIC and MBC values ranging from 1024−3072 μg/mL. The EOA also had weak activity against *Acinetobacter baumannii* and *Klebsiella pneumonia*, which can cause serious infections, with MIC and MBC values ranging from 1024−2048 μg/mL. This study presents the first report on the chemical composition and antimicrobial activity of the EOA. Our results support the traditional use of wide chive as a spice and medicine to prevent food rot and infectious diseases. Therefore, the rhizome of wide chive should be further studied and applied as a potential antimicrobial agent in the functional food industry.

Table 1: Chemical composition of the essential oil of *Allium hookeri*.

No	Rt	Compounds	RI_{cal}	RI_{lit}	%
1	7.12	Diallyl sulfide	855	848	0.3
2	9.90	Methyl propyl disulfide,	930	936	0.5
3	11.49	Dimethyl trisulfide	967	960	0.8
4	16.62	Diallyl disulfide	1078	1083	28.4
5	17.95	Dipropyl disulfide,	1106	1098	5.0
6	21.61	3-Vinyl-1,2-dithiacyclohex-4-ene	1183	1180	0.2
7	26.86	Di-2-propenyl trisulfide,	1297	1296	31.8
8	27.98	Dipropyl trisulfide,	1323	1328	8.4
9	28.63	(*E*)-3,5-Diethyl-1,2,4-trithiolane	1338	1333	2.1
10	32.95	2-Hexyl-5-methyl-3(2*H*)-furanone	1440	1434	1.9
11	36.88	Di-2-propenyl tetrasulfide,	1541	1538	3.1
12	39.39	Tetradecanal	1615	1614	0.3
13	43.98	Diisobutyl phthalate	1874	1874	0.9

RT refers to retention time in minutes. RI_{cal} refers to the retention index experimentally calculated using C_5-C_{30} alkanes. RI_{lit} refers to the retention index taken from NIST.

Table 2: Antimicrobial activity of the essential oil of *Allium hookeri* rhizome.

Microbial strain	EOA			Positive control[b]	
	IZD [a] (mm)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)
MRSA098	10±0	512	1024	0.5	1
S. aureus	13±0	1024	1024	0.25	0.5
A. baumannii	10.3±0.6	1024	2048	0.5	1
E. coli	NA	NA	ND	ND	ND
K. pneumoniae	11.7±0.6	1024	1024	256	512
P. aeruginosa	11.7±0.6	1024	3072	0.25	1
A. fumigatus	32.7±1.1	32	64	512	1024
C. albicans	34.3±0.6	32	32	0.5	1

[a] All values were performed in triplicate and expressed as means ± SD. [b] Positive control: Vancomycin for *S. aureus* and MRAS098; Fluconazole for *A. baumannii* and *C. albicans*; Cefotaxime for *E. coli*; Amikacin for *K. pneumonia*; Ciprofloxacin for *P. aeruginosa*; Tigecycline for *A. fumigatus*. NA means "not active"; ND means "not detected"

Experimental

Plant Material and extraction of essential oil: The rhizomes of *Allium hookeri* Thwaites were collected form Menglun town, Xishuangbanna Dai Autonomous Prefecture on October, 2013. The rhizomes were air-dried and powdered by a laboratory mill. The EOA was collected by simultaneous distillation extraction (SDE) and stored at 0°C in the dark until further use. Diethyl ether was used as the solvent to extract the EOA simultaneously when the rhizome sample was steam distilled. The EOA and solvent were collected together, and then the diethyl ether was removed using a rotary evaporator at 45°C to give the EO.

Gas chromatography/mass spectrometry (GC/MS) analysis: The analysis of the EOA was performed using an Agilent Technologies 7890A GC, equipped with a HP-5 MS capillary column (30 mm, 0.25 mm, 0.25µm film thickness) and a mass spectrometer 5975C (Agilent Technologies, USA). MS were taken at 70 eV with a mass range of *m/z* 45–500. Helium was used as the carrier gas, at a flow rate of 1 mL/min. Injector and detector (MS transfer line) temperatures were both 250°C. Column temperature was gradually increased from 40°C to 160°C at a 3°C /min rate, then increased to 250°C at a 10°C /min rate, and then held for 10 min. A diluted sample of 0.2 µL of the EOA was injected manually.

Identification of the components: The components were identified on the basis of retention index {RI, determined with reference to a homologous series of *n*-alkanes (C_5–C_{30}), under identical experimental conditions}, and by comparison of their mass spectra with those recorded in the NIST 08 database (National Institute of Standards and Technology, Gaithersburg, MD, USA). The percentage composition of individual components was computed by the normalization method from the GC peak areas, assuming an identical mass response factor for all compounds.

Microbial strains and culture media: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC Y0109) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (China); *Aspergillus funigatus*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and a methicillin-resistant strain of *S. aureus* (MRSA 098) were provided by Kunming General Hospital. Standard Mueller–Hinton agar and broth (MHA and MHB), and Sabouraud agar and broth (SA and SB) (Tianhe Microbial Agents Co., Hangzhou, China) were used as the bacterial and fungal culture media in testing the inhibition zones, MICs and MBCs.

Antimicrobial screening: The EOA was dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20 mg/mL, and sterilized by filtration through 0.45 µm Millipore filters. The agar well diffusion method, as described previously [5a], was used to determine the inhibitory zones of the EOA. Under aseptic conditions, 20 µL of suspensions containing 1.5×10^9 colony forming units (CFU)/mL of bacteria and 1.0×10^6 CFU/mL of fungi were seeded on either MHA or SA, respectively. The wells (6 mm in diameter) were cut from the agars, and 50 µL of the EOA solution was delivered into them. After incubation for 24 h at 37°C, the diameters of the inhibition zones, measured in mm, were examined. MICs and MBCs were determined for the tested sensitive microbial strains according to the procedures reported previously [5b, 5c]. The MICs and MBCs were both determined for the starting test strains at 1.0×10^6 CFU/mL for bacteria and 1.0×10^4 CFU/mL for fungi, incubated at 35°C for 24 h, and were examined for growth in daylight. For MIC assay, the serial dilution method [5a] was used for the determination of the MICs of the EOA. Briefly, 100 µL of an appropriate medium, the EOA solutions and the tested strains were dispensed onto a 96-well plate. The microtitre plates were incubated at 35°C for 24 h. MIC values were the lowest concentration of a given sample that completely inhibited the visible microbial growth. For MBC assay, 10 µL samples taken from the clear wells of the microbroth susceptibility studies were placed onto the surfaces of either MHA or SA plates to determine the MBC. The MBC was defined as the concentration of sample that resulted in >99.9% killing of the bacterium relative to the concentration of bacterium that was presented in the test wells at 0 h [5c]. The MICs and MBCs of amikacin, fluconazole, vancomycin, tigecycline, ciprofloxacin and cefotaxime were also determined in parallel experiments in order to control the sensitivity of the standard test organisms. All tests were performed in triplicate.

Acknowledgments - The authors gratefully acknowledge the financial support by the Special Grant for Basic Work of Science and Technology from the Ministry of Science and Technology, China (grant No. 2012FY110300). Ms Chun-Fen Xiao is appreciated for her help in plant identification. We also wish to thank the Central Laboratory of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences for technical support in this study.

References

[1] (a) Chen XQ, Liang SY, Xu JM, Tamura MN. (**2000**) *Flora of China*, Lilicete. 73-263; (b) Block E, Ahmad S, Catalfamo JL, Jain MK, Apitz-Castro R. (**1986**) The chemistry of alkyl thiosulfinate esters. 9. Antithrombotic organosulfur compounds from garlic: structural, mechanistic, and synthetic studies. *Journal of the American Chemical Society*, ***108***, 7045-7055; (c) Aviello G, Abenavoli L, Borrelli F, Capasso R, Izzo AA, Lembo F, Romano B, Capasso F. (**2009**) Garlic: empiricism or science? *Natural Product Communications*, ***4***, 1785-1796.

[2] (a) Kyung KH. (**2012**) Antimicrobial properties of *Allium* species. *Current Opinion in Biotechnology*, ***23***, 142-147; (b) Ye CL, Dai DH, Hu WL. (**2013**) Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.). *Food Control*, ***30***, 48-53.

[3] (a) Hu GH, Sheng C, Mao RG, Ma ZZ, Lu YH, Wei DZ. (**2013**) Essential oil composition of *Allium tuberosum* seed from China. *Chemistry of Natural Compounds*, ***48***, 1091-1093; (b) Lopes D, Godoy RLO, Goncalves SL, Koketsu M, Oliveira AM. (**1997**) Sulphur constituents of the essential oil of nira (*Allium tuberosum* Rottl.) cultivated in Brazil. *Flavour and Fragrance Journal*, ***12***, 237-239.

[4] (a) Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I. (**2009**) Chemical composition and antimicrobial activity of wild garlic *Allium ursinum* of Bulgarian origin. *Natural Product Communications*, ***4***, 1059-1062; (b) Pino JA, Fuentes V, Correa MT. (**2001**) Volatile constituents of Chinese chive (*Allium tuberosum* Rottl. ex Sprengel) and rakkyo (*Allium chinense* G. Don). *Journal of Agricultural and Food Chemistry*, ***49***, 1328-1330.

[5] (a) Dickson RA, Houghton PJ, Hylands PJ, Gibbons S. (**2006**) Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of *Mezoneuron benthamianum* Baill., *Securinega virosa* Roxb. &Wlld. and *Microglossa pyrifolia* Lam. *Phytotherapy Research*, ***20***, 41-45; (b) CLSI: Clinical and Laboratory Standards Institute (**2006**) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard-Seventh Edition. Document M7-A7. CLSI, Wayne, Pa, USA; (c) NCCLS: National Committee for Clinical and Laboratory Standards (**1999**) Methods for determining bactericidal activity of antimicrobial agents; Approved Guideline; Document M26-A. CLSI (formerly NCCLS), Wayne, Pa, USA.