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Short Communication

Chemical Composition of Volatile Oil from Two Emergent Plants and Their Algae Inhibition Activity

Hong-Qiang Wang[1, 2], Feng Liang[1], Ning Qiao[1], Jin-Xia Dong[1], Lie-Yu Zhang[3]*, Yi-Fei Guo[1]

[1]School of Municipal and Environmental Engineering, Henan University of Urban Construction, Pingdingshan, Henan province 467036, PR China
[2]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province 430072, PR China
[3]Chinese Research Academy of Environmental Sciences, Beijing 100012, PR China

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Abstract

The volatile oils of two emergent plants (*Typha latifolia* and *Arundo donax*) were extracted with alcohol and then fractionated with some chemical reagent. Chemical composition of volatile oils were analyzed and identified by gas chromatograph-mass spectrometry (GC-MS). The volatiles appeared to form a complex mixture of 27 identified constituents, containing mainly fatty acids, ester, aldehyde, sterol, ketone, phenol, etc. Allelopathic activities of volatile oils on *Microcystis aeruginosa* were also determined. The results indicated that volatile oils from two emergent plants inhibited the growth of *M. aeruginosa* with inhibition rates of 43.3% and 47.9%, respectively, when the concentration of extracts was 50.0 mg/L.

Keywords: allelopathic activity, volatile oil, *Typha latifolia*, *Arundo donax*, *Microcystis aeruginosa*

Introduction

Eutrophication has been a major water quality problem all over the world [1]. During the past decades many efforts have been made to combat eutrophication of shallow lakes by various measures. Recent studies have demonstrated that macrophytes with allelopathic potential may play an important role in the restoration of eutrophic lakes [2]. In other words, the growth of harmful algal blooms can be controlled by using allelochemical excreted from some macrophytes [3, 4]. Allelopathy has been described for many macrophytes, such as *Phragmites communis* [5], *Vallisneria spiralis* [6], *Myriophyllum spicatum* [7], *Stratiotes aloides* [8], and *Potamogeton maackianus* [9, 10]. Macrophytes can produce a wide variety of secondary metabolites such as polyphenols, terpenes, ester, fatty acid, and ketone to effectively inhibit the growth of blue-green algae. Volatile organic compounds such as major allelochemicals in some aquatic plants have been reported. For example, dihydroactinidiolide from *Vallisneria spiralis* showed inhibition activity against *Microcystis aeruginosa* [6]. Shao et al. [11] also showed that β-ionone had strong inhibitory effects on *M. aeruginosa*. Ozaki et al. [12] reported that volatile organic compounds have lytic effects on Microcystis. In addition, Harada et al. [13] also found that β-ionone can cause cellular lysis and a decrease of Chl *a* content on Microcystis. It is known that cellular lysis and a decrease of Chl *a* are common phenomena of algal cell death [11].

Release of volatile organic compounds can help plants acclimatize to abiotic stress, contributing to thermotoler-

*e-mail: zhanglieyu@163.com

ance and the removal of reactive oxygen species, and there is a growing appreciation that chemical emissions may have wider importance for plant biology [14].

These results indicate that algal growth could be controlled by using some macrophytes with allelopathic potential. The objective of this study was to investigate the components of the volatile oils from *Typha latifolia* and *Arundo donax* as well as their allelopathic effect on the growth of *M. aeruginosa*.

Materials and Methods

Chemicals

Anhydrous sodium sulfate (AR), alcohol (AR), diethyl ether (AR), and dimethyl sulfoxide (DMSO, AR) were purchased from Chemical Reagent Company, Shanghai, China. Methanol (HPLC) was purchased from Tedia Company, INC, USA.

Macrophytes and Extraction of Chemicals

T. latifolia and *A. donax* were collected from Pingxi Lake, Pingdingshan, China. Plant materials were washed free of debris with regular water and later by deionized water, then dried and powdered. An appropriate amount of the powdered sample was soaked with alcohol for 72 h at room temperature, then filtered with GF/C glass fibre filters (47 mm, 1.2 μm, purchased from Whatman Maidstone, UK) with reducing pressure using a vacuum pump, subsequently collected the filtrates. The alcohol filtrates were evaporated to be close to dryness by rotary evaporator at 39ºC, then appropriate amounts of ultrapure water were added to the alcohol filtrates, finally fractionated with diethyl ether three times. The diethyl ether filtrates were first dried with anhydrous sodium sulfate and then evaporated to dryness by rotary evaporator at 39ºC. The diethyl ether extracts were stored at 4ºC until being used for GC-MS analysis and biological assay.

Bioassay

Axenic *M. aeruginosa* were obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences. The algae were cultured in sterilized BG11 medium (pH 7.4) at 25ºC and light intensity of 2500 lux, 12:12 h light:dark cycle. The algae were cultured for 4 days to reach the exponential phase with the density of 10^5-10^6 cells/mL, which were used for the assay of growth inhibition. The growth medium of all cultures was BG11 [15].

The concentration-response relationships between the allelochemicals and the tested organisms were studied in 50 mL flasks containing 20 mL test solution, to which 10^6 cells·mL^{-1} of *M. aeruginosa* were inoculated. The tested organisms were exposed, in triplicate, to one concentration level and a control, respectively. The final concentrations of compounds in the test solution were 50 mgL^{-1} for volatile oils from *T. latifolia* and *A. donax*. The 50% inhibition concentration of every volatile oil based on cell density of the tested organisms (EC_{50}) were determined after exposure for 72 h. The stock solutions of volatile oils were prepared with DMSO, which in test solution was lower than 0.2% (v/v). The test results indicated that the concentrations of DMSO added had no effect on the growth of the tested organisms.

Table 1. Composition of the volatile oil from *T. latifolia* and *A. donax*.

Rent time (min)	Compounds	*T. latifolia*	*A. donax*
7.31	2-metoxy-4-vinyl-phenol		
7.54	4-hydroxybenzaldehyde		
7.90	vanillin		
8.25	dimethyl phthalate		
8.79	lauric acid	–	
9.11	3,4,5-trimethoxyphenol	–	
9.5	syringaldehyde	–	
9.95	myristic acid		–
10.24	phytane	–	
10.37	P-hydroxyl ethyl cinnamate		–
10.41	3,7-dimethyloct-6-en-l-yl acetate		–
10.45	6,10,14-trimethyl-2-pentadecanone		–
11.01	hexadecanoic acid		
11.10	dibutyl phthalate		
11.18	palmitic acid ethyl ester		
11.78	phytol		–
11.86	linoleic acid		
11.96	stearic acid		–
11.99	ethyl linoleate		–
12.03	linolenic acid ethyl ester		
12.11	ethyl stearate		
12.89	4,8,12,16-tetramethylheptadecan-4-olide		–
13.68	unknown		–
21.84	campesterol	–	
22.51	stigmasterol	–	
23.85	γ-sitosterol	–	
24.07	stigmastanol	–	
27.58	stigmast-4-ene-3-one		

"–" undetectable

Table 2. Inhibitory effects of the volatile oil from *T. latifolia* and *A. donax* on growth of *M. aeruginosa*.

	T. latifolia	*A. donax*
Inhibition ratio	43.3%	47.9%

Identification of Organic Acid

The dried volatile oils were analyzed by gas chromatograph-mass spectrometry (GC-MS) (Agilent computerized system consistiing of a 6890 gas chromatograph coupled to a Agilent 5973N quadrupole mass spectrometer) using an HP-5MS capillary column (30 m × 0.25 mm × 0.25 mm id). The oven temperature was programmed at 60ºC (initial temperature maintained 1 min) to reach 260ºC at a rate of 4ºC/min, kept constant 20 min. Helium was used as a carrier gas with a flow rate of 1 mL/min. Mass fragments of the components were compared to the mass fragmentation data contained in the NIST 02.

Result and Discussion

Composition of the Volatile Oil from *T. latifolia* and *A. donax*

The components of the volatile oils from *T. latifolia* and *A. donax* were listed in Table 1. 27 compounds were analyzed and identified by GC-MS, in which fatty acids, ester, aldehyde, sterol were primary compositions. Compared components of the volatile oils from *T. latifolia* and *A. donax*, the amount and components of volatile oils were different in two macrophytes. There were differences in the amount and components of volatile oil, which might be due to the differences of plant species.

Allelopathic Effects of Volatile Oils on Algae

The allelopathic effects of volatile oils from *T. latifolia* and *A. donax* were explored by bioassay of *M. aeruginosa*. The results showed that volatile oils from these plants inhibited the growth of *M. aeruginosa* with inhibition rates of 43.3% and 47.9%, respectively, when the concentration of extracts was 50.0 mg/L.

The role of allelopathy in aquatic systems has received increasing attention as a potential means of controlling algal blooms [2]. Yang et al. [16] reported that vanillin at a concentration of 40 mg/L had obvious inhibition on the growth of *Alexandrium tamarense*. Five allelopathic compounds (lauric acid, myristic acid, hexadecanoic acid, linoleic acid, stearic acid) can inhibit the growth of *M. aeruginosa*, *Chlorella pyrenoidosa* Chick, and *Scenedesmus obliques* Kütz [17] and *Selenastrum capricornutum* [18] (Table 3). These compounds were also found in *T. latifolia* and *A. donax*.

T. angustifolia had a strong allelopathic effect on *Bolboschoenus fluviatilis* (river bulrush), reducing the longest leaf length and root, shoot, and total biomass of *B. fluviatilis* [20]. Aliotta et al. [19] reported that *T. latifolia* contains six compounds (β-sitosterol, (20s)24-methylenlophenol, stigmast-4-ene-3,6-dione, α-linolenic, linoleic, and an unidentified C18:2) with inhibitory activity to the growth of some microalgae. Compared to the results reported by Aliotta et al. [19], linoleic acid and γ-sitosterol were detected. Other compounds did not find it in the study. There was a difference in components of compounds, which might be due to the differences of research methods.

Hong and Hu [21] reported the effects of aquatic extract of *Arundo donax* Linn. (*A. donax*) on the growth of four freshwater algae (*M. aeruginosa*, *Selenastrum capricornutum*, *S. obliquus*, and *C. pyrenoidosa*) to control algal blooms. Hong et al. [22] isolated and identified gramine (N,N-dimethyl-3-amino-methylindole) from *Arundo donax*, gramine with 0.47 $mg \cdot L^{-1}$ of medium effective concentration to inhibit the growth of *M. aeruginosa*. After gramine exposure, esterase and total dehydrogenase activities were increased firstly but decreased later. In contrast with the controls, the cells exposed to gramine showed apparent ultrastructure alterations with thylakoids in breakage, phycobilins in decrease; lipid and cyanophycin granules were abundant at first but dissolved afterwards, DNA in fragementation [23]. The results indicated that the allelopathic effects of extract of *A. donax* L. on algae were strain-specific and *A. donax* L. may control the toxic cyanobacterium *M. aeruginosa*. Based on the observation of algal morphology and the measurement of algal density and cell size, the results showed that allelochemicals extracted with all three solvents inhibited *M. aeruginosa*. These results indicate that the production of metabolites by *T. latifolia* and *A. donax* might control harmful algal growth.

Table 3. EC_{50} (mg/L) of five allelochemicals on toxic *M. aeurginosa*, *C. pyrenoidosa* Chick, *S. obliques* Kütz (the fifth day), and *S. capricornutum* (the third day).

Compounds	*M. aeruginosa*	*C. pyrenoidosa* Chick	*S. obliquus* Kütz	*S. capricornutum*
Lauric acid	4.563	6.265	5.813	10.36
Myristic acid	15.498	16.003	15.977	18.65
Hexadecanoic acid	18.234	19.876	20.146	35.46
Linoleic acid	0.047	0.089	0.059	17.27
Stearic acid	19.849	20.849	20.754	40.27
Reference	[17]	[17]	[17]	[18]

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