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ALLELOPATHIC ACTIVITY OF ETHYL ACETATE EXTRACTS FROM TYPICAL EMERGENT PLANTS AGAINST *MICROCYSTIS AERUGINOSA* KÜTZ.

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Abstract

Some macrophytes have been reported to contain anti-cyanobacterial compounds, which can be used for nuisance cyanobacterial growth control by releasing allelochemicals. This study aimed at identifying allelochemicals of ethyl acetate extracts from *Pontederia cordata* L., *Alternanthera philoxeroides* (Mart.) Griseb., *Acorus calamus* L., *Typha latifolia* L. and to investigate their inhibitory effects on the cyanobacteria *Microcystis aeruginosa* Kütz. A series of analyses of ethyl acetate extracts from four aquatic plants by gas chromatograph-mass spectrometry (GC-MS) revealed that macrophytes contained fatty acids, sterol, ester, ketone, ether, alkane. Some identified compounds (hexadecanoic acid, linoleic acid, stearic acid, α-linolenic acid, asarone) have been reported to significantly inhibit the growth of algae. The inhibitory effect of the ethyl acetate extracts was also explored by bioassay of *M. aeruginosa*. Results indicated that ethyl acetate extracts from four plants inhibited to the growth of *M. aeruginosa* with the inhibition rate of 40.7, 30.5, 40.9 and 33.6%, respectively, when the concentration of extracts was 40.0 mg/l.

Algal blooms occur in many regions all over the world. Algal blooms can have negative impacts on freshwater systems including the release of toxins that are lethal to animals and humans (Pakdel *et al*. 2013). The cyanobacteria *Microcystis aeruginosa* Kütz. is one of the most common species found in algal blooms (Zhang *et al.* 2015). Therefore, the control and elimination of cyanobacteria blooms have become a significant goal in the restoration and protection of lake ecosystems.

Macrophytes with allelopathic potential can stabilize clear-water states in shallow eutrophic lakes by releasing allelopathic compounds that reduce epiphyton and phytoplankton biomass (Inderjit *et al*. 2005). Field evidence and laboratory studies indicate that allelopathy occurs in all aquatic habitats (marine and freshwater), and that all primary producing organisms (cyanobacteria, micro- and macroalgae as well as angiosperms) are capable of producing and releasing allelopathic active compounds (Gross 2003). So far, many allelochemicals have been studied and identified for controlling harmful algal blooms. For example, polyphenols substances secreted from *Myriophyllum spicatum* inhibited the growth of *M. aeruginosa* (Nakai *et al.* 2000) and the organic acids from the aquatic plants strongly inhibited growth of *M. aeruginosa* (Wang *et al*. 2010). Considering these findings showed the possibility of contorl of cyanobacteria by using appropriate allelochemicals.

Pontederia cordata, *Alternanthera philoxeroides*, *Acorus calamus* and *Typha latifolia* were several kinds of typical emergent plants. The allelopathic effects of *P. cordata*, *A. philoxeroides*, *A. calamus* and *T. latifolia* have been reported by some workers (Zhang *et al*. 2007, Jarchow and

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Cook 2009, Tian *et al*. 2011, Zhang *et al*. 2015). However, the composition of allelochemicals from *P. cordata, A. philoxeroides, A. calamus* and *T. latifolia* were still unknown. This study aimed at identifying the allelochemicals from *P. cordata, A. philoxeroides, A. calamus* and *T. latifolia* by gas chromatograph-mass spectrometry (GC-MS), and their allelopathic effects on *M. aeruginosa* were discussed.

Pontederia cordata, A. philoxeroides, A. calamus and *T. latifolia* were collected from Guanqiao experimental base at the Institute of Hydrobiology, Chinese Academy of Sciences. Plant materials were washed free of debris with regular water and later by deionized water, then, were dried and powdered. Powdered sample (5 g) was soaked in 100 ml of ethyl acetate for 24 hrs 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 ethyl acetate filtrates were first dried with anhydrous sodium sulfate and then evaporated to dryness by rotary evaporator at 39°C. The ethyl acetate extracts were stored at 4 °C until used for GC-MS analysis and biological assay. Fig. 1 shows different steps used in this study.

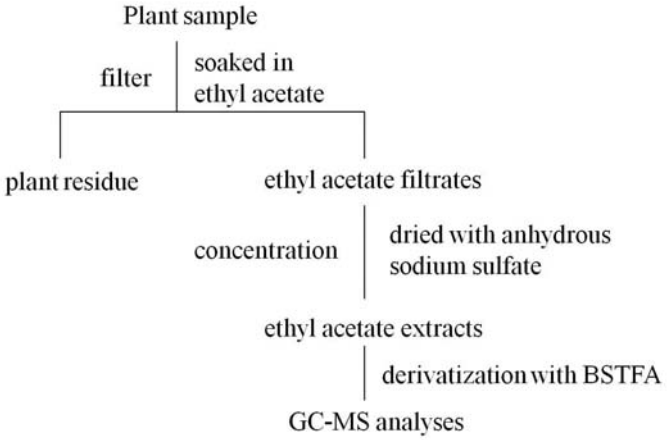

Fig. 1. Flow diagram of ethyl acetate extracts from *Pontederia cordata, Alternanthera philoxeroides, Acorus calamus* and *Typha latifolia*.

Axenic *M. aeruginosa* (FACHB 905) was 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 25°C and light intensity of 2500 lux, 12 : 12 hrs 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 (Rippka *et al*. 1979).

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 of *M. aeruginosa* were inoculated. The tested organisms were exposed, in triplicate, to one concentration levels and a control. The final concentrations of compounds in the test solution were 40 mg/l for ethyl acetate extracts from *P. cordata, A. philoxeroides, A. calamus* and *T. latifolia*. The inhibition ratio of every ethyl acetate extracts based on the cell density of the tested organisms were determined after exposure for 72 hrs. The stock solutions of ethyl acetate extracts 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.

From the Table 1, it could be seen that 22 compounds were detected in the ethyl acetate extracts of *P. cordata*, *A. philoxeroides*, *A. calamus* and *T. latifolia*. These three allelochemicals (linoleic acid, α-linolenic acid, and stearic acid) were also detected from the ethyl acetate extracts of *P. cordata*, *A. philoxeroides*, *A. calamus* and *T. latifolia*. Which has been proved to have obvious inhibition on the growth of *M. aeruginosa* (Zhang *et al*. 2009). While the amount and components of compounds were different in *P. cordata*, *A. philoxeroides*, *A. calamus* and *T. latifolia* in which fatty acids, sterol, ester, ketone, ether, and alkane were primary compositions. There was difference in the amount and components of extracts which might be due to the differences of plant species. Fatty acids were the main allelochemicals, the inhibition mechanism had been reported in some of the literature. For example, Wu *et al.* (2006) found that fatty acids primarily affect the plasma membranes, leading to a change in membrane permeability and dissociation of phycobilins from the thylakoids. Severe damage to the plasma membranes would give rise to a disruption of the stressed cells.

Table 1. The analytical results of ethyl acetate extracts from *P. cordata, A. philoxeroides, A. calamus* and *T. latifolia* by GC-MS.

Rent time (min)	Compounds	*P. cordata*	*A. philoxeroides*	*A. calamus*	*T. latifolia*
7.04	Glycerol	+	+	+	+
7.46	Unknown	+	+	+	+
11.13	Tyrosol	-	-	+	-
11.95	Asarone	+	+	+	+
13.83	Unknown	-	+	-	-
16.26	Unknown	+	+	+	-
17.50	14-methyl-pentadecanoic acid methyl ester	-	+	-	-
17.85	n-hexadecanoic acid	-	-	-	+
18.66	Unknown	+	+	+	+
19.14	Phytol	-	+	-	+
19.34	(Z,Z,Z)-9,12,15-octadecatrien-1-ol	+	+	+	+
19.57	Unknown	+	+	+	+
19.79	Linoleic Acid	+	+	+	+
19.83	α-linolenic acid	+	+	+	+
19.95	Stearic Acid	+	+	+	+
20.24	Tricosane	+	+	+	+
28.04	Cholesterol	+	-	-	-
30.08	Unknown	+	-	+	-
30.77	Stigmasterol	+	+	+	-
31.33	γ- sitosterol	-	-	-	+
32.15	β-sitosterol	+	-	+	+
34.90	Stigmast-4-en-3-one	+	-	-	+

"+"detectable, "–"undetectable

The results indicated that ethyl acetate extracts from *P. cordata*, *A. philoxeroides*, *A. calamus* and *T. latifolia* inhibited the growth of *M. aeruginosa* with the inhibition rate of 40.7, 30.5, 40.9 and 33.6%, respectively, when the concentration of extracts were 40.0 mg/l.

Some workers have utilized the allelopathic effects of certain aquatic plants as a means of biological control of the growth of undesirable algae and weeds in aquatic ecosystems (Jin *et al*. 2005). He and Ye (1999) studied the inhibitory effect of *Acorus tatarinowii* on algae growth, in addition to the competitions of light and mineral nutrients between *A. tatarinowii* and algae, the machanism of this inhibitory effect is mainly due to the excretion of some organic substances from the root system and rhizome of *A. tatarinowii* which may injure and abate the algae cells. When the algal cells were treated with cultured water of *A. tatarinowii*, chlorophyll 'a' was found to be destroyed, and the photosynthetic rate of algae markedly decreased, under fluorescence microscope it was seen that algal cells turned bright red to bluish green. This also explained the reason of emergent plants (*P. cordata*, *A. philoxeroides*, *A. calamus* and *T. latifolia*) could inhibit the growth of algae in the natural water bodies.

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References

Gross EM 2003. Allelopathy of Aquatic Autotrophs. Crit. Rev. Plant Sci. **22**(3-4): 313-339.

He CQ and Ye JX 1999. Inhibitory effects of *Acorus tatarinowii* on algae growth. Acta Ecologica Sinica **19**(5): 754-758.[In Chinese]

Inderjit, Weston LA and Duke SO 2005. Challenges, achievements and opportunities in allelopathy research. J. Plant Interact. **1**(2): 69-81.

Jarchow ME and Cook BJ 2009. Allelopathy as a mechanism for the invasion of *Typha angustifolia*. Plant Ecol. **204**(1): 113-124.

Jin Q, Dong SL and Wang CY 2005. Allelopathic growth inh*ibition of Prorocentrum micans* (Din*ophyta) by Ulva pertusa and Ulva linza* (Chlorophyta) in laboratory cultures. Eur. J. Phycol. **40**(1): 31-37.

Nakai S, Inoue Y, Hosomi M and Murakami A 2000. *Myriophyllum spicatum*-released allelopathic polyphenols inhibiting growth of blue-green algae *Microcystis aeruginosa*. Water Res. **34**(11): 3026-3032.

Pakdel FM, Sim L, Beardall J and Davis J 2013. Allelopathic inhibition of microalgae by the freshwater stonewort, *Chara australis* and a submerged angiosperm, *Potamogeton crispus*. Aq. Bot. **110**: 24-30.

Rippka R, Deruelles J, Waterbury JB, Herdman M and Stanier RY 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. **111**(1):1-61.

Tian RN, Sun XX, Wei Y and Wang SG 2011. Effects of hydrophytes on the growth of *Microcystis aeruginosa*, *Scenedesmus obliqnus* and *Chlorella vulgaris*. Chinese Journal of Ecology **30**(12): 2732-2738.[In Chinese]

Wang HQ, Cheng SP, Zhang SH, He F, Liang W, Zhang LP, Hu CY, Ge FJ and Wu ZB 2010. Chemical composition in aqueous extracts of *Potamogeton malaianus* and *Potamogeton maackianus* and their allelopathic effects on *Microcystis aeruginosa*. Polish J. Environ. Stud. **19**(1): 213-218.

Wu JT, Chiang YR, Huang WY and Jane WN 2006. Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. Aquat. Toxicol. **80**(4): 338-345.

Zhang SH, Chang JJ, Cao JY and Yang CL 2015. Comparative studies on growth and physiological responses of unicellular and colonial *Microcystis aeruginosa* to *Acorus calamus*. Bull. Environ. Contam. Toxicol. **94**(2): 225-231.

Zhang TT, Chen CP, He M, Wu AP and Nie LW 2007. Allelopathic effects of several higher aquatic plants on algae. Journal of Biology **24**(4): 32-36. [In Chinese]

Zhang TT, Zheng CY, He M, Wu AP and Nie LW 2009. Inhibition on algae of fatty acids and the structure-effect relationship. China. Environ. Sci. **29**(3): 274-279. [In Chinese]

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