Chemical Composition in Aqueous Extracts of *Najas Marine* and *Najas Minor* and their Algae Inhibition Activity

Hongqiang WANG[1,2], Huijie ZHU[1], Kui ZHANG[1], Liping ZHANG[2],Zhenbin WU[2*]

[1]*Department of Environmental and Municipal Engineering, Henan University of Urban Construction, Pingdingsha 467036, P.R. China*

[2] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, P.R. China*

Email:wanghq77@yahoo.com.cn

Abstract: Using allelochemicals, which were produced by some macrophytes, to inhibit the growth of some harmful algae has been reported. The ethyl acetate fractions of aqueous extracts from two submerged macrophytes *Najas marine* and *Najas minor* were analyzed by gas chromatograph-mass spectrometry (GC–MS), and the allelopathic effect of the ethyl acetate fraction was also explored by bioassay of *Microcystin aeruginosa*.The results showed that the ethyl acetate fraction had activity to inhibit the growth of *M. aeruginosa*. The conclusion of the study provided important information for recovering eutrophic waters by submerged macrophytes.

Keywords: Allelopathic activity; *Najas marine*; *Najas minor*; Organic acid; *Microcystis aeruginosa*

1. Introduction

Increasing human activities, particularly urbanisation, agricultural and industrial activities, have accelerated eutrophication in many lakes and rivers worldwide. Harmful algae blooms in eutrophic water bodies have caused serious problems, such as fisheries, water supply, and recruitment[1].

Chemical interactions between aquatic plants and microalgae play an key role in control of the algal growth in aquatic ecosystems due to excreting second metabolites by the macrophytes[2]. Organic acids with allelopathic activity are present in many aquatic plants[3-6]. Under certain conditions, these compounds are released into surrounding water to inhibit the growth of algae[4]. In some research papers, a variety of organic acids have been isolated from some submerged macrophytes and characterized as allelochemicals, which play an important role in control of the algal growth in aquatic ecosystems[5,6]. In previous work, we have researched the chemical composition of organic acids from two submerged macrophytes(*Potamogeton malaianus* and *Potamogeton maackianus*) and their allelopathic effects on *Microcystis aeruginosa* [7], which is often a dominant cyanobacterial species in summer phytoplankton of eutrophic shallow lakes.

There are some reports about antialgal activities of *Najas minor* and *Najas marine*. For example, He *et al*. [8] reported that the allelopathic effects of *P. malaianus* and *N. minor*, on growth, photosynthesis and antioxidant systems of *Scenedesmus obliquus* by coexistence experiments. The results indicated that the two macroph- ytes *N. minor* and *P. malaianus* had significant allelopathic effects on *S. obliquus*. Gross *et al.* [9]showed that the allelopathic activity of *N. marine* on various filamentous cyanobacteria as target species. The reports about organic acids as allelochemicals in aqueous extracts from *N. marine* and *N. minor* were rare to be found on literature.

In order to compare the effect of different species of submerged macrophytes on *M. aeruginosa*, the composition of organic acids from two common *Najas* L. species (*N. marine* and *N. minor*) and their allelopathic effects on *M. aeruginosa* were investigated in the present paper.

2. Experimental

2.1 Chemicals

Ethyl acetate and n-hexane (HPLC grade) were purchased from Tedia Company, INC, USA. *N,O*-bis-(trimethylsilyl)trifluoroacetamide(BSTFA) were purchased from Sigma-Aldrich Company, USA. Dimethyl sulfoxide (DMSO, AR), and anhydrous sodium sulfate(AR) were purchased from Chemical Reagent, Shanghai, China.

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2.2 Preparation of Aqueous Extracts and Fractionation

N. marine and *N. minor* was collected from the pond of Wuhan Botanical Garden, Chinese Academy of Science, Wuhan, China. Plant materials were washed free of debris by tap water and later by deionized water, then, were dried and powdered. Appropriate amount of the powdered sample for were soaked in 300 mL of distilled water for 48 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 filtrate for further fractionations. The Fig 1 shows all the steps used in the study. The aqueous extracts were fractionated according to Xian *et al*. [3].

2.3 Bioassay

Axenic *M. aeruginosa* were obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences. *M. aeruginosa* was cultured in sterilized BG11 medium (pH 7.4) at temperature of 25℃, under light intensity of 2500 lux and 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 [10]. Assay of algal growth inhibition was performed as per U.S EPA [11]method.

Excel 2003 software -were used for the analysis of the data.

2.4 Identification of Organic Acids

The dried ethyl acetate extracts were derivatized by adding 0.1 mL *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The mixture was heated at 80℃ oven for 2 h and derivatized samples submitted to GC-MS(Agilent 5973, USA) analysis. Mass fragments of the components were compared to the mass fragmentation data contained in the NIST 02.

3. Results and Discussion

More than 20 compounds were detected in the extracts of submerged macrophytes (Table 1) using GC-MS. Organic acids were primary compositions.While the amount and components of organic acids were different in two plants.

The allelopathic effects of extracts from submerged macrophytes were explored by bioassay of *M. aeruginosa*. The results showed that the extracts from the plants inhibited to the growth of *M. aeruginosa* with the inhibition rate of 53.1% and 47.5% for *N. marine* and *N. minor*, respectively, when the concentration of extracts were 60mg/L.

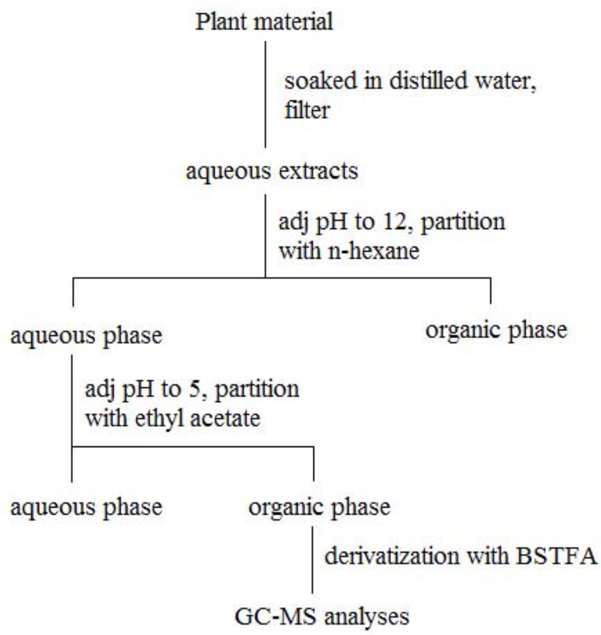

Figure 1. Flow diagram for extraction of organic acids from *Najas marine* and *Najas minor*

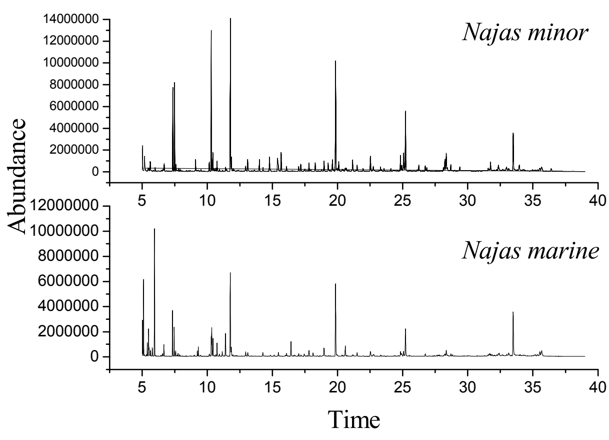

Figure 2. Total ion chromatogram of ethyl acetate fraction of aqueous extracts from *Najas marine* and *Najas minor*

4. Discussion

In this study, strong polar organic acids of aqueous extracts from *N. marine* and *N. minor* were isolated and characterized. The results indicated that more than 20 fatty acids, phenolic acids and hydroxy fatty acids were detected in the aqueous extracts of the plants. Compared

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Table 1 Chemical compositions of aqueous extracts of *Najas marine* and *Najas minor*

Rent time (min)	Compounds	Najas marine	Najas minor
5.50	Acetic acid		
5.64	2-methyl-butanoic acid		–
5.79	3-methyl-butanoic acid		–
7.58	Phenol		
9.24	3-Hydroxybutanoic acid		–
9.31	2-Hydroxy-3-methylbutyric acid		–
10.16	Phenethyl alcohol	–	
10.35	2-Hydroxylhexanoic acid		–
10.43	Benzoic acid		
10.48	catechol	–	
11.15	Phenylacetic acid		–
11.40	Succinic acid		
14.28	2-Hydroxy-2-(4-methoxyphenyl)acetic acid	–	
16.09	4-Hydroxyphenethyl alcohol	–	
17.19	4-Hydroxybenzoic acid	–	
17.81	3-Hydroxydecanoic acid		
19.85	4-Hydroxyphenylpropionic acid		
20.61	Azelaic acid		–
21.52	Myristic acid	–	
22.53	3-(4-hydroxy-3-methoxyphenyl)propan-oic acid		
24.84	unknown		
24.94	unknown	–	
25.09	3-Hydroxytetradecanoic Acid		
25.26	Palmitic acid		
26.24	6,7-Dihydroxycoumarin	–	
26.74	Margaric acid	–	
28.14	9,12-octadecadienoic acid	–	
28.24	unknown	–	
28.36	Octadecenoic acid		
28.70	Octadecanoic acid	–	

"–"undetectable

to the components of aqueous extracts from *P. maackianus* and *P. malaianus*[7], there are some compounents were only detected in the aqueous extracts of *N. marine* and *N. minor, although* most detected organic acids were similar. Furthermore the highest content compound detected in this study was not palmitic acid.

The results of bioassay showed that the ethyl acetate fraction of aqueous extracts had activity to inhibit the growth of *M. aeruginosa*.

The antialgal effects of phenolic acids were reported by some literature. Gross *et al.* [12] have shown that *Myriophyllum spicatum* released the allelopathic compounds hydrolyzable tannin (eugeniin), and its derivatives, ellagic and gallic acids. Leu *et al.* [13] found that β-1,2,3-tri-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose (tellimagrandin II)from the aquatic angiosperm *M. spicatum* is an effective inhibitor of microalgal exoenzymes, and tellimagrandin II has at least two modes of action, inhibition of exoenzymes and inhibition of PSII. Multiple target sites are a common characteristic of many potent allelochemicals.

Comparing with phenolic compounds extracted from plant tissue, the exuded phenolic compounds played a great role to inhibit the growth of *M. aeruginosa*. Because of their high water solubility, phenolic compounds are easily released into the surrounding water by aquatic plants than other classes of potential allelochemicals, which makes the hypothesis reasonable that phenolic compounds can be partly responsible for the reduction of algal growth in natural aquatic ecosystems[4]. So, further research is needed to investigate the characterized componds whether released into natural aquatic ecosystem by aquatic plants.These conclusions may provide key information for controlling the algal bloom in eutrophic water bodies by submerged macrophytes.

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