Inhibitory effects of golden thread (*Coptis chinensis*) and berberine on *Microcystis aeruginosa*

Shulin Zhang, Bo Zhang, Kezhi Xing, Xiumei Zhang, Xiuping Tian and Wei Dai

ABSTRACT

The effects of 40 Chinese herbs on *Microcystis aeruginosa* growth were monitored spectrophotometrically. Golden thread (*Coptis chinensis*) exhibited the best inhibitory effects. Cell density of *M. aeruginosa* decreased with the increasing concentrations of golden thread and the prolongation of exposure time. Decreases in protein content, carbohydrate content, and chlorophyll a content were observed in a golden thread concentration-dependent manner after 96 h exposure. Changes in cell density, protein content, carbohydrate content, and chlorophyll a content of *M. aeruginosa* exposed to berberine, the main component of golden thread, were also investigated. It was observed that berberine exhibited the same inhibitory effects on *M. aeruginosa.* The results suggested that golden thread could inhibit *M. aeruginosas* growth effectively, and berberine might be the main allelochemical implementing the inhibitory effects of golden thread.

Key words | allelochemical, berberine, golden thread, inhibitory effects, *Microcystis aeruginosa*

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INTRODUCTION

Algae blooms, and cyanobacterial blooms in particular, have been observed in aquaculture waters all over the world. Harmful blooms can result in water anoxia, secretion of algal toxins as well as several other adverse effects (Pitois *et al.* 2001). To avoid the menace posed to the aquatic environment by algae bloom s, physicochemical, chemical and biochemical methods have been employed to prevent bloom occurrence (Kolmakov 2006).

Allelopathy, any direct or indirect, inhibitory or stimulatory effect produced by an organism on another organism through the release of chemical compounds into the environment, is a new effective method of water blooms control (Hong *et al.* 2008). It was indicated that the inhibitory effects of *Hydrilla verticillata* and *Ceratophyllum demersum* on *Microcystis spp.* were realized through excreting substances into water (Wang *et al.* 2006; Xian *et al.* 2007). Nakai *et al.* (2006) found that rotting-reed solution (RRS) could inhibit *M. aeruginosa* growth, and the inhibitory effects were correlated with its dissolved organic carbon concentrations. Moreover, culture liquid or exudates of other aquatic macrophyte such as *Potamogeton maackianus*, *Potamogeton pectinatus* L, and *Acorus calamus* had inhibitory effects on the growth of *M. aeruginosa* (Mulderij *et al.* 2005; Zhang *et al.* 2006; Wu *et al.* 2007). In addition to aquatic macrophyte, terrestrial plant also exhibited allelopathic effects on algae. The presence of decomposing barley straw in water could reduce the growth of a range of algal species under field and laboratory conditions (Welch *et al.* 1990; Newman & Barrett 1993; Ball *et al.* 2001). Compared with other terrestrial plant, the Chinese medicinal plant is easier to produce allelochemicals. Zhou *et al.* (2007) studied the inhibitory activity of five Chinese herbs on red-tide causing alga, *Alexandrium tamarense*. The results showed that golden thread (*Rhizoma coptidis*) and areca seed (*Semen arecae*) had the best inhibitory effects. Very little work had been done in using

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Table 1 | Inhibitory effects of Chinese herbs on *M. aeruginosa* growth

		Absorption value				
Herb	**Concentration (W/V)**	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
Golden thread	0.05%	0.24 ± 0.01	0.23 ± 0.00	0.13 ± 0.02	0.12 ± 0.01	0.07 ± 0.02
Golden thread	0.10%	0.23 ± 0.00	0.24 ± 0.01	0.13 ± 0.00	0.09 ± 0.01	0.03 ± 0.01
Areca	0.05%	0.24 ± 0.01	0.26 ± 0.01	0.21 ± 0.02	0.18 ± 0.00	0.14 ± 0.01
Areca	0.10%	0.24 ± 0.00	0.26 ± 0.02	0.19 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
Ash bark	0.05%	0.24 ± 0.01	0.20 ± 0.02	0.24 ± 0.01	0.25 ± 0.01	0.24 ± 0.01
Ash bark	0.10%	0.24 ± 0.02	0.22 ± 0.01	0.23 ± 0.02	0.24 ± 0.01	0.25 ± 0.01
Stemona	0.05%	0.24 ± 0.00	0.24 ± 0.02	0.25 ± 0.01	0.24 ± 0.02	0.25 ± 0.01
Stemona	0.10%	0.25 ± 0.02	0.22 ± 0.00	0.24 ± 0.01	0.24 ± 0.02	0.25 ± 0.01
Southernwood	0.05%	0.24 ± 0.01	0.25 ± 0.01	0.25 ± 0.02	0.25 ± 0.01	0.25 ± 0.01
Southernwood	0.10%	0.24 ± 0.00	0.21 ± 0.02	0.25 ± 0.02	0.25 ± 0.01	0.24 ± 0.01
Garden burnet	0.05%	0.22 ± 0.02	0.26 ± 0.02	0.24 ± 0.00	0.25 ± 0.01	0.24 ± 0.02
Garden burnet	0.10%	0.22 ± 0.02	0.23 ± 0.00	0.22 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
Control		0.24 ± 0.02	0.24 ± 0.02	0.29 ± 0.01	0.34 ± 0.02	0.346 ± 0.01

Values are represented as mean ± SD, $n = 3$.

Chinese herbs to control algae bloom. In this study, to estimate the potential of Chinese herbs on algae bloom control, the effects of forty Chinese herbs on *M. aeruginosa* growth were monitored spectrophotometrically. Moreover, the inhibitory effects of golden thread and berberine, as the major component of golden thread, on cell density, protein content, carbohydrate content, and chlorophyll a content of *M. aeruginosa* were investigated, respectively.

MATERIALS AND METHODS

Materials

Microcystis aeruginosa was provided by the Institute of Hydrobiology, Chinese Academy of Sciences. Alga was grown in 2,000 mL conical flasks containing 1,500 mL BG11 medium under 12:12 LD cycle with a light density of 2,500 ± 500 Lux at 25 ± 1°C. The initial algae concentration was 1×10^7 cells mL^{-1}. To reduce any effects related to minor differences in photon irradiance, the flasks were shaken slightly 3 times each day and rearranged randomly. The Chinese herbs were purchased from a pharmacy as dehydrated products. Berberine was purchased from the Northeast General Pharmaceutical Factory, China.

Preparation of herb extracts and berberine solution

Herb extracts were prepared according to the method of Zhou *et al.* (2007). Herbs were mixed with distilled water in a ratio of 1 g herb per 10 ml water, then boiled in covered beakers for 20 min and filtered with a screen, and the final volume was readjusted to match the starting volume. Berberine was dissolved in heated distilled water to a concentration of 10% (w/v). The prepared 10% herb extracts and berberine solution were kept at 4°C.

Inhibitory effects assessment of Chinese herbs on *M. aeruginosa* growth

The effects of forty Chinese herbs on the growth of *M. aeruginosa* were assessed by measuring algal culture

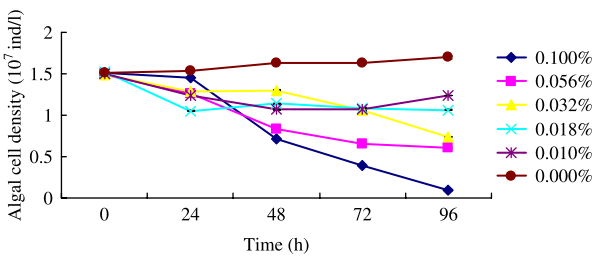

Figure 1 | Effects of various concentrations of golden thread on algal cell density of *M. aeruginosa*.

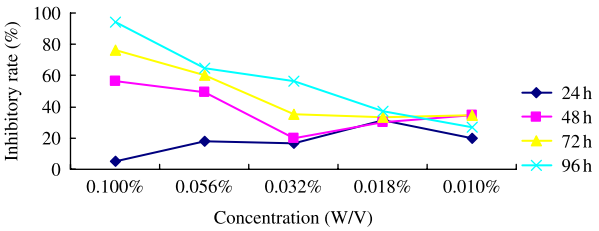

Figure 2 | Inhibitory rate of various concentrations of golden thread on *M. aeruginosa*.

absorbance on a UV-Spectrophotometer (Thermo Electron, USA). The tested Chinese herbs included ginger, peppermint, gardenia, golden thread, phellodendron, baical skullcap, lightyellow sophora root, ash bark, southernwood, figwort root, houttuynia thunb, dyers woad leaf, forsythia, flos lonicerae, indigowoad, rangoon creeper, cyrtomium fortunei, areca seed, stemonae, mugwort leaf, garden burnet, tangerine peel, pepper, allicin, rhubarb, Chinese prickly ash, huang shen, dandelion, liquorice, fructus mume, angelica, gallnut, atractylodes, schisandra chinensis, catnip, anemarrhena, atractylodes macrocephala koidz, pulsatilla chinensis, and fructus chebulae. The most suitable wavelength to use for monitoring culture growth was 625 nm. Three herb concentrations (0, 0.05%, 0.10%) were chosen for algal exposure experiment and each concentration was conducted in triplicate. The media prepared without herb extracts was taken as the control group. Samples were taken 0 h, 24 h, 48 h, 72 h, and 96 h after addition of the herb extracts. To reduce erroneous results, samples were taken 1 cm below the water surface with movement. Measures were taken to avoid the resuspension of sunken algae cells. Each sampling was replicated three times and averaged.

The inhibitory effects of golden thread and berberine on *M. aeruginosa*

Golden thread concentrations added in culture medium were chosen as follows: 0, 0.01%, 0.018%, 0.032%, 0.056% and 0.10% (w/v) with three replicate flasks per concentration. The concentration gradients of berberine added in culture medium were designed as follows: 0, 0.005, 0.010, 0.020, 0.030 and 0.040% (w/v) with three replicate flasks per concentration. Algal cell number was counted using a 0.1 ml counting chamber under a microscope ($\times 40$). Samples were removed from the cultures at 24 h intervals (0, 24, 48, 72, and 96 h) to determine the following indices.

- Inhibitory rate (IR), the decrease of intact algae in suspension, was calculated with the following: $IR(\%) = (N_0 - N_S)/N_0 \times 100$, where N_0 = control algal cell density (ind l^{-1}); Ns = treatment algal cell density (ind l^{-1});
- Protein content was measured using Folin-phenol Reagent method;
- Carbohydrate content was determined according to phenol-sulfuric method; and
- Chlorophyll a content was measured spectrophotometrically with the following:

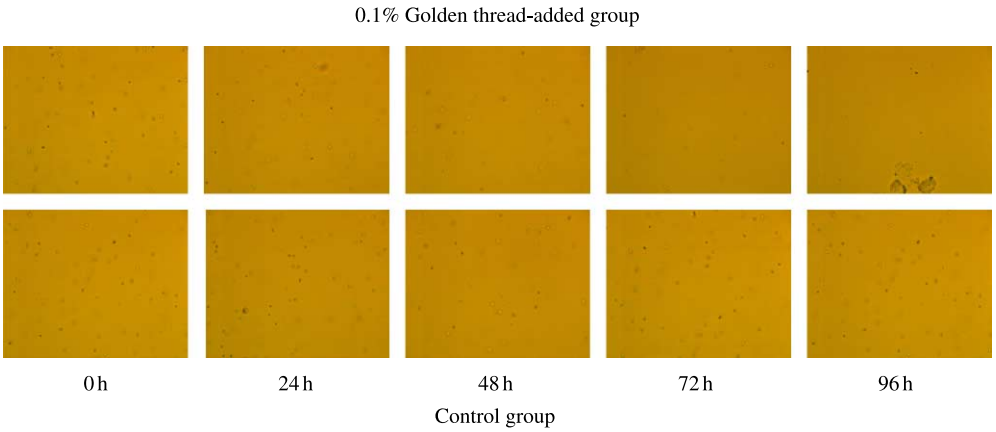

Figure 3 | *M. aeruginosa* growth in control and 0.1% golden thread-added groups.

Table 2 | Effects of various concentrations of golden thread on chlorophyll a content, protein content and carbohydrate content of *M. aeruginosa* after 96 h exposure

Concentration (W/V)	Chlorophyll a content (μg/L)	Mass fraction of protein (%)	Mass fraction of carbohydrate (%)
0.100%	128.70 ± 7.8^{f}	14.18 ± 0.27^{e}	5.14 ± 0.06^{e}
0.056%	596.34 ± 7.89^{e}	29.07 ± 2.43^{d}	17.37 ± 1.12^{d}
0.032%	708.28 ± 5.71^{d}	33.52 ± 4.60^{cd}	22.61 ± 0.99^{c}
0.018%	742.84 ± 9.27^{c}	36.71 ± 1.13^{c}	22.38 ± 1.27^{c}
0.010%	1097.60 ± 16.07^{b}	44.24 ± 4.29^{b}	25.66 ± 1.06^{b}
0.000%	1429.88 ± 6.77^{a}	75.80 ± 5.31^{a}	36.32 ± 1.07^{a}

Values are represented as mean ± SD, $n = 3$; Values sharing the same letters or no letters are not significantly different ($P > 0.05$), whereas those with different letters are significantly different ($P < 0.05$).

- Pchll-a $= (11.85 \times (A_{664} - A_{750}) - 1.54 \times (A_{647} - A_{750}) - 0.08 \times (A_{630} - A_{750})) \times V_0/V \times$ L, where A = absorbance of chlorophyll a; V_0 = volume of acetone used for extraction (ml); V = liters of water filtered; L = path length of cuvette (cm).

Statistical analysis

The results were expressed as mean ± SD. One-way analysis of variance (ANOVA, SPSS version 10.0) followed by LSD's multiple range test was used to examine whether there were any significant differences among treatments. Statistical significance was established at $P < 0.05$.

RESULTS

Inhibitory effects assessment of Chinese herbs on *M. aeruginosa* growth

Among forty tested Chinese herbs, golden thread had the best inhibitory effects on *M. aeruginosa* growth, followed by areca, ash bark, stemona, southernwood and garden burnet (Table 1). The rest herbs had no or stimulatory effects on *M. aeruginoas* growth.

The inhibitory effects of golden thread on *M. aeruginosa*

Figure 1 showed that cell density of *M. aeruginosa* decreased with the increasing concentrations of golden thread and prolongation of exposure time. Among all the tested concentrations, 0.1% golden thread exhibited the best inhibitory effects on *M. aeruginosa* growth, 48 h IR reached 56.28% and 96 h IR was 96.46% (Figure 2). There were no suspension algal cells in culture after 96 h exposure to 0.1% golden thread and culture became bright yellow, as the color of golden thread extract. Residual body of killed algal cell deposited on the bottom of conical flask (Figure 3). Decreases in chlorophyll a content, protein content and carbohydrate content of *M. aeruginosa* were observed in a golden thread concentration-dependent manner after 96 h exposure (Table 2).

The inhibitory effects of berberine on *M. aeruginosa*

Berberine exhibited a concentration-dependent inhibitory effect on cell density of *M. aeruginosa*. Cell density of *M. aeruginosa* also decreased with the prolongation of exposure time (Figure 4). 24 h IR all arrived at about 50% in the 0.040%, 0.030% and 0.020% berberine-added groups and 96 h IR reached to 94.90%, 93.62% and 91.86%, respectively (Figure 5). It was observed that the intact algal cell could not be observed in culture after 96 h exposure to 0.02% berberine, which indicating that the effective inhibition of berberine on *M. aeruginosa* growth (Figure 6). Chlorophyll a content, protein content and carbohydrate

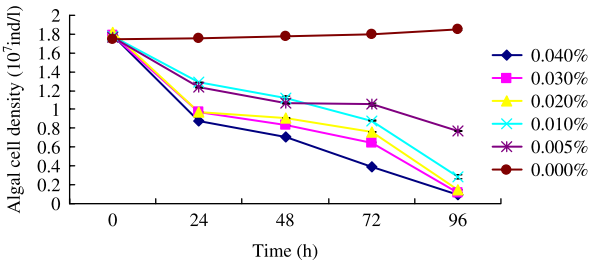

Figure 4 | Effects of various concentrations of berberine on algal cell density of *M. aeruginosa*.

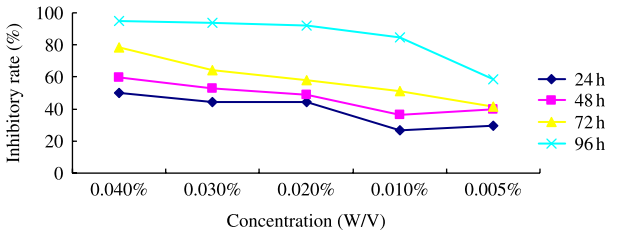

Figure 5 | Inhibitory rate of various concentrations of berberine on *M. aeruginosa*.

content of *M. aeruginosa* decreased with the increasing concentrations of berberine after exposed for 96 h (Table 3).

Comparision inhibitory effects between golden thread and berberine

Similar inhibitory effects were observed between 0.1% golden thread and 0.01% berberine groups. 0.056% golden thread and 0.005% berberine also exhibited similar inhibition on *M. aeruginosa* (Table 4).

DISCUSSION

Microalgae growth could be inhibited or stimulated by Chinese herbs. Zhou & Huang (2003) found angelica, ginseng and platycodon grandiflorum could accelerate the growth of *Pavlova viridis*. It was found that golden thread and areca seed inhibited *Alexandrium tamarense* growth effectively (Zhou *et al.* 2007). Consistent with the above results, we found different Chinese herbs had different effects on *M. aeruginosa* growth, either inhibitory of stimulatory. Golden thread had the most pronounced inhibitory effects on *M. aeruginosa* growth. Zhou *et al.* (2007) found 48 h IR of 0.096% golden thread was highest at 99.3% while 72 h IR value showed a decrease. The reason might be attributed to recovery and resuspenion of settlement algae cells that were not killed by golden thread. In this study, cell density decreased sharply with the prolongation of exposure time after 24 h, and 96 h IR

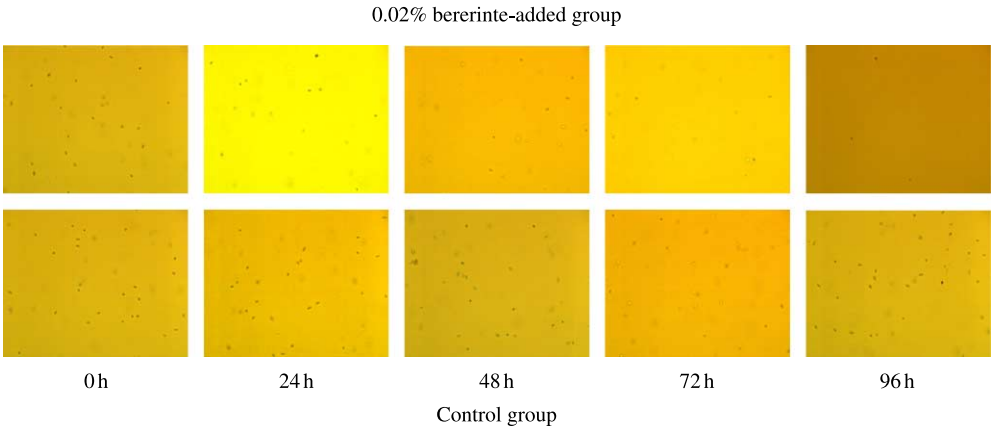

Figure 6 | *M. aeruginosa* growth in control and 0.02% bererinte-added groups.

Table 3 | Effects of various concentrations of berberine on chlorophyll a content, protein content and carbohydrate content of *M. aeruginosa* after 96 h exposure

Concentration (W/V)	Chlorophyll a content (μg/L)	Mass fraction of Protein (%)	Mass fraction of carbohydrate (%)
0.040%	148.28 ± 4.04^{e}	9.17 ± 1.69^{d}	7.07 ± 0.45^{c}
0.030%	157.75 ± 0.83^{e}	9.63 ± 1.20^{cd}	7.97 ± 0.45^{c}
0.020%	172.84 ± 7.57^{d}	10.64 ± 0.69^{cd}	7.67 ± 0.47^{c}
0.010%	310.35 ± 4.36^{c}	11.74 ± 1.39^{c}	7.07 ± 0.59^{c}
0.005%	678.68 ± 11.50^{b}	29.77 ± 0.83^{b}	14.24 ± 0.67^{b}
0.000%	1594.99 ± 10.45^{a}	59.47 ± 1.15^{a}	28.56 ± 0.78^{a}

Values are represented as mean ± SD, $n = 3$; Values sharing the same letters or no letters are not significantly different ($P > 0.05$), whereas those with different letters are significantly different ($P < 0.05$).

Table 4 | Comparision inhibitory effects between golden thread and berberine on *M. aeruginosa*

Concentration (W/V)		96 h inhibitory rate (%) Cell density	Chlorophyll a content	Carbohydrate content	Protein content
Golden thread berberine	0.10%	96.46 ± 0.01	91.63 ± 0.02	86.92 ± 0.00	80.84 ± 0.01
	0.01%	84.76 ± 0.02	80.50 ± 0.00	75.50 ± 0.02	80.22 ± 0.03
Golden thread berberine	0.056%	59.80 ± 0.00	58.29 ± 0.02	51.28 ± 0.02	59.05 ± 0.02
	0.005%	58.39 ± 0.01	57.44 ± 0.00	50.42 ± 0.01	49.97 ± 0.01

Values are represented as mean ± SD, $n = 3$; Values sharing no letters are not significantly different ($P > 0.05$).

reached 94.46%. This suggests that *M. aeruginosa* was killed by golden thread.

It has been demonstrated that golden thread and berberine both exhibited diverse antibacterial activities and had wide antimicrobial spectrums (Hu *et al.* 2000; Choi *et al.* 2007; Fan *et al.* 2008; Yan *et al.* 2008). *Microcystis aeruginosa* belongs to prokaryote and has a similar structure to bacteria. Golden thread and berberine inhibited *M. aeruginosa* growth and the inhibition mechanisms might be the same as that involved in antibacterial activity.

Changes in the contents of three key biochemical compounds (protein, carbohydrate and chlorophyll a) of *M. aeruginosa* exposed to golden thread and berberine were observed in this study. Protein is of main importance for algal cell division, and chlorophyll a is the main pigment transforming the light energy into chemical energy, which directly represents the photosynthetic ability of algae. Decreases in protein content, carbohydrate content and chlorophyll a content of *M. aeruginosa* were observed with the increasing concentrations of golden thread and berberine, respectively. It suggests that golden thread/berberine could inhibit biosynthesis and metabolism of these chemical compounds. Decreases in chlorophyll a content might be due to golden thread-induced/berberine-induced inhibition of enzyme activities or destruction of chloroplast structure and function. The inhibition of algal cell photosynthesis resulted from the decreased Chlorophyll a content might lead to decreases in protein and carbohydrate synthesis. Furthermore, decreases in protein content and carbohydrate content could result in inhibition of *M. aeruginosa* growth.

Berberine is the main component of gold thread. Similar inhibitory effects on *M. aeruginosa* was observed between 0.1% golden and 0.01% berberine groups as well as between 0.056% golden thread and 0.005% berberine groups. This suggested that berberine might be the main allelochemical produced by golden thread and implemented inhibitory effects on *M. aeruginosa* growth.

CONCLUSIONS

Golden thread and berberine both could inhibit *M. aeruginosa* growth without rebound. Berberine might be the allelochemical implementing the inhibitory effect produced by golden thread. It was demonstrated that golden thread and berberine had a promising future in using as bloom inhibitor. Further studies needed to clarify the inhibition mechanisms involved.

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REFERENCES

Ball, A. S., Vincent, D., Robinson, J. & Williams, M. 2001 Algal growth control by a barley straw extract. *Bioresour. Technol.* **77**(2), 177–181.

Choi, U. K., Kim, M. H. & Lee, N. H. 2007 Optimization of antibacterial activity by Gold-Thread (*Coptidis Rhizoma* Franch) against Streptococcus mutans using evolutionary operation-factorial design technique. *J. Microbiol. Biotechnol.* **17**(11), 1880–1884.

Fan, D. L., Xiao, X. H. & Ma, X. J. 2008 Calorimetric study of the effect of protoberberine alkaloids in *Coptis chinensis* Franch on *Staphylococcus aureus* growth. *Thermochim. Acta* **480**, 49–52.

Hong, Y., Hu, H. Y. & Li, F. M. 2008 Physiological and biochemical effects of allelochemical ethyl 2-methyl acetoacetate (EMA) on cyanobacterium *Microcystis aeruginosa*. *Ecotoxicol. Environ. Saf.* **71**(2), 527–534.

Hu, J. P., Takahashi, N. & Yamada, T. 2000 *Coptidis rhizoma* inhibits growth and proteases of oral bacteria. *Oral Dis.* **6**(5), 297–302.

Kolmakov, V. I. 2006 Methods for prevention of mass development of the cyanobacterium *Microcystis aeruginosa* Kutz emend Elenk. in aquatic ecosystems. *Mikrobiologiia* **75**(2), 149–153.

Mulderij, G., Mooij, W. M., Smolders, A. J. P. & Donk, E. 2005 Allelopathic inhibition of phytoplankton by exudates from Stratiotes aloides. *Aquat. Bot.* **82**(4), 284–296.

Nakai, S., Zhou, S. & Hosomi, M. 2006 Allelopathic growth inhibition of cyanobacteria by reed. *Allelopathy J.* **18**(2), 277–285.

Newman, J. R. & Barrett, P. R. F. 1993 Control of *Microcystis aeruginosa* by decomposing barley straw. *J. Aquat. Plant Manage.* **31**, 203–206.

Pitois, S., Jackson, M. H. & Wood, B. J. 2001 Sources of the eutrophication problems associated with toxic algae: an overview. *J. Environ. Health* **64**(5), 25–32.

Wang, L. X., Zhang, L., Zhang, Y. X., Jin, C. Y., Lu, C. M. & Wu, G. R. 2006 The inhibitory effect of *Hydrilla verticillata* culture water on *Microcystic aeruginosa* and its mechanism. *Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao* **32**(6), 672–678.

Welch, I. M., Barrett, P. R. F., Gibson, M. T. & Ridge, I. 1990 Barley straw as an inhibitor of algal growth I: studies in the Chesterfield Canal. *J. Appl. Phycol.* **2**(3), 231–239.

Wu, Z. B., Zhang, S. H., Wu, X. H., Cheng, S. P. & He, F. 2007 Allelopathic interactions between *Potamogeton maackianus* and *Microcystis aeruginosa*. *Allelopathy J.* **20**(2), 327–338.

Xian, Q. M., Chen, H. D., Zhou, H. X. & Yin, D. Q. 2007 Allelopathic activity and nutrient competition between *Ceratophyllum demersum* and *Microcystis aeruginosa*. *Allelopathy J.* **19**(1), 227–232.

Yan, D., Jin, C., Xiao, X. H. & Dong, X. P. 2008 Antimicrobial properties of berberines alkaloids in *Coptis chinensis Franch* by microcalorimetry. *J. Biochem. Biophys. Methods* **70**, 845–849.

Zhang, W. H., Zhou, L. F., Wu, X. G. & Song, L. R. 2006 Allelopathic effect of *Acorus calamus* on *Microcystis aeruginosa*. *China Environ. Sci.* **26**(3), 355–358.

Zhou, L. H., Zheng, T. L., Wang, X., Ye, J. L., Tian, Y. & Hong, H. S. 2007 Effect of five chinese traditional medicines on the biological activity of a red-tide causing alga-Alexandrium tamarense. *Harmful Algae* **6**, 354–360.

Zhou, Y. H. & Huang, H. L. 2003 Effects of three kinds of Chinese herb on growth and chlorophyll-a levels of *Pavalova viridis*. *Fish Sci.* **22**(4), 10–13.