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Effects of Allelochemicals from *Ficus microcarpa* on *Chlorella pyrenoidosa*

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ABSTRACT

This study was performed in order to isolate and identify unknown allelochemicals from Ficus microcarpa, *and to investigate the inhibitory to bloom-forming of green alga* Chlorella pyrenoidosa. *Through gradient elution, fraction C2, whose inhibition of alga growth in diverse extracts was the strongest was shown to cause significant reductions of maximum quantum yield, as well as electron transport rates of* C. pyrenoidosa. *The study data also showed that the increase of fraction C2 concentration decreased the activity of total superoxide dismutase (SOD), but increased the activities of catalase (CAT) and malondialdehyde (MDA) content. These results demonstrate that the active fraction C2 not only induced the photoinhibition or photodamage of PSII reaction centers, but also triggered the synthesis of reactive oxygen species which may change cell membrane penetrability, thereby leading to the eventual death of* C. pyrenoidosa. *Furthermore, the gas chromatography/mass spectrometry (GC/MS) analyses showed that the most potential allelochemical in active fraction C2 was 2-Propyl phenol, which may exhibit potent allelopathy.*

Key words: Allelochemical, Phenols, Photosynthesis, *Ficus microcarpa*, *Chlorella pyrenoidosa*

INTRODUCTION

Allelopathy is the release of organic compound by plants or bacterial species that affects other plants or bacterial species, which is regarded as a form of interference competition (Yang et al. 2011). Allelochemicals from macrophytes or other organisms which inhibit microalgal growth have gained great interest due to their environmental potential as algaecides to control harmful algal blooms (HABs). HABs occurring in eutrophic water bodies have been a great threat to the effective utilization of water resources such as fisheries and water supplies in recent years. A variety of methods have been recommended for removing or inhibiting HABs, but most are impractical due to high cost or subsequent secondary pollution (Anderson 1997; Achnine et al. 1999). As a low-cost and environment-benign method, the use of allelopathy to control HABs has become a hot research topic recently (Hong et al. 2008a).

Many types of effective allelochemicals originating from plants which possess bloom-forming algae growth suppression effects have been isolated and identified. According to previous studies, N-phenyl-2-naphthylamine extracted from water hyacinth has been reported as one type of strong anti-algal allelochemical (Sun et al. 1993). Nakai reported that *Myriophyllum spicatum*

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inhibited *Selenastrum capricornutum* and *Microcystis aeruginosa* coexisting in a culture (Nakai 1999), along with polyphenols (Nakai 2000) and fatty acids (Nakai 2005) from *Myriophyllum spicatum* exhibited growth inhibition effects. The allelopathic compound isolated from *Phragmites communis*, identified as ethyl 2-methylacetoacetate (EMA) (Li and Hu 2005a), showed strong inhibition activity on the growth of *Chlorella pyrenoidosa* and *Microcystis aeruginosa* (Li and Hu 2005b). They also found that 3-(dimethylaminomethyl)indole (i.e. gramine, an alkaloid) from *Arundo donax L.* was an inhibitory allelochemical on *Microcystis aeruginosa*, and proved it as one of the strongest antialgal allelochemicals originating from aquatic plants (Hong et al. 2010).

Previous approaches have mainly focused on the isolation of allelopathic compounds from submerged plants (Nakai and Hosomi 2002; Gross 2003; Mulderij et al. 2003), emerged macrophytes (He and Wang 2001; Li and Hu 2005; Hong et al. 2010) and floating plants (Sun et al. 1993). In addition to aquatic macrophytes, a small number of terrestrial plants also exhibited allelopathic effects on algae. The presence of decomposing barley straw (Ball et al. 2001) and *Lantana camara* (Kong et al. 2006) reduced the growth of some algal species under field or laboratory conditions. Compared with other terrestrial plants and aquatic macrophytes, woody plants with a wide range of raw material sources may more easily produce a larger amount of allelochemicals (Pohjamo et al. 2003), but little work has been done in using woody plants to control algae bloom.

Another important point is that the target systems of allelochemicals in algae are unclear. Allelochemicals are likely to alter a variety of physiological processes of algae, such as ion and water uptake, cell division, respiration, photosynthesis, enzyme function, signal transduction, etc. (Inderjit and Duke 2003; Hong et al. 2008). Photosynthesis is one of the most important metabolic activities of algae, as it is the main source of biomass and energy for the support ecosystem in crust (Wang et al. 2008). Evidence has shown that the photosystem of phytoplankton is inhibited or damaged under different types of stress, including high temperature (Sayed and EI-Shahed 2000), high light (Banet et al. 1999), salt stress (Lu et al. 2002), nutritional deficiency (Lurling et al. 2006), UV-B damage (Wang et al. 2008) and heavy metal (Mallick and Mohn 2003). Algal cells will reduce energy transfer and usage. Fluorescence intensity from photosystems, electron transport rates and effective quantum yield were diminished as well. The inhibition or damage of photosynthesis appears to be a widespread mode of action for chemically different aquatic allelochemicals (Körner and Nicklisch 2002). Some allelochemicals isolated from aquatic macrophytes have been shown to significantly inhibit the activity of photosynthesis or antioxidant enzymes in several species of algae (Sukenik et al. 2002; Qian et al. 2009). It has also been reported that the photosystem or enzyme function of phytoplankton is probably an important target of some allelochemicals. The present study, which addresses both of the questions above, will provide insight into the mechanism of woody plant allelopathy in aquatic ecosystems.

Our previous research revealed that the extracts from leaves of *Ficus microcarpa (F. microcarpa)* are capable of significantly inhibiting the growth of *C. pyrenoidosa* in southern China (Li et al. 2010). However, the allelochemicals and mechanisms underlying the anti-algae of *F. microcarpa* extracts are still not unraveled. In this study the allelochemicals from *F. microcarpa* extracts were isolated and identified, and the inhibition mechanism of *C. pyrenoidosa* growth was also investigated by photosynthesis and antioxidant enzymes measurement, as the result of exposure *C. pyrenoidosa* to allelochemicals.

MATERIALS AND METHODS

Plant materials and bioassay alga preparation

C. pyrenoidosa was obtained from the Freshwater Algae Culture of Hydrobiology Collection (Wuhan, China) and cultivated in the following nutrient solution: ($NaNO_3$ (250 mg), $FeCl_3$ (16.2 mg), $K_2HPO_4 \cdot 3H_2O$ (75 mg), Na_2EDTA (200 mg), $MgSO_4 \cdot 7H_2O$ (75 mg), H_3BO_3 (2.86 mg), $CaCl_2 \cdot 2H_2O$(25 mg), $MnCl_2 \cdot 4H_2O$ (1.81 mg), KH_2PO_4 (175 mg), $ZnSO_4 \cdot 7H_2O$ (0.22 mg), NaCl (25 mg), $CuSO_4 \cdot 5H_2O$ (0.079 mg), $FeCl_3 \cdot 6H_2O$ (0.5 mg) and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.039 mg) in 1000 mL of distilled water), at 25 ± 1^{o}.C, and exposed to approximately 50 μM photons$\cdot m^{-2} \cdot s^{-1}$ light intensity until the concentration of algae reached about 10^5 cells$\cdot mL^{-1}$. *Ficus microcarpa* plants were collected from Xiamen, China.

Isolation and identification of antialgal compounds

The antialgal compounds were isolated and identified in view of methods with minor modifications from Xian et al. (2006). Leaves of *F. microcarpa* (1000 g) were extracted with ultrasonic-assisted extraction in 10 L 95% (v/v) ethanol. After 60 min, the solvent was removed from the extract through vacuum filtration. The extract was suspended in distilled water, partitioned sequentially with 0.5 L petroleum ether, $CHCl_3$ and EtOAc, then *n*-BuOH and five fractions (marked A-E) were obtained. Next, the EtOAc fraction with the strongest allelopathic potential on *C. pyrenoidosa* was subjected to the silica gel chromatography column with 150 mL of $CHCl_3$-MeOH (1:0, 3:1, 1:1, 1:3, 0:1,v/v). Five fractions (C1-C5) without solvent were obtained, among which the second fraction with highly inhibitory activity on *C. pyrenoidosa* was recrystallized three times with MeOH.

The constituents of fraction C2 were analyzed by gas chromatography-mass spectrometry (GC-MS) (Agilent 7890A/5975C) equipped with an HP-5 column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas, and the temperature for injection port and transmission line was 280 ºC. The temperature programming was set with an initial oven temperature of 50 ºC and held for 3 min, then programmed to increase to 170 °C at a rate of 20 °C/min, then kept constant for 2 min, and the final temperature of the oven was 280 ºC with rate at 10 ºC·min^{-1}. A 1 μL sample was injected with splitless mode. Mass spectra were recorded over a 30-500 amu range with electron impact ionization energy at 70 eV.

Bioassay

Assay of *C. pyrenoidosa* growth inhibition was performed by the US EPA (ISO 8692, 1989) and Hong et al. (2010) method with minor modifications. The potential allelopathic of the isolated fractions (fractions A-E and C1-C5) from *F. microcarpa* Leaves, which were pre-dissolved in *dimethyl sulphoxide* (DMSO), were evaluated at different concentrations by algal bioassays with *C. pyrenoidosa.* A volume of 100 mL algal medium, which had been sterilized by autoclaving for 0.5 h, was inoculated with algae and fractions in a 250 mL Erlenmeyer flask, using three replicates. The control group was prepared without the fraction. Algae was allowed to grow for 7 d, with the growth monitored every 24 h using a microscope and a haemocytometer to count cell number at the stationary phase. Note: Pre-experiments proved that when the proportion of DMSO added was less than 1 % (v/v), the growth of *C. pyrenoidosa* was not affected, so the final proportion of DMSO added did not exceed 1% (v/v) in the later experiments.

The percentage of growth inhibition rate (*IR*) at the specific test substance concentration was calculated as follows:

$$IR(\%)=(1-N_e/N_t)\times 100,$$

where N_e and N_t are the numbers of algal cells in the treatment groups (added with extract, fractions or potential allelochemicals) and the controls, respectively (unit: cell·mL^{-1}). Positive *IR* shows inhibitory effects on algal growth.

Determination of photosynthetic activity

Variable fluorescence on the algal samples was measured by a pulse amplitude modulated fluorometer (Phyto-PAM Walz, Effeltrich, Germany). Before fluorescence measurement, treated algal samples were adapted to darkness to obtain an oxidized steady state of PSII. The initial fluorescence (F_o) and maximal fluoroscence (F_m) was determined at the beginning of each measurement, and the variable fluorescence (F_v) was obtained. F_s (steady-state fluorescence) was achieved by illuminating *C. pyrenoidosa* for 2 min with an actinic light at 64 μmol photons·m^{-2}·s^{-1}, a light intensity similar to that used during cultivation. The time of actinic light exposure was optimized before operation. Then a saturating light pulse was produced to obtain the F_m (maximal fluorescence in the light adapted state). According to Genty et al. (1989), the maximum quantum yield (F_v/F_m) of the charge separation of PSII in actinic light was calculated as follows: $F_v/F_m = (F_m - F_o)/ F_m$.

Rapid light curves (RLCs) were constructed by exposing the samples for 20 s to each of 12 increasing actinic light levels and by calculating the relative electron transport rate (ETR) from the delivered actinic irradiance and the effective quantum yield of PSII by ETR=PAR·YII·0.84·0.5. PAR denotes the incident photosynthetic active radiation, in which 0.84 is a widely accepted absorptivity value and the factor 0.5 corrects for an assumed equal distribution of the absorbed quanta between PSII and PSII (Björkman and Demmig 1987). The light response of microalgae was

characterized by fitting the model of Platt et al. (1980) to the ETR versus PAR curves and by estimating ETRm (maximum ETR).

Measurement of enzymatic antioxidant

Total superoxide dismutase (SOD) activity was determined by the method of Giannoplities and Ries (1977). One unit of SOD was defined as the amount of enzymes resulting in 50% inhibition of the photochemical reduction of nitroblue terazolium (NBT). The assay for catalase (CAT) activity was based on its ability to decompose H_2O_2, with the absorbance of supernatant at 240 nm (Rao et al. 1996). One unit of CAT was defined as the decrease of absorbance at 240 nm, up to 0.1 in 1 min. Lipid peroxidation product as malondialdehyde (MDA) was measured spectrophotometrically using the thiobarbituric acid (TBA) method (Heath and Parker 1968).

RESULTS AND DISCUSSION

Effects of *F. microcarpa* extracts on the growth of *C. Pyrenoidosa*

The antialgal activities of the five fractions obtained by ethanol extract from leaves of *F. microcarpa* were evaluated by algal bioassays with *C. pyrenoidosa* (Fig. 1A). All data used in this study were tested by means of ANOVA. The strongest inhibitory effect occurred on the fraction C, and the average percentage inhibition of growth rate was over 80% beginning on the second day ($p<0.05$). In addition, the fractions C and D also exhibited quick inhibitory effects on algal growth, implying their potential use for algal-bloom control. In contrast, the inhibition rate of fraction A was poor, even lower than that of the control beginning on the third day, suggesting that fraction A stimulated the algal growth. The stimulation effect of fraction A, what we referred to as 'Hormesis', was similar to the results involving other plant extracts and allelochemicals (Nakai et al. 1996; Körner and Nicklisch 2002; Mulderij et al. 2007; Hong et al. 2010). Fraction C showed the strongest inhibitory activity on the growth of algae, followed by fraction D, while fractions A and B boosted the algal growth inversely. Therefore, it is shown that the inhibitory fractions and potential allelochemicals must be isolated and characterized.

The further separation of fraction C was processed by silica gel column chromatography. Five fractions (C1-C5) were obtained, and their antialgal activities were studied (Fig. 1B). The results demonstrated that all of the fractions possessed inhibitory activities on algal growth after 5 d, and the antialgal activities were **C2** > **C1** > **C5** > **C3** > **C4**. Fraction C2 showed highest inhibitory activity ($p<0.05$), and was chosen for further study.

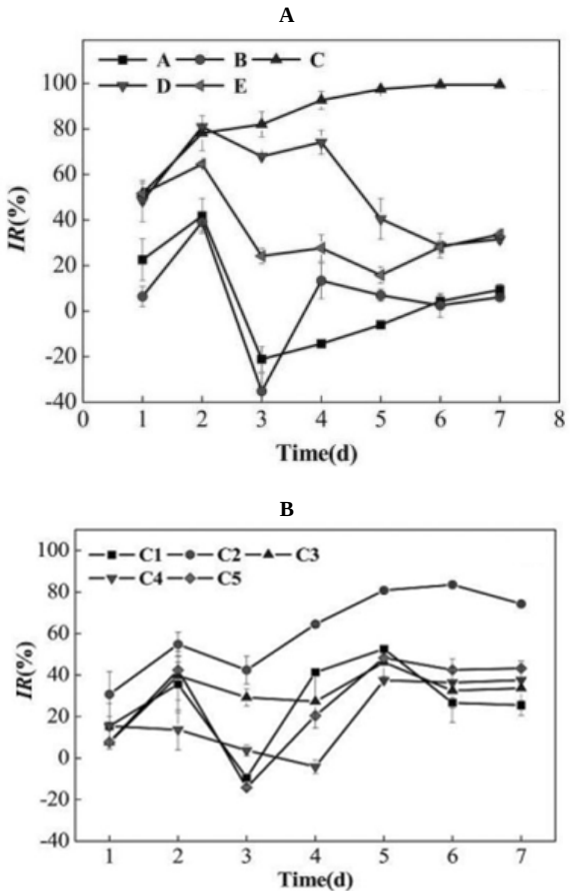

Figure 1 - Effects of fractions A-E **(A)** and C1-C5 **(B)** on the growth of *Chlorella pyrenoidosa.*

In order to verify the inhibitory effects of fraction C2 on the growth of *C. pyrenoidosa*, the dose-response relationships between the active compounds were examined (Fig. 2). It was demonstrated that the inhibitory effects of fraction C2 was concentration-dependent to degree certain extent. Although four test concentrations had all strong inhibitions on *C. pyrenoidosa*, the antialgal effects of the components with high concentration was slightly better than those with low concentration. The inhibition rate reached almost 100% when exposed to 0.4 $g \cdot L^{-1}$ after 5 d, while the growth condition of algae cells in the control

group was good. Under microscope, there was hardly any survival of algae cells, and the growth of subsequent algae cells showed no recovery phenomenon. At an initial *C. pyrenoidosa* density of 2×10^5 cells·mL^{-1}, the $EC_{50,\ 96h}$ of the allelochemicals was 0.065 g·L^{-1}.

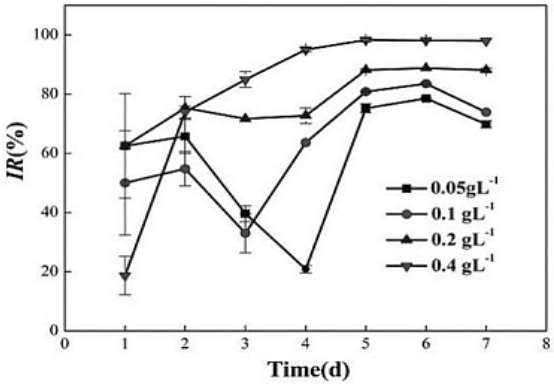

Figure 2 - Effects of fraction C2 on the growth of *Chlorella pyrenoidosa.*

Effects of *Ficus microcarpa* fraction C2 (FFC) on the photosynthetic activity of *C. pyrenoidosa*

FFC significantly decreased the photosynthetic activity of *C. pyrenoidosa*, as shown in Figure 3. Increasing the FFC concentration resulted in an increase in the inhibition of photosynthetic quantum yield. It was on the first day that the inhibition of the yield was most significant, and particularly worth noting was that the yield of the 0.4 g·L^{-1} FFC treatment was only 0.0365, only 1/20 of the control (0.73). As the exposure time was extended, the yield inhibition of the other treatments gradually decreased, except for the 0.4 g·L^{-1} FFC treatment, the yield of which remained low for all seven days.

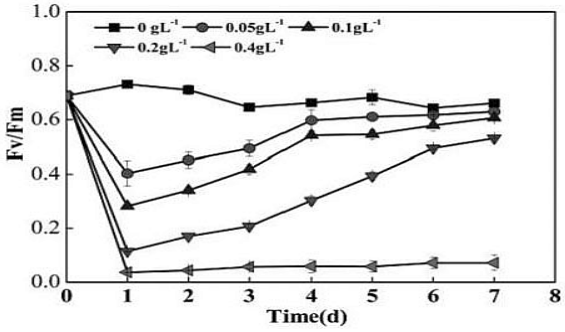

Figure 3 - Changes of photosynthetic activity of *Chlorella pyrenoidosa* in response to FFC.

The maximum quantum yield (F_v/F_m) provides an accurate approximation of the maximum photochemical efficiency of photosystem II (PSII) (Titlyanov et al. 2007). The decrease of the apparent yield suggests that the primary acceptor of PSII, Q_A, is fully reduced by the actinic light, and that there is a transient accumulation of reduced pheophytin during saturating light pulses (Goh et al. 1999). These observations suggest that the decrease of F_v/F_m is caused by the separation of LHCII from the PSII core complexes. The inhibition of electron flow from Q_A to Q_B is similar to that found with the effect of tricolorin A on chloroplasts (Achnine et al. 1999), or is due to the reduction of Q_A followed by the reduction of P_{680} by Z without the direct participation in situ of oxygen evolving complex (OEC) (Strasser 1997). Hernandez-Terrones et al. (2003) reported that the Q_A-reoxidation was slowed down by both DCMU and trachyloban-19-oic acid at low concentration, in a concentration-dependent manner.

The photosynthetic activity of *C. pyrenoidosa* recovered to different degrees after 1 d for exposure adaptation. With regard to the low treatment concentration groups, the activities returned to the normal state, as did the control, while the high treatment concentration group (e.g. 0.4 g·L^{-1}) did not. The results implied that the high concentration of FFC may cause permanent damage to the photosynthetic apparatus.

Comparing the maximum electron transport rates (ETRm) in five concentrations of allelochemicals, clear differences were found between the control and FFC treatments (Fig. 4). The decrease of ETRm, which is a characteristic of photosystems representing the capacity to convert light energy into chemical energy, may correlate with the immobilization of the chloroplast and inhibition of the photosynthetic capacity (Zhu et al. 2010).The ETRm of *C. pyrenoidosa* was inhibited from the first day to the fourth day at 0.05~0.4 g·L^{-1} of FFC, respectively, and the ETRm decrease was concentration-dependent. The results indicated that the ETRm of *C. pyrenoidosa* appeared to be affected significantly on the first day at the same biomass density (p<0.05). After 5 d, the ETRm of the 0.05 and 0.1g·L^{-1} treatments were slightly lower than that in the control (p>0.05). However, the ETRm of the 0.4 g·L^{-1} FFC treatment was no more than 13.8 at 7 d, much lower than the control group (89.35~135.55, p<0.05).

Polyphenolic allelochemicals produced by higher plants can pass through cell membranes and

interrupt the electron transfer chain due to interference with nonheme iron (Leu et al. 2002; Dziga et al. 2007). The ETR provided evidence that the treatment had less efficient energy transfer and usage rate than the control group, and a striking decrease was observed especially clearly in high concentrations of allelochemicals. The difference in energy transfer and usage may explain why the treatment had a lower growth rate than the control group (Wang et al. 2005). This tendency mimicked irreversible photosynthesis inactivation caused by the disorganization of PSII reaction centers (photodamage).

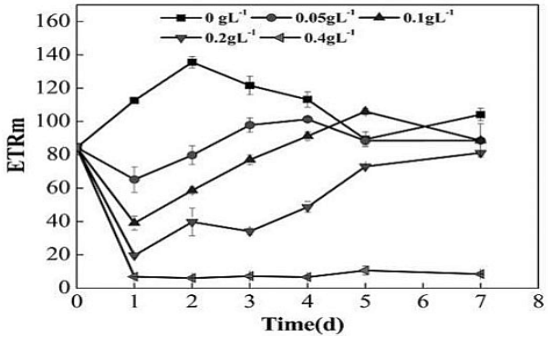

Figure 4 - Effects of FFC on maximum electron transport rate (ETRm) for *Chlorella pyrenoidosa.*

Effects of FFC on SOD, CAT activities and MDA content of *C. pyrenoidosa*

When the cells of *C. pyrenoidosa* were exposed to FFC, SOD activity decreased with the increasing allelochemical concentration (Fig. 5 A). After 24 h of exposure to a concentration series of FFC (with the exception of 1.0 $g \cdot L^{-1}$), the SOD activity did not change significantly. As the exposure time was extended to 96 h, the decrease became more marked above 0.6 $g \cdot L^{-1}$. In the algal cells exposed to 0.6, 0.8 and 1.0 $g \cdot L^{-1}$ of FFC, SOD activity gradually reduced from 5.41, 5.18 and 3.47 $U \cdot 10^{-7}$ cells at 24 h to 1.81, 0.81 and 1.56 $U \cdot 10^{-7}$ cells at 96 h, only 26.88, 12.04 and 23.23% of the control after 96 h ($p<0.05$).

It is well known that most types of environmental stress affect the production of active oxygen species in plants and cause oxidative stress (Smirnoff 1993). Recent studies have suggested that some allelochemicals, which act as an environmental stress, can increase the production of $O_2^{-}\bullet$ in cells (Hong et al. 2008). Therefore, oxidant damage may be an important mechanism for the allelopathy of *F. microcarpa* on *C. pyrenoidosa* cells. In addition, $O_2^{-}\bullet$ derived from the initial electron donation is a precursor of reactive oxygen species, and limits the reactivity of molecular oxygen (O_2). Once the unfavorable $O_2^{-}\bullet$ has been produced, the oxidation of reactive oxygen species will propagate in a variety of other biological molecules, including H_2O_2, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA (Shao et al. 2009).

SOD can specially catalyze the conversion of $O_2^{-}\bullet$ into H_2O_2 and O_2, acting as the first line of defense against the potential toxicity of superoxide radical. (Bernroitner et al. 2008; Zamocky et al. 2008). CAT also plays an important role in protecting cells from oxidative damage, by catalyzing the H_2O_2 into H_2O and O_2 (Bernroitner et al. 2008; Zamocky et al. 2008). In this study, the decrease of SOD activity in *C. pyrenoidosa* may directly reflect the degradation of cellular detoxification to $O_2^{-}\bullet$ leaking mainly from the energy centers. This may be due to the acute contact damage from this high concentration of *F. microcarpa* allelochemicals exposed to *C. pyrenoidosa* cells.

CAT activity is shown in Figure 5B. After 24 h of exposure, FFC caused the activity to increase, but only SOD activities in the algal cells exposed to 0.8, 1 $g \cdot L^{-1}$ of allelochemicals had significant effects relative to the controls ($p<0.05$). CAT activity in the algae exposed to high concentrations of FFC appeared to increase compared with those exposed to low concentrations. The increase was more apparent when the exposure time extended to 96 h. The highest activity of CAT was about 12.22 times that of the controls at 96 h.

The increase in CAT activity through *F. microcarpa* allelochemicals exposure suggests that the cellular resistance to oxidative stress was initiated to cope with the overproduction of H_2O_2. An increase in the activity of CAT implies an increase in H_2O_2 production. In the present study, SOD activity was reduced, thus the increase in H_2O_2 must be derived from amine oxidase, peroxidase and oxalate oxidase which could also supply H_2O_2 in addition to SOD (Wojtaszek et al. 1997). The enhancement of CAT activity presented in this study suggested that *C. pyrenoidosa* cells encountered oxidant stress when exposed to *F. microcarpa* allelochemicals, supporting the hypothesis that oxidant damage is an important mechanism for the allelopathy of *F.*

microcarpa allelochemicals to microalgae, such as *C. pyrenoidosa*.

The MDA, an indicator of lipid peroxidation, showed a concentration-dependent change in response to allelochemicals exposure at 96 h (Fig. 5C). At this exposure time, the significant increases in MDA content of treatment groups appeared at 0.6, 0.8 and 1.0 $g \cdot L^{-1}$ of FFC, while the marked decreases occurred at 0.4 and 1.0 $g \cdot L^{-1}$. From 0 to 1.0 $g \cdot L^{-1}$ of allelochemicals at 24 h of exposure, MDA content transitioned from a decrease to an increase, then to a decrease again.

The cell membrane is the target for many antimicrobial agents (Sun et al. 2004). The lipid membranes containing polyunsaturated fatty acids are most susceptible to oxidation (Floyd 1990), and MDA is the oxidative product of unsaturated fatty acids. Next, the MDA concentration in *C. pyrenoidosa* cells under *F. microcarpa* allelochemicals stress was determined. Compared with the control, the MDA content was significantly higher at allelochemical concentrations over 0.6 $g \cdot L^{-1}$ using 96 h of the treatment, demonstrating that the polyunsaturated fatty acids underwent oxidative damage.

In addition, the autoxidation, occurring in the reaction of the hydroxy of allelochemicals and transition metal ion (Cu^{2+}, Fe^{3+}) in the medium, produce H_2O_2, which may make directly oxidative damage to DNA and induce programmed cell death (Slater 1995; Ross 2006; Dziga 2007). This kind of hypothesis may be supported in more studies in the future.

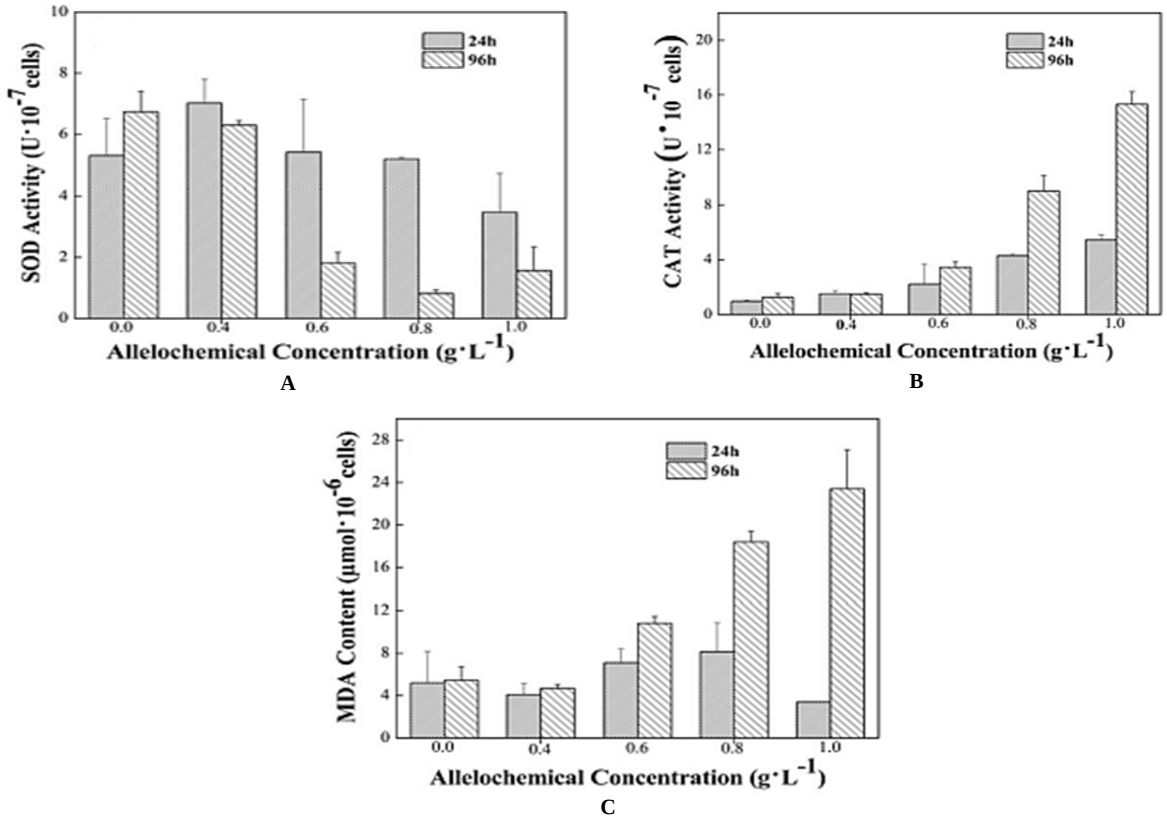

Figure 5 - Effects of FFC on SOD **(A)**, CAT **(B)** and MDA **(C)** of *Chlorella pyrenoidosa.*

Identification of antialgal compounds

Increasing the FFC concentration increased the inhibition, suggesting that the FFC may contain growth inhibitory substances and possess allelopathic potential. The analysis with GC-MS identified six constituents from FFC (Fig. 6A). As illustrated in Figure 6B), the mass spectral pattern of the peak occurring at 12.774 min and the main component corresponded to 2-*Propyl phenol.*

Allelopathic effects of plants on algal growth have been studied for several decades, and a variety of antialgal allelochemicals have been isolated and identified, including sesquiterpene lactones, organic acids, quinines, coumarins, flavonoids,

tannins and phenols (Rice 1984; Nakai et al. 2001; Kato-Noguchi 2003). Greca et al. (1998) reported some antialgal phenols and polyphenols isolated from *Zantedeschia aethiopica*. Nakai et al. (2000) showed that *M. spicatum* released tellimagrandin II, ellagic, gallic and pyrogallic acids and (+)-catechin, and that each compound produced an inhibitory effect. Xian et al. (2006) studied inhibitory activity of aquatic extracts of submerged macrophytes on *M. aeruginosa* and found that allelopathic activity was affected by the content of phenolic acids which was lower in *Vallisneria spiralis*. Similarly, our results have shown that the antialgal allelochemical isolated from *F. microcarpa* was 2-*Propyl phenol*, which may have been responsible for inhibiting the growth of *C. pyrenoidosa.* It has yet to be reported that 2-*Propyl phenol* is a potent allelopathic substance in *F. Microcarpa.*

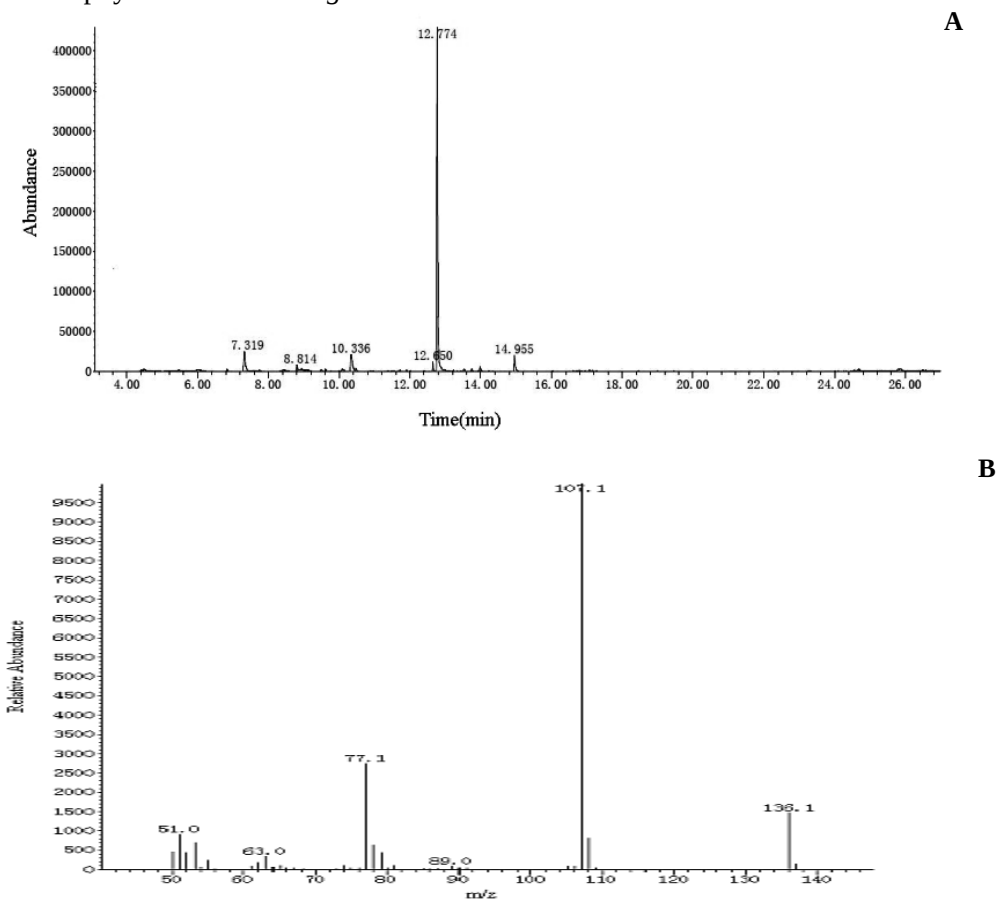

Figure 6 - Results of GC-MS analysis of Fraction C2: **(A)** Gas chromatogram; **(B)** mass spectrum of compound with a retention time of 12.774 min.

CONCLUSIONS

In this study, the unknown allelochemical FFC, isolated from *Ficus microcarpa*, showed inhibition of *Chlorella pyrenoidosa* growth, through inducing the photoinhibition or photodamage of PSII reaction centers and triggering the synthesis of reactive oxygen species. Furthermore, we identified the primarily active fraction of FFC as 2-*Propyl phenol*, which may be a major influence in the effective inhibition of the growth, photosynthetic activities and antioxidant enzymes activities of *C. pyrenoidosa.* However, more studies examining the effects of pure allelochemical (i.e. 2-*Propyl phenol*) on algae are required in the future. In addition, it was found that *Ficus microcarpa* extracts presented the 'Hormesis' phenomenon acting on *C. pyrenoidosa* growth, but we suggest that the action modes of pure allelochemicals are distinct from the active fraction.

ACKNOWLEDGMENTS

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