Inhibitory effects of extracts from *Cinnamomum camphora* fallen leaves on algae

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**ABSTRACT**

Natural allelochemicals are considered as a source of algaecides. To uncover the anti-algal activity of *Cinnamomum camphora* fallen leaves and promote their usage as algaecides, the composition of their water and methanol extracts was analyzed, and the inhibitory effects of extracts on the growth of *Microcystis aeruginosa* and *Chlamydomonas reinhardtii*, and chlorophyll (Chl) content and photosynthetic abilities in *C. reinhardtii* were investigated. Twenty-five compounds were detected in the water extracts, mainly including terpenoids, esters, alcohols, and ketones. Compared to water extracts, there were more compounds and higher concentration in methanol extracts. Both water and methanol extracts inhibited the growth of the two algae, and 15 mg·ml⁻¹ methanol extracts killed the algal cells after 48 h. The levels of Chl a and Chl b, as well as maximum quantum yield of photosystem II photochemistry (Fv/Fm) in *C. reinhardtii* cells reduced gradually with increasing the concentration of extracts, while the maximum quantum yield of non-photochemical de-excitation (φD₂) increased gradually. At the same concentration, methanol extracts showed stronger inhibitory effects than water extracts, due to their higher number of compounds and higher concentration. Therefore, *C. camphora* fallen leaves have a potential value as an algaecide.

**Key words** | algaecide, *Cinnamomum camphora*, methanol extract, photosynthesis, water extract

**INTRODUCTION**

With the increasing input of nutrients mainly nitrogen (N) and phosphorus (P) to waters, eutrophication becomes more serious (Cloern 2001) and promotes the excessive growth of cyanobacteria and green algae, and even blooms. Algae blooms can lower the water quality and cause a series of ecological problems (Dodds et al. 2009; Qin 2009). Algae release an abundance of volatile organic compounds (VOCs), which frequently lead to an unpleasant, earthy–musty odor in the water. Geosmin and 2-methyl borneol are considered as the main compounds to cause the odor (Fujise et al. 2010). In addition, these VOCs can inhibit other algae growth by inducing photosynthetic pigment degradation and inhibiting photosynthesis (Zhao et al. 2016; Xu et al. 2017; Ye et al. 2018). Besides VOCs, algae can also produce lots of toxins, including microcystin, hepatotoxins, neurotoxins, neosaxitoxins, anatoxin-a, etc. (Codd 2000; Frangópulos et al. 2004). Previous studies have reported that algal toxins showed inhibitory effects on the growth of other algae (Sanna et al. 2004; Li & Li 2012), aquatic plants (Pflugmacher 2002), zooplankton (Abrantes et al. 2006), and even fishes (Guzmán-Guillén et al. 2015). Moreover, they are a potential hazard to human health through the usage of the water for drinking and recreation (Hoeger et al. 2007).

For the benefit of ecosystems and human health, extensive methods have been developed to control the growth of undesired algae, including the usage of yellow loess (Choi et al. 1999), biquaternary ammonium salt (Liu et al. 2004), TiO₂ (Kim & Lee 2005), copper sulfate (Costas & Lopez-Rodas 2006; Song & Wang 2015), sediment capping

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(Huang et al. 2011), phosphorus inactivation (Lürling & van Oosterhout 2015), and also biomitigation such as with viruses (Garry et al. 1998) and bacteria (Park et al. 1998; Cai et al. 2011). During the experiments, these methods seem to be efficient in controlling the algal growth, but they may bring potential disaster to the environment (Jeong et al. 2000; Song & Wang 2015) and have high financial costs (Kim & Lee 2015; Huang et al. 2011; Lürling & van Oosterhout 2015) once they are used in the field.

Plant allelochemicals are natural compounds, which effectively inhibit neighbor plant growth and can be degraded in nature (Zuo et al. 2011; Zhang et al. 2012). They have been considered as a source of potential agents for algacides (Zhou et al. 2008; Ni et al. 2012; Pęczula 2013). Some plants have been found to have inhibitory effects on algal growth, e.g., extracts from Rhizoma coptidis and Semen arecae on Alexandrium tamarense (Zhou et al. 2007), garlic solution on A. tamarense, A. satoanum, A. cate-nella and Scrippsiella trochoidea (Zhou et al. 2008), and grape extracts on Chlamydomonas reinhardtii (Zuo et al. 2015). When Microcystis aeruginosa cells were treated with the extracts from Artemisia annua (Ni et al. 2012) and Iris wilsonii (Chen et al. 2012), a remarkable inhibition was found on the cell growth, and the anti-algal activity compounds are artemisinin in A. annua, and phenolics and tannin in I. wilsonii. Although lots of plants have inhibitory effects on algae, the usage of plant wastes from agricultural production as algacides was more economically favorable and environmentally friendly, such as rice hulls (Chung et al. 2007), barley straws (Grover et al. 2007; Pęczula 2013), and grape pruning wastes (Zuo et al. 2015).

Cinnamomum camphora (L) Presl is an evergreen landscaping and forestation tree species, drops old leaves in May to June, and is widely planted in the south of China. This species synthesizes abundant secondary metabolites, mainly terpenoids, which can repel herbivore attack and resist fungal infection (Frizzo et al. 2000; Chen & Dai 2012; Yang et al. 2014), indicating that the plants may have the inhibitory effects on algae. To develop effective algacides using C. camphora wastes, the components of the extracts from C. camphora fallen leaves were analyzed, and the inhibitory effects of the extracts on the growth of typical species of cyanobacteria (M. aeruginosa) and green algae (C. reinhardtii) were investigated. Meanwhile, the variation of photosynthetic pigment and photosynthetic abilities in C. reinhardtii were measured to uncover the inhibitory mechanism of C. camphora extracts on photosynthesis.

MATERIAL AND METHODS

Cell cultures

*M. aeruginosa* FACHB-912 provided by Freshwater Algae Culture Collection at the Institute of Hydrobiology, China, and *C. reinhardtii* strain CC-125 wild type mt+ [137c] from Dr E. H. Harris (Duke University, Durham, NC, USA) were grown in BG-11 (Rippka et al. 1979) and tris-acetate-phosphate (TAP) (Gorman & Levine 1965) medium, respectively. They were kept in 16 h light (30 μmol-m⁻²-s⁻¹)/8 h dark, with temperature at 25 °C. They were used for experiments when their density reached the mid-logarithmic phase. The cell density was determined by using the blood cell counting plate, with each value being the means of six repeats.

Preparation of *C. camphora* fallen leaf extracts

Fallen leaves from *C. camphora* were collected in Zhejiang A & F University (30°15’ N, 119°45’ E) in May to June. The leaves were dried using a drying oven at 60 °C, and smashed with a pulverizer. The pulverized materials of 10 g were extracted with 100 ml distilled water and 50% methanol, respectively, at 25 °C for 48 h. The water- and methanol-extracted solution was centrifuged at 5,000 r·min⁻¹ for 6 min, and then its concentration was 100 mg·ml⁻¹.

Treatments with fallen leaf extracts

The fallen leaf extracts were added into the BG-11 medium to treat *M. aeruginosa* (6 × 10⁶ cells·ml⁻¹), and into TAP medium to treat *C. reinhardtii* (2 × 10⁶ cells·ml⁻¹), with the concentration of 1, 5, 10 and 15 mg·ml⁻¹, respectively. The medium was added into the same amount of distilled water or 50% methanol as the control for water extract treatment and methanol extract treatment, respectively. The live cell density of the two algae were determined by using neutral red staining method (Wang et al. 2007) after 24 h and 48 h treatment, and the chlorophyll (Chl) content per 10⁶ cells and Chl fluorescence parameters per 10⁶ cells in *C. reinhardtii* were determined after 48 h.

Determination of Chl content

*C. reinhardtii* cells of 5 ml were collected by centrifugation and the pellets were resuspended in 5 ml 80% acetone. After removal of insoluble materials by centrifugation, the
Chl content was determined following Arnon’s method (Arnon 1949).

**Measurement of photosynthetic efficiency**

According to our previous method (Zuo et al. 2012), 10 ml *C. reinhardtii* culture was collected by centrifugation and suspended in 10 µl of the same culture medium. The resuspended cells were pipetted on a piece of filter paper to form 0.5 cm² spots. After incubation in darkness for 15 min, their Chl fluorescence was measured by a non-modulation Chl-fluorescence analyzer (Yaxin-1161, Yaxinliyi Science and Technology Ltd Co., Beijing, China) following the procedure of Strasser et al. (1995). Maximum quantum yield of photosystem II (PSII) photochemistry (Fv/Fm) and maximum quantum yield of non-photoschemical de-excitation (φD0) were calculated using the formula given by Strasser et al. (1995).

**Analysis of fallen leaf extracts**

The methanol extracts of 20 ml were distilled to 8 ml at 50 °C (lower than the boiling point of methanol at 64.5 °C) using a rotary evaporator to remove the methanol. The distilled extracts were supplemented to 20 ml using distilled water to keep the extract concentration at 100 mg·ml⁻¹. Six millilitres of removed methanol extracts and water extracts were separately extracted with 1 ml ethyl acetate, and analyzed by gas chromatography mass spectrometry (GC-MS). The GC (7890B, Agilent Technologies Company, CA, USA) was run with a 30 m × 0.25 mm × 0.25 μm HP-5MS capillary column. The temperature of the column was programmed to increase from 50 °C to 180 °C at a rate of 20 °C·min⁻¹ and kept for 4 min. Then it was increased to 220 °C at a rate of 10 °C·min⁻¹ and kept for 15 min. The MS (5977B, Agilent Technologies Company, CA, USA) was run under the following conditions: electron ionization mode of ionization energy at 70 eV and source temperature at 230 °C, mass range between m/z 28 and m/z 450, interface temperature at 250 °C and quadrupoles temperature at 150 °C. The qualitative and quantitative analyses of the GC/MS data were obtained from NIST/EPA/NIH Mass Spectral Library (NIST 14) (National Institute of Standards and Technology, Gaithersburg, USA). D-Limonene, eucalyptol, terpinene, linalool, camphor and E-nerolidol were used as the standard samples to calculate the concentration of the corresponding compounds in the extracts. The concentration of sesquiterpenoids (C15) was calculated referring to E-nerolidol, while the concentration of monoterpenoids (C10) and other compounds was calculated referring to camphor.

**Calculations and statistical analyses**

The response index (RI) was calculated according to the method described by Williamson & Richardson (1988).

\[
RI = \begin{cases} 
1 - \frac{C}{T} & \text{when } T \geq C \\
\frac{T}{C} - 1 & \text{when } T < C
\end{cases}
\]

where C and T are control response and treatment response, respectively. The positive values of RI indicate stimulation by the treatments relative to the controls, while negative values indicate inhibition.

Statistical analyses of one-way analysis of variance and drawing figures were performed with Origin 8.0 (Origin Lab, USA).

**RESULTS**

**Composition of *C. camphora* fallen leaf extracts**

There were 25 compounds in the water extracts from *C. camphora* fallen leaves, mainly including terpenoids, esters, alcohols, and ketones. Among these components, linalool, camphor and 6-epi-shyobunol were the main compounds, with the concentration of 510.6, 666.7 and 161.3 µmol·l⁻¹, respectively. Compared to water extracts, 12 new compounds were detected in the methanol extracts, including ethyl linalool (66.2 µmol·l⁻¹), coumaran (47.0 µmol·l⁻¹), Z-9-tetradecenal (28.0 µmol·l⁻¹), spathulenol (115.9 µmol·l⁻¹), α-santalol (119.7 µmol·l⁻¹), isolongifolol (343.6 µmol·l⁻¹), palustrol (47.2 µmol·l⁻¹), isoaromadendrene epoxide (33.1 µmol·l⁻¹), β-santalol (75.5 µmol·l⁻¹), platambin (53.1 µmol·l⁻¹), methyl linolenate (56.3 µmol·l⁻¹), and octadecanolate acetate (43.2 µmol·l⁻¹). Not only compound types, but the concentration of most of the compounds in methanol extracts was higher than that in water extracts, and their total concentration was three-fold that in water extracts (Table 1).

**Effects of *C. camphora* extracts on algal cell multiplication**

When *M. aeruginosa* cells were treated with water extracts from *C. camphora* fallen leaves at 1, 5, 10 and 15 mg·ml⁻¹ for 24 h, the cell multiplication was markedly inhibited, with the RI of −0.22 (P < 0.01), −0.36 (P < 0.01), −0.39 (P < 0.01) and −0.43 (P < 0.01), respectively. After 48 h,
## Table 1 | The main compounds in *C. camphora* fallen leaf extracts

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Compounds</th>
<th>Formula</th>
<th>Concentration (μmol·l⁻¹)</th>
<th>Water extracts</th>
<th>Methanol extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.515</td>
<td>3-Methyl-2-pentanone</td>
<td>C₆H₁₂O</td>
<td>4.3 ± 0.4</td>
<td>34.0 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>4.756</td>
<td>Isobutyl acetate</td>
<td>C₆H₁₄O₂</td>
<td>11.0 ± 0.7</td>
<td>77.8 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>5.029</td>
<td>Mesityl oxide</td>
<td>C₆H₁₀O</td>
<td>17.8 ± 2.0</td>
<td>59.6 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>5.237</td>
<td>Butyl acetate</td>
<td>C₆H₁₂O₂</td>
<td>3.4 ± 0.6</td>
<td>19.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>7.530</td>
<td>D-Limonene</td>
<td>C₁₀H₁₆</td>
<td>10.7 ± 7.2</td>
<td>165.6 ± 37.2</td>
<td></td>
</tr>
<tr>
<td>7.569</td>
<td>Eucalyptol</td>
<td>C₁₀H₁₈O</td>
<td>10.6 ± 4.6</td>
<td>146.3 ± 15.2</td>
<td></td>
</tr>
<tr>
<td>7.928</td>
<td>(S)-Linalool oxide</td>
<td>C₁₀H₁₈O₂</td>
<td>31.6 ± 4.7</td>
<td>24.5 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>8.162</td>
<td>Linalool</td>
<td>C₁₀H₁₈O</td>
<td>510.6 ± 64.1</td>
<td>395.8 ± 35.1</td>
<td></td>
</tr>
<tr>
<td>8.589</td>
<td>Camphor</td>
<td>C₁₀H₁₈O</td>
<td>666.7 ± 54.8</td>
<td>985.4 ± 73.0</td>
<td></td>
</tr>
<tr>
<td>8.746</td>
<td>α-Terpineol</td>
<td>C₁₀H₁₈O</td>
<td>59.7 ± 6.2</td>
<td>91.0 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>8.854</td>
<td>Hotrienol</td>
<td>C₁₀H₁₈O₂</td>
<td>99.2 ± 5.3</td>
<td>87.8 ± 14.4</td>
<td></td>
</tr>
<tr>
<td>8.922</td>
<td>α-Terpineol</td>
<td>C₁₀H₁₈O</td>
<td>101.7 ± 12.9</td>
<td>186.6 ± 14.0</td>
<td></td>
</tr>
<tr>
<td>9.479</td>
<td>2,6-Dimethyl-1,7-octadien-3,6-diol</td>
<td>C₁₀H₁₈O₂</td>
<td>2.6 ± 0.3</td>
<td>86.1 ± 12.0</td>
<td></td>
</tr>
<tr>
<td>10.017</td>
<td>Ethyl linalool</td>
<td>C₁₂H₂₂O</td>
<td>−a</td>
<td>66.2 ± 12.4</td>
<td></td>
</tr>
<tr>
<td>10.702</td>
<td>Coumaran</td>
<td>C₁₅H₂₄</td>
<td>−a</td>
<td>47.0 ± 12.5</td>
<td></td>
</tr>
<tr>
<td>11.180</td>
<td>Z-9-Tetradecenal</td>
<td>C₁₂H₂₂O</td>
<td>−a</td>
<td>28.0 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>11.350</td>
<td>Epiglobulol</td>
<td>C₁₅H₂₆O</td>
<td>10.7 ± 0.7</td>
<td>9.6 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>11.850</td>
<td>E-Nerolidol</td>
<td>C₁₅H₂₆O</td>
<td>7.0 ± 0.5</td>
<td>1,096.1 ± 74.6</td>
<td></td>
</tr>
<tr>
<td>12.345</td>
<td>Cis-Lanceol</td>
<td>C₁₅H₂₄O</td>
<td>34.7 ± 11.8</td>
<td>87.4 ± 17.0</td>
<td></td>
</tr>
<tr>
<td>12.213</td>
<td>Spathulenol</td>
<td>C₁₅H₂₄O</td>
<td>−a</td>
<td>115.9 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>12.310</td>
<td>Caryophyllene oxide</td>
<td>C₁₅H₂₄O</td>
<td>6.5 ± 1.1</td>
<td>116.9 ± 20.0</td>
<td></td>
</tr>
<tr>
<td>12.428</td>
<td>Bihydro-β-ionone</td>
<td>C₁₅H₂₄O</td>
<td>36.5 ± 7.2</td>
<td>197.0 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>13.218</td>
<td>6-Epi-shyobunol</td>
<td>C₁₅H₂₆O</td>
<td>161.3 ± 9.6</td>
<td>646.4 ± 21.2</td>
<td></td>
</tr>
<tr>
<td>13.997</td>
<td>α-Santalol</td>
<td>C₁₅H₂₄O</td>
<td>−a</td>
<td>119.7 ± 14.4</td>
<td></td>
</tr>
<tr>
<td>14.449</td>
<td>Isolongifolol</td>
<td>C₁₅H₂₄O</td>
<td>−a</td>
<td>343.6 ± 43.1</td>
<td></td>
</tr>
<tr>
<td>14.757</td>
<td>Dehydrosaussurea lactone</td>
<td>C₁₅H₂₄O₂</td>
<td>8.3 ± 4.2</td>
<td>93.8 ± 23.2</td>
<td></td>
</tr>
<tr>
<td>14.994</td>
<td>Palustrol</td>
<td>C₁₅H₂₆O</td>
<td>−a</td>
<td>47.2 ± 11.4</td>
<td></td>
</tr>
<tr>
<td>15.435</td>
<td>(E)-Atlantone</td>
<td>C₁₅H₂₆O</td>
<td>10.5 ± 2.9</td>
<td>121.5 ± 20.6</td>
<td></td>
</tr>
<tr>
<td>15.536</td>
<td>Saussurea lactone</td>
<td>C₁₅H₂₂O₂</td>
<td>1.2 ± 0.5</td>
<td>15.9 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>15.644</td>
<td>Cedrenol</td>
<td>C₁₅H₂₂O</td>
<td>11.4 ± 7.7</td>
<td>30.5 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>15.705</td>
<td>Proximadiol</td>
<td>C₁₅H₂₆O₂</td>
<td>20.2 ± 1.2</td>
<td>−a</td>
<td></td>
</tr>
<tr>
<td>16.021</td>
<td>Isoaromadendrene epoxide</td>
<td>C₁₅H₂₄O</td>
<td>−a</td>
<td>33.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>16.283</td>
<td>β-Santalol</td>
<td>C₁₅H₂₆O₂</td>
<td>−a</td>
<td>75.3 ± 8.5</td>
<td></td>
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<tr>
<td>16.390</td>
<td>Platambin</td>
<td>C₁₅H₂₆O₂</td>
<td>−a</td>
<td>53.1 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>18.070</td>
<td>1,8-Bimethyl-8,9-epoxy-4-isopropylspiro[4.5]decan-7-one</td>
<td>C₁₅H₂₄O₂</td>
<td>18.0 ± 1.4</td>
<td>93.7 ± 16.6</td>
<td></td>
</tr>
<tr>
<td>19.459</td>
<td>Methyl linolenate</td>
<td>C₁₅H₂₂O₂</td>
<td>−a</td>
<td>36.3 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>21.285</td>
<td>Octadecanol acetate</td>
<td>C₂₀H₄₀O₂</td>
<td>−a</td>
<td>43.2 ± 10.5</td>
<td></td>
</tr>
</tbody>
</table>

Data are means of three independent experiments ± standard deviation.

No compound was found.
the inhibitory effects alleviated, but significant \( P < 0.01 \) inhibition was also detected in the treatment at 5, 10 and 15 mg·ml\(^{-1}\) (Figure 1(a)). Compared to water extracts, methanol extracts showed stronger inhibition at the same concentration. In the treatment with methanol extracts at 15 mg·ml\(^{-1}\), the cells were killed completely (Figure 1(b)).

When \( C.\) reinhardtii cells were treated with \( C.\) camphora water and methanol extracts, the cell multiplication was inhibited significantly, and the inhibition enhanced with prolonging the treatment time. Similar to \( M.\) aeruginosa, \( C.\) reinhardtii cells were also killed completely in the treatment with methanol extracts at 15 mg·ml\(^{-1}\) (Figure 2).

**Impacts of \( C.\) camphora extracts on Chl levels**

The content of Chl \( a \) in \( C.\) reinhardtii cells reduced in the treatment with \( C.\) camphora water extracts, and the RI was \(-0.04, \ -0.09 \ (P < 0.05), \ -0.32 \ (P < 0.01) \) and \(-0.50 \ (P < 0.01) \), respectively, at 1, 5, 10 and 15 mg·ml\(^{-1}\). At the same concentration, methanol extracts showed stronger impacts on the content of Chl \( a \) in contrast to water extracts (Figure 3(a)). Similar reduction was also found in the content of Chl \( b \) (Figure 3(b)).

**Effects of \( C.\) camphora extracts on Chl fluorescence parameters**

\( C.\) camphora extracts remarkably reduced the Fv/Fm in \( C.\) reinhardtii cells, with the RI of \(-0.14 \ (P < 0.01), \ -0.35 \ (P < 0.01), \ -0.51 \ (P < 0.01) \) and \(-0.65 \ (P < 0.01) \), respectively, in water extracts at 1, 5, 10 and 15 mg·ml\(^{-1}\), and \(-0.34 \ (P < 0.01), \ -0.55 \ (P < 0.01) \) and \(-0.72 \ (P < 0.01) \), respectively, in methanol extracts at 1, 5 and 10 mg·ml\(^{-1}\) (Figure 4(a)). However, \( C.\) camphora extracts significantly \( P < 0.01 \) promoted the increase of \( \phi D_o \) in \( C.\) reinhardtii cells, and methanol extracts showed stronger effects compared to water extracts (Figure 4(b)).
DISCUSSION

An abundance of terpenoids that comprise terpanes and their derivatives were detected in the extracts from Cinnamomum genus (Kaul et al. 2003; Lee 2009; Ragasa et al. 2015; Monteiro et al. 2017; Taha & Eldahshan 2017; Tomazoni et al. 2017; Wei et al. 2017; Yuan et al. 2017). For C. camphora, the main compounds in the extracts from fresh leaves were terpenoids, especially oxygenated monoterpenes, and linalool and camphor were the two compounds with higher amount (Hamidpour et al. 2015; Yang et al. 2014; Tomazoni et al. 2017). In addition, in our previous studies, monoterpenes and oxygenated monoterpenes were the main VOCs released from the plants, and the emission amount of oxygenated monoterpenes was higher than that of monoterpenes, especially at high temperature (Zuo et al. 2017a). Similarly, amounts of terpenoids were found in the extracts from C. camphora fallen leaves, mainly including monoterpenoids and sesquiterpenoids, and the oxygenated terpenes were the main components (Table 1).

Among the extracts of water, methanol, ethyl acetate, hexane and dichloro-methane from fenugreek seeds, the methanol and ethyl acetate extracts showed the highest content of extracted compounds, indicating that organic compounds were more likely to be dissolved by methanol and ethyl acetate (Belguith-Hadriche et al. 2015). When grape pruning wastes (stems and leaves) were extracted by water and methanol, more compounds, mainly including terpenoids and phenolic compounds, were detected from the methanol extracts (Zuo et al. 2015). Similar results were also found in the extracts from C. camphora fallen leaves (Table 1).

When M. aeruginosa was treated with the extracts from A. annua, Conyza canadensis and Erigeron annuus, the cell

Figure 3 | Effects of C. camphora extracts on the levels of chlorophyll a (a) and chlorophyll b (b) in C. reinhardtii cells. *Compared to the control, the significant difference at $P < 0.05$ level. **Compared to the control, the significant difference at $P < 0.01$ level. The cells were killed completely in the treatment with methanol extracts at 15 mg·ml$^{-1}$, so the chlorophyll content was not measured. Data are means of four replicates ± standard error.

Figure 4 | Effects of C. camphora extracts on Fv/Fm (a) and φD0 (b) in C. reinhardtii cells. **Compared to the control, the significant difference at $P < 0.01$ level. The cells were killed completely in the treatment with methanol extracts at 15 mg·ml$^{-1}$, so the Fv/Fm and φD0 were not measured. Data are means of four replicates ± standard error.
growth was inhibited markedly, and their main components, terpenoids, may be the main anti-algal active ingredients (Ni et al. 2011). When *M. aeruginosa* cells were exposed to artemisinin, a terpenoid identified from *A. annua*, their soluble protein content decreased obviously, and superoxide dismutase activity and abscisic acid content increased remarkably (Ni et al. 2012). *C. reinhardtii* cell growth and photosynthesis were inhibited after the cells were treated with grape extracts with terpenoids and phenolic compounds as the main components (Zuo et al. 2015). Meanwhile, abundant VOCs including lots of terpenoids released from *M. flos-aquae* cells under non-N condition can inhibit *Chlorella vulgaris* cell growth (Xu et al. 2017), and from *M. aeruginosa* under non-P condition can inhibit *C. reinhardtii* cell growth (Ye et al. 2018). In this study, the inhibitory effects of *C. camphora* extracts on the growth of *M. aeruginosa* and *C. reinhardtii* should be caused by the terpenoid components (Figure 1), and the methanol extracts showed stronger effects due to their higher number of compounds and higher concentration (Table 1).

Previous studies have reported that linalool and camphor can inhibit the growth of several microorganisms, such as *Campylobacter* spp. (Duarte et al. 2016), *Candida albicans* (Zore et al. 2011), *Schistosoma japonicum* (Yang et al. 2014), *Microsporum canis* and *M. gypseum* (Silva et al. 2017), *Escherichia coli*, *Staphylococcus aureus* and *C. albicans* (Cuillas et al. 2017), by affecting membrane integrity and arrest of cell cycle (Zore et al. 2011). Meanwhile, linalool enhanced antifeedant activity against agricultural pests (Rani et al. 2014) and showed antitumor activity (Miyashita & Sadzuka 2015), and camphor treated several diseases, such as infection, inflammation, congestion, and pain (Hamidpour et al. 2015). The two compounds showed higher concentration in *C. camphora* extracts, which may be the main compounds to inhibit algal growth. In exposure to eucalyptol and limonene, *C. vulgaris* and *C. reinhardtii* cell growth reduced, due to the degradation of photosynthetic pigments and decrease of PSII efficiency (Zhao et al. 2016; Zhou et al. 2016; Xu et al. 2017). The two compounds existed in *C. camphora* extracts, and should play inhibitory roles on the growth of *M. aeruginosa* and *C. reinhardtii* in addition, there were other terpenoids in *C. camphora* extracts, which might also contribute to the inhibitory effects.

In algae, Chl is an essential photosynthetic pigment and functions by capturing light and transducing it to biochemical energy during photosynthesis. Its content in *C. reinhardtii* cells declined after the cells were exposed to grape extracts (Zuo et al. 2015). When *C. vulgaris* and *C. reinhardtii* were treated with eucalyptol and limonene, their Chl including divinyl-pheophytin *a*, divinyl-Chl *a*, divinyl-Chl *b*, monovinyl-protochlorophyllide *a*, monovinyl-Chl *b*, and monovinyl-Chl *a* were degraded, and the degradation enhanced with increasing the compound concentration (Zhao et al. 2016; Zhou et al. 2016). This indicated that terpenoids can induce photosynthetic pigment degradation, which may be the reason for the decline of Chl levels in *C. reinhardtii* cells treated with *C. camphora* extracts (Figure 2).

Photosynthesis is the most fundamental biological process supporting algal growth and nutrient uptake. The characteristics of Chl fluorescence provide an insight into PSII photochemical efficiency and the damage level of the photosynthetic apparatus to a certain extent (Maxwell & Johnson 2000). Terpenoids or plant extracts containing amounts of terpenoids can block the PSII quantum production and electron transport in algae, and promote the absorbed solar energy dissipating as heat (Ni et al. 2012; Yang et al. 2012; Zuo et al. 2015; Zhao et al. 2016; Zhou et al. 2016; Zuo et al. 2017b; Xu et al. 2017). Similarly, the extracts from *C. camphora* fallen leaves inhibited the Fv/Fm in *C. reinhardtii* cells, and increased φD0 (Figure 3).

**CONCLUSIONS**

Water and methanol extracts from *C. camphora* fallen leaves can inhibit *M. aeruginosa* and *C. reinhardtii* cell growth by inducing Chl degradation and reducing photosynthesis, and methanol extracts showed stronger inhibitory effects than water extracts at the same concentration, due to their greater number of compounds and higher concentration. This indicates that *C. camphora* fallen leaves have a potential value as an algaecide, and the algaecide is suitably extracted by methanol.

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