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Algicidal properties of extracts from *Cinnamomum camphora* fresh leaves and their main compounds

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ABSTRACT

Plant allelochemicals are considered as the source of effective, economic and friendly-environmental algaecides. To uncover the anti-algal activities of *Cinnamomum camphora* fresh leaves and their main algicidal agents, we investigated the inhibitory effects of water and methanol extracts from *C. camphora* fresh leaves on *Microcystis aeruginosa* and *Chlamydomonas reinhardtii* cell growth, analyzed the composition of the water and methanol extracts, and determined the main compounds in extracts on the growth of the two algae and their anti-algal mechanism from photosynthetic abilities. Water and methanol extracts from *C. camphora* fresh leaves can inhibit *M. aeruginosa* and *C. reinhardtii* cell growth, and methanol extracts showed stronger inhibitory effects, due to their more compounds and higher molar concentration. There were 23 compounds in the water extracts, mainly including terpenoids, esters, alcohols, and ketones. Compared to the water extracts, 9 new compounds were detected in the methanol extracts, and the molar concentration of total compounds in methanol extracts increased by 1.3 folds. Camphor, α-terpineol and linalool were 3 main compounds in the water and methanol extracts. Their mixture (1: 3: 6) and individual compound showed remarkable inhibition on *M. aeruginosa* and *C. reinhardtii* cell growth. The degradation of photosynthetic pigments and the reduction of maximum quantum yield of photosystem II (PSII) photochemistry, coefficient of photochemical quenching as well as apparent electron transport rate in *C. reinhardtii* cells aggravated gradually with increasing the concentration of the mixture and individual compound, while the non-photochemical dissipation of absorbed light energy increased gradually, which led to the decline of photosynthetic abilities. This indicated that camphor, α-terpineol and linalool were 3 main algicidal agents in *C. camphora* fresh leaf extracts, and they inhibited algal growth by inducing photosynthetic pigment degradation and declining PSII efficiency. Therefore, *C. camphora* fresh leaf extracts and their main components have potential utilization values as algaecides.

1. Introduction

Eutrophication is a natural process concerning all habitats, and becomes more serious with the increasing input of nutrients, mainly nitrogen (N) and phosphorus (P), caused by human activities in the past decades. Eutrophication promotes the excessive growth of phytoplankton and leads to algal blooms (Azevedo et al., 2002; Smayda, 2004). In recent decades, occurrences of algal blooms have increased around the world. The main algae causing the blooms are cyanobacteria, and sometimes green algae (Azevedo et al., 2002; Hudnell and Dortch, 2008; Kravtsova et al., 2014).

Algal blooms cause a series of ecological problems and lower the water quality, due to the emission of secondary metabolites including volatile organic compounds (VOCs) and algal toxins (Dodds et al., 2009; Xu et al., 2017; Zuo et al., 2018a). Algae release a wide spectrum of VOCs, mainly including sulfocompounds, alkanes, terpenoids, benzenes, alcohols, aldehydes, ketones, and esters (Walsh et al., 1998; Zuo et al., 2012a, 2012b; Ye et al., 2018), which have inhibitory effects on the growth of other algae (Xu et al., 2017; Zuo et al., 2018b). Meanwhile, these VOCs cause unpleasant, earthy-musty odor in waters, and geosmin and 2-methyl borneol are considered as the main odor compounds (Huang et al., 2007; Fujise et al., 2010). The water odor

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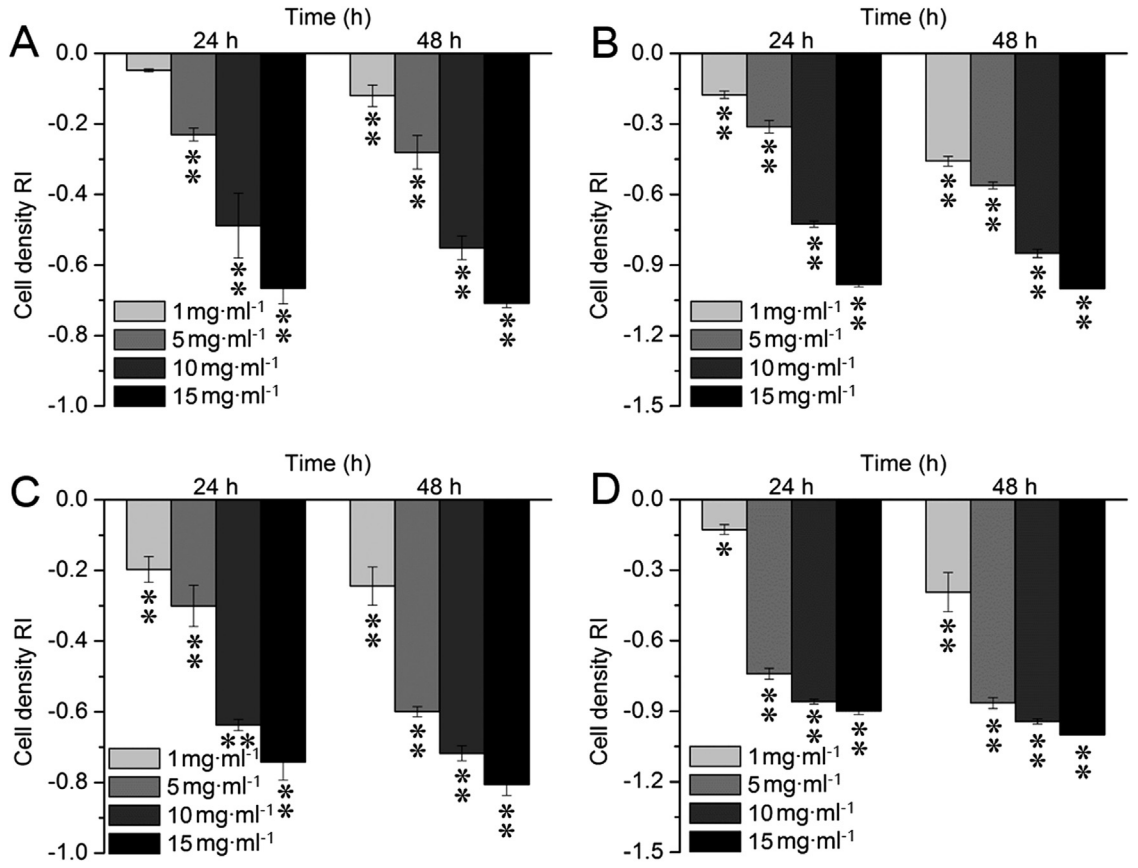

Fig. 1. Effects of water (A and C) and methanol (B and D) extracts from *C. camphora* fresh leaves on *M. aeruginosa* (A and B) and *C. reinhardtii* (C and D) cell growth. *: Compared to the control, significant difference at $P < 0.05$ level. **: Compared to the control, significant difference at $P < 0.01$ level. Data are means of four independent experiments ± SE.

dramatically impacts water supplies and even induces drinking water crisis (Zhang et al., 2010). Algal toxins are another kind of secondary metabolites, including microcystin, neurotoxins, anatoxin-a, hepatotoxins, neosaxitoxins, etc. (Codd, 2000; Frangópulos et al., 2004). These toxins can poison other aquatic organisms, such as algae, zooplankton, aquatic plants and fishes (Pflugmacher, 2002; Li and Li, 2012; Guzmán-Guillén et al., 2013). In addition, they may threaten human health when the waters polluted by the toxins are used for drinking and recreation and plants and fishes polluted are used as food (Hoeger et al., 2007).

For the benefits of aquatic ecological system and human health, lots of physical, chemical and biological methods have been developed to control undesired algae. In physical method, the algae are artificially salved during their blooms, but it cannot be practiced widely, due to the high consumption of time and fees and hardly elimination. Some chemicals, such as biquaternary ammonium salt (Liu et al., 2004), TiO_2 (Kim and Lee, 2005), polyaluminium chloride (Lürling and van Oosterhout, 2013), and copper sulfate (Costas and Lopez-Rodas, 2006; Song and Wang, 2015), kill and/ or control the algae quickly, but they can poison other aquatic organisms. Algal viruses (Garry et al., 1998) and bacteria (Zhou et al., 2016) easily mutate, which leads to their uncontrollability once released into the environment. These potential defects limit the wide usage of the 3 methods in the wild. Therefore, it is important to develop a new generation of algaecide that should be effective, economic and friendly-environmental in controlling undesired algae.

Plant allelochemicals can effectively inhibit algal growth and be degraded in the nature, which are considered as the potential source of new algaecide. Extracts from 5 Chinese herbs such as *Rhizoma coptidis*, *Semen arecae*, *Isatis tinctoria*, *Sophora flavescens*, and *Houttuynia cordata* can inhibit the growth of *Alexandrium tamarense*, and the extracts from the front two species showed the strongest inhibitory effects with low concentration and fast reaction (Zhou et al., 2007). Meanwhile, garlic solution also inhibited the growth and activities of 3 species of *Alexandrium* (*A. tamarense*, *A. satoanum* and *A. catenella*), as well as *Scrippsiella trochoidea* (Zhou et al., 2008). The extracts from barley and rice straw (Choe and Jung, 2002; Su et al., 2014), *Conyza canadensis* and *Erigeron annuus* (Ni et al., 2011), *Artemisia annua* (Ni et al., 2011, 2012), *Iris wilsonii* (Chen et al., 2012), *Fructus ligustri* Lucidi (Wu et al., 2014) and *Dracontomelon duperreanum* (Wang et al., 2016) had remarkable inhibitory effects on the growth of *Microcystis aeruginosa*, with decreases in the membrane integrity and chlorophyll (Chl) fluorescence in the treatment with rice straw, *F. ligustri* and *D. duperreanum* extracts. In the extracts, phenolics and tannin were considered as the main anti-algal compounds in *I. wilsonii* (Chen et al., 2012), and artemisinin was in *A. annua*, with inhibitory effects on soluble protein content in *M. aeruginosa* (Ni et al., 2012). In previous studies, we found that the extracts from grape leaves and stems can inhibit the growth of *Chlamydomonas reinhardtii* cells with decreases of photosynthetic pigments and abilities, and the leaf extracts showed stronger inhibitory effects in contrast to stem extracts (Zuo et al., 2015). In plant extracts, the anti-algal compounds play inhibitory roles on algal growth and are the basic functional compounds for developing new algaecides. Unfortunately, the identified anti-algal compounds are limited and expensive for practical application (Jančula and Maršálek, 2011). Therefore, reasonably priced anti-algal compounds have practical significance in controlling algal blooms and need to be identified.

Cinnamomum camphora (L.) Presl is an evergreen tree species and is widely planted in the south of China for landscaping and forestation. This species synthesizes an abundance of terpenoids to repel herbivore