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# Allelochemicals Extracted from *Eleocharis dulcis* and their Inhibitory Effects on *Microcystis aeruginosa*

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## Abstract

Allelochemicals are considered to be effective substances for controlling the outbreak of harmful cyanobacteria. In this study, *Eleocharis dulcis* peel fractions with an inhibitory effect on *Microcystis aeruginosa* were isolated by extraction and purification. The extracted organic compounds were identified by gas chromatography-mass spectrometry (GC-MS), and the allelopathic effect and mechanism underlying the inhibition of *M. aeruginosa* were explored. The results demonstrated that the exogenous compound, eriodictyol, corresponding to one of the detected flavonoids ($C_{15}H_{12}O_6$) could inhibit the growth of *M. aeruginosa*, indicating that the flavonoid $C_{15}H_{12}O_6$ is one of the allelochemicals contained in *E. dulcis*. Levels of chlorophyll a (Chl-a), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and reduced glutathione (GSH) of cell health in *M. aeruginosa* were analysed. Our results show that the damage to the antioxidant enzymes of algal cells caused by eriodictyol could be a main contributing factor to the decrease in the number of algal cells and even cell death.

**Keywords:** *Eleocharis dulcis*; *Microcystis aeruginosa*; Allelochemicals; Extraction; Eriodictyol

## Introduction

Cyanobacterial bloom is a common problem following water eutrophication [1-4]. *Microcystis aeruginosa* (*M. aeruginosa*) is a typical aquatic cyanobacterium that can produce algal toxins, which pose a significant threat to the survival of aquatic organisms and humans [5-7]. China has suffered severely due to toxic cyanobacterial bloom [8,9]. It is reported that the microcystin (MC) concentration in untreated water in Lake Taihu (the third largest freshwater lake in China) was 4.8-44.00 μg/L [10], much higher than the recommended safe value (1 μg/L) for human exposure set by the World Health Organization [11]. To counter this threat, effective strategies have been suggested that control the release of algal toxins by controlling the growth of *M. aeruginosa* need to be employed. Plants release a series of secondary metabolites into the environment to maintain their growth advantage relative to other species and as a way to obtain more nutrient resources [2,12]. These metabolites can have allelopathic inhibitory effects on other organisms in the same aquatic environment. Wang et al. found that *M. aeruginosa* that was exposed to *Dracontomelon duperreanum* defoliation extract might experience a decrease in cell volume or slow growth [13]. The inhibitory effect of macrophytes on algal species belonging to different phyla have been studied by many authors as reviewed [14]. Most of these studies reported that cyanobacteria are more sentive to macrophyte allelochemicals compared to other groups such as *chlorophyta*, *cryptophyta*, *xanthophyta*, *bacillariophyta* and *Euglenphyta.*

*Eleocharis dulcis* (*E. dulcis*) is one of the most common economic crops in southern China, with a strong ability to absorb nitrogen, phosphorus and other nutrients [15]. Among all root and tuber economic crops, *E. dulcis* has the highest phosphorus content in the bulbs. At present, studies on the use of *E. dulcis* to control eutrophication have focused on the construction of aquatic ecosystems by ecological floating beds to absorb nitrogen and phosphorus in water bodies [16]. As a common emergent plant, *E. dulcis* has been widely used in constructed wetlands. Overall and Parry found that *E. dulcis* grew well in the constructed wetlands, with an average diameter of 5 mm and a length of 2 m [17]. However, few researchers have studied the use of the allelopathic effect of *E. dulcis* to inhibit the growth of *M. aeruginosa*. The extraction and purification of the allelochemicals in *E. dulcis* has been especially understudied.

Organic acids and esters have been confirmed by many researchers as important allelochemicals in plants. However, for sterols, some studies have shown a lack of effect on the allelopathic activity on algal inhibition. Aliotta et al. separated a series of active substances (linolenic acid, linoleic acid and unsaturated 18-carbon fatty acids) that could inhibit the growth of *Chlorella* spp. and *Anabaena* spp. from *Typha latifolia* [18]. Schrader found that the obtained unsaturated fatty acids, lysine, and ferulic acid, as well as other active substances from the degradation of barley straw inhibited the growth of *M. aeruginosa* [19]. Zhang et al. conducted a comparative analysis of 17 types of fatty acids on the growth of toxin-producing *M. aeruginosa*, *Chlorella pyrenoidosa*, and *Scenedesmus obliquus* [20]. The results found that fatty acids with a higher degree of unsaturation and shorter carbon chains possessed a stronger inhibitory effect on the above three algal species. Hu et al. isolated and identified 13 types of organic acids, such as nonanoic acid, from *Potamogeton malaianus*, and found that all types of fatty acids had different degrees of inhibitory effects on *Selenastrum capricornutum* [21]. The isolated sterols had no significant inhibitory effect upon *Selenastrum capricornutum* and also had no effect on the algal inhibition activity of fatty acids. This is consistent with the results of Greca et al. who extracted ten steroids from *Zantedeschia aethiopica* [22] and by Aliotta et al. who separated two sterols from the ethyl acetate extract of *Pistia stratiotes* [23]. Li et al. isolated ethyl-2-methyl acetoacetate (EMA) from reed, and confirmed that EMA had a strong

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inhibitory effect on *M. aeruginosa* and *Chlorella pyrenoidosa* [24]. Deng et al. confirmed a good correlation between dibutyl phthalate and cetyltrimethylammonium bromide mass concentrations and their inhibitory effect on *M. aeruginosa* [25]. Choe et al. found that silvery wormwood, barley straw, rice straw and chrysanthemum could release substances that inhibit algal growth during the rotting process [26]. These substances mainly included adipic acid, dioctyl phthalate, and bis(2-ethylhexyl)phthalate. These esters were considered to be the main active substances, although phenols also played a certain role in algal inhibition.

In this study, individual compounds were isolated and purified from *E. dulcis* extract. Using the obtained extracts, algal growth inhibition tests were conducted. The inhibitory activity of isolated allelochemicals was tested against *M. aeruginosa*. Furthermore, the identified allelochemicals was used to confirm the effect of the extract on the growth of *M. aeruginosa*, to provide a basic framework for exploring the techniques used in the biological control of *M. aeruginosa*.

## Materials and Methods

### Materials

***E. dulcis* samples:** Fresh *E. dulcis* samples were purchased from Changsha, Hunan Province. Intact *E. dulcis* bulbs of uniform size were selected and used in the experiments. The *E. dulcis* samples were washed three times with ultrapure water and the *E. dulcis* peel was collected, dried in a forced-air drying oven, and ground in a grinder to give *E. dulcis* powder.

**Algal strains:** *M. aeruginosa* (No. FACHB-905) was purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences. Cultivation was conducted using MA medium according to Li et al. [27] in an artificial climate chamber, with a light intensity of 2500 Lux, a light-dark cycle of 12 h/12 h, a temperature of 25 ± 1°C and a relative humidity of 75%.

**Extraction and separation of compounds present in *E. dulcis* peel and a comparison of their ability to inhibit algal growth**

First, 900 g of *E. dulcis* powder was immersed in 10 L of 70% ethanol solution, and the mixture was divided into five fractions in glass containers, which were sealed by Parafilm. After incubating the mixture in a water bath at 50°C for 1 h, the glass containers were placed into a digital ultrasonic cleaner, sonicated at a power of 100 W for 1.5 h, and then allowed to sit in a water bath at 50°C for 1 h. Subsequently, vacuum filtration was performed, and the filtrate was evaporated under reduced pressure in a rotatory evaporator (100 r/min). After evaporation, the paste was transferred to a vacuum oven and dried until the weight did not change to obtain a brownish black extract (106 g), i.e., the crude ethanol extract of *E. dulcis* peel.

Based on the polarity order of organic solvents, petroleum ether, ethyl acetate, chloroform and *n* butanol were added in succession to extract the crude extract of *E. dulcis* peel [28,29]. Each extraction was repeated three times to obtain four organic solvent extracts. The four extracts were dried separately in a vacuum oven until the weight did not change to give petroleum ether extract, ethyl acetate extract, chloroform extract and *n*-butanol extract.

The four solid extracts were dissolved with ultrapure water. *M. aeruginosa* was used as the test object, and algal growth inhibition tests were conducted to determine the efficacy of these four extracts in inhibiting algal growth. Algal inhibiting ring experiments were applied to test algal growth inhibition efficiency of extracts according to the method of Meurant (1991). A filter paper containing algal agent was placed in the solid culture medium, and the ability of algal growth inhibition was determined by measuring the diameter of the blank region of the algae cell death in the solid medium. After comparing the inhibition zone diameters obtained using different extracts, the extract with the optimal inhibition on algal growth was selected for subsequent thin layer chromatography (TLC) [30,31] and column chromatography experiments for separation and purification.

### Separation and purification of the extract with optimal algal inhibition activity

**TLC experiments:** A GF254 silica gel plate (size 50 mm × 100 mm × 0.25 mm) was washed three times with ultrapure water and absolute ethanol, and then dried and activated in a vacuum oven at 110°C for 1 h. The activated silica gel plate was placed in a desiccator to cool down to room temperature. The extract with the strongest algal inhibition activity was selected from the algal growth inhibition test. This extract was then dissolved by adding an appropriate amount of acetone, and spotted onto the GF254 silica gel plate with a spotting glass capillary tube. According to the literature, after several pre-experiments, petroleum ether, ethyl acetate, chloroform, *n*-butanol, acetone, isopropanol, and methanol were selected as pure developing solvents. Binary mixtures of solvents, ethyl acetate/chloroform, ethyl acetate/isopropyl alcohol, and ethyl acetate/methanol at a volume ratio of 10:1, and mixtures of chloroform/acetone and chloroform/ethanol at a volume ratio of 5:1 were selected as binary developing solvents. Solvent mixtures consisting of ethyl acetate/chloroform/methanol at volume ratios of 5:1:1 and 10:1:1 and chloroform/acetone/methanol at a volume ratio of 5:1:1 were selected as ternary developing solvents. After the components were developed by the solvent mixture, the number of separate resolvable bands in the TLC was measured by iodine vapour staining. The quality of various solvents as eluents were compared, and the solvent with the largest number of distinct TLC bands was selected for column chromatography.

**Column chromatography for separation and purification:** The extract with the optimal algal inhibition activity was isolated and purified by column chromatography [32]. A column was wet-packed with 100-300 mesh silica for column chromatography and nitrogen gas was used to aid elution. The eluate was collected in fractions and analysed using TLC. Different separation components were distinguished by iodine vapour staining, and the fractions with small ratios to the front ($R_f$) values were combined. The combined fractions were then placed in a vacuum oven and dried to yield a solid material.

**Secondary column chromatography for separation and purification:** Small amounts of the dried products separated were dissolved by adding ultrapure water to conduct algal growth inhibition tests. The abilities of all fractions on inhibiting algal growth were compared, and the extract with the strongest algal inhibition activity was selected as the initial sample for further column chromatography. Different eluents (solvent mixtures of ethyl acetate and chloroform at volume ratios of 15:1, 12:1, 9:1, 4:1, and 2:1 were used in the second purification step using column chromatography, and solid products were obtained after eluting, collecting, combining the fractions, and drying. Small amounts of dried product were dissolved by adding ultrapure water to conduct another algal growth inhibition test. The abilities of all fractions to inhibit algal growth were compared, and the extract with the strongest algal inhibition activity was selected for gas chromatography-mass spectrometry (GC-MS) analysis.

### Structural identification of the active ingredient in *E. dulcis*

### responsible for algal inhibition

GC-MS analysis was performed using a GC-MS system (GCMS-QP2010, Shimadzu Corporation, Japan) on the product that was obtained after two separations and purifications (the fraction with the best algal inhibition activity).

The chromatographic column was an HP-1 type with dimensions of 30 m × 0.25 mm × 0.17 μm. The carrier gas was high purity grade helium (purity ≥ 99.999%) and was maintained at a constant pressure at 220 kPa. The carrier gas flow rate was 1.0 mL/min. The inlet temperature was 280°C, and samples were injected using a split injection method with a split ratio of 20:1 and an injection volume of 0.5 μL. The initial oven temperature was maintained at 70°C for 2 min, programmed at 8.0°C /min to 220°C (that was held for 3 min), and then programmed at 15.0°C /min to 280°C, which was held for 30 min.

The ion source temperature was 230°C while the chromatography-mass spectrometer interface temperature was 280°C. The ionisation method used was electron ionisation (EI) which was conducted at 70 eV. A full scan mode was employed with a scanning range of 30-450 amu, a scanning speed of 20000 amu/s and an acquisition frequency of 50 Hz.

### Determination of the effects of the allelochemicals in *E. dulcis* on the physiological indicators in algae

The algal density, chlorophyll, malondialdehyde, antioxidant enzyme system, and non-enzyme system of *M. aeruginosa* were determined based on the methods reported in our previous study Choe and Jung.

### Data processing

All data were processed using Microsoft Office 2016, and analysed using SPSS 19. Significant differences between the data sets were analysed using single factor analysis of variance (ANOVA) and the Tukey test, with $p<0.05$ as a significant difference. Graphs were plotted in Origin 8.0 software, and the data in the graphs are expressed as a mean ± standard deviation.

## Results and Discussion

### Separation of crude ethanol extract

Based on the order of polarity, four commonly used organic solvents were used to extract fractions from the crude ethanol extract of *E. dulcis* peel. The obtained four extracts were applied for inhibiting the growth of *M. aeruginosa*. The experimental results are shown in Figure 1, ethyl acetate extract exhibited the highest activity against *M. aeruginosa* (15.6 mm), compared to petroleum ether, chloroform and *n*-butanol extracts ($p<0.05$). Therefore, the ethyl acetate extract was selected as the starting material for subsequent TLC and column chromatography separation and purification.

### TLC separation of the extract

The solvent in 2.3.1 was selected as the developing solvent for TLC experiments, and the number of separation spots obtained from the iodine vapour staining method was used as the criterion to compare and analyze the separation effects of different solvents, in order to select the eluent used for column chromatography. The experimental results are shown in Table 1. According to the experimental results in Table 1, the mixed solvents of ethyl acetate, chloroform, methanol in different volume ratios were selected as the eluents for subsequent separation of compounds in the ethyl acetate extract using column chromatography.

**. Separation and purification of compounds in the ethyl acetate extract using column chromatography:** The compounds in the ethyl acetate extract of *E. dulcis* peel were further separated and purified. Fractions with similar $R_f$ values were combined, and five fractions were obtained after combining similar fractions. These were labelled as 1, 2, 3, 4, and 5, respectively, and their algal inhibition performance was determined using an algal growth inhibition test. The experimental results are shown in Figure 2a. As can be seen from Figure 2a, fraction 2 had the strongest inhibition on the growth of *M. aeruginosa*, with an average algal inhibition zone diameter of 21.3 mm. Therefore, fraction 2 was selected as the starting material for the second separation and purification steps.

**Secondary column chromatography for separation and purification:** Fraction 2, which was obtained from the previous column chromatography step and had the highest ability in inhibiting algal growth, was purified by secondary column chromatography. TLC analysis was carried out on each fraction, and fractions with similar $R_f$ values were combined to yield five fractions, which were labelled as 6, 7, 8, 9, and 10, respectively. The algal growth inhibition performance of each fraction was determined by an algal growth inhibition test. The experimental results are shown in Figure 2b. As shown in Figure 2b, fraction 7 had the strongest inhibition effect on the growth of *M. aeruginosa*, with an average algal inhibition zone diameter of 24.5 mm. Therefore, according to the results of the comparison of algal inhibition zone diameters, fraction 7 was selected as the starting material for the following structural identification steps.

**Identification of active components in the fraction with the highest algal inhibition activity:** Fraction 7, which was obtained from the two column chromatography separations and had the highest inhibiting effect on algal growth, was dissolved, filtered through a 0.22 μm filter, centrifuged, and the supernatant was collected. Structural identification was conducted according to the conditions described in 2.4 on a GC-MS system. The identification results are shown in Figure 3. More than ten types of organic compounds with possible allelopathic effects were identified from the GC-MS results, and they were mainly

| Solvent | Volume ratio | Number of spots |
|---|---|---|
| Petroleum ether | - | 1 |
| Ethyl acetate | - | 2 |
| Chloroform | - | 2 |
| N-butanol | - | 2 |
| Acetone | - | 2 |
| Isopropyl alcohol | - | 1 |
| Methanol | - | 1 |
| Ethyl acetate: chloroform | 10:1 | 3 |
| Ethyl acetate: isopropyl | 10:1 | 2 |
| Ethyl acetate: methanol | 10:1 | 5 |
| Chloroform: acetone | 5:1 | 2 |
| Chloroform: methanol | 5:1 | 3 |
| Ethyl acetate: chloroform: methanol | 5:1:1 | 8 |
| Ethyl acetate: chloroform: methanol | 10:1:1 | 7 |
| Chloroform: acetone: methanol | 5:1:1 | 4 |

**Table 1:** TLC results using different solvents.

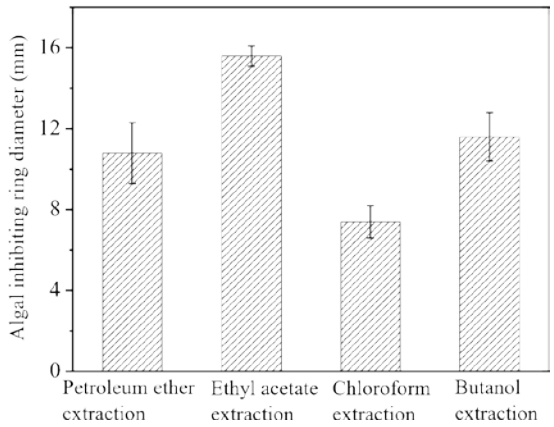


**Figure 1:** Effect of Different Extracts on the Growth of *M. aeruginosa*. The Labeling (Different Letters) Indicates Significant Differences ($p<0.05$).

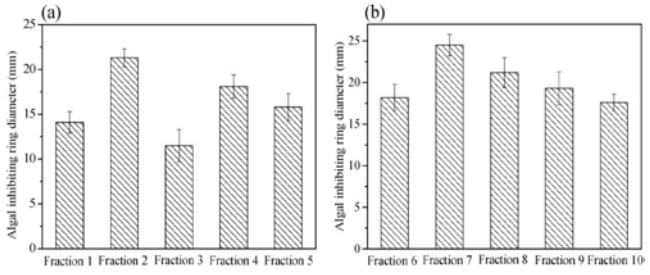


**Figure 2:** Inhibitory Effects of Different Fractions on M. Aeruginosa (a) Fractions Obtained After Chromatographic Separation and Purification, (b) Fractions Obtained After Secondary Chromatographic Separation and Purification.

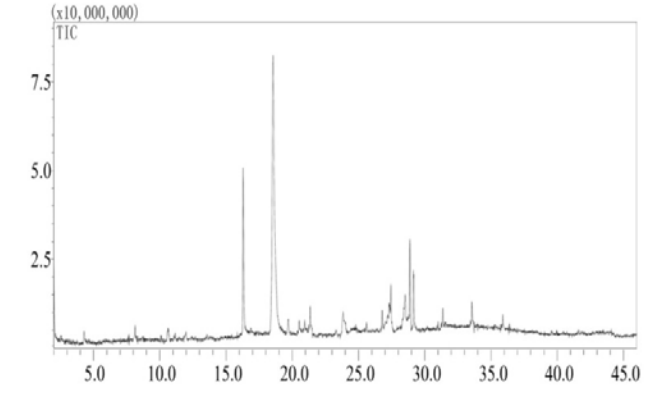


**Figure 3:** GC-MS Chromatogram of Fraction 7.

steroids, organic acids, esters and flavonoids. Similar results were reported by Jia et al., which confirmed that *E. dulcis* peel contained sterols, esters, flavonoids, and organic acids. Among the four organic compounds identified in this study, organic acids and esters have been confirmed by many researchers as important allelochemicals in plants. For sterol compounds, some studies have shown that sterols do not affect the allelopathic activity on algal inhibition. Most studies on flavonoids have focused on the antibacterial activity, and the allelopathic effect of flavonoids on algal inhibition has been rarely reported and still needs further verification. Therefore, the algal inhibition activity of the flavonoid compounds identified in this study was further analysed.

The GC-MS results indicated that the organic compound $C_{15}H_{12}O_6$ was the most abundant and matched the results more closely (96.7%) than all the other flavonoids. The comparison of the chemical structures suggested that the flavonoid $C_{15}H_{12}O_6$ identified by GC-MS analysis was basically the same as that of 3',4',5,7-tetrahydroxyflavanone (eriodictyol) as shown in Figure 4. Guo obtained the extract of *E. dulcis* peel using 75% ethanol by filtration under reduced pressure, and 50% ethanol was added to prepare a pigment solution followed by spectral scanning at a range of 250-700 nm. The results confirmed that flavonoids were the main antioxidant component in the extract of *E. dulcis* peel. [33] analysed fresh *E. dulcis* samples using GC-MS and found that *E. dulcis* contained eriodictyol.

**Verification of the algal inhibition ability of the allelochemicals present in *E. dulcis***

In combination with the analysis of the compounds identified in GC-MS and results from other researchers, it was speculated that the flavonoid compound $C_{15}H_{12}O_6$ was one of the allelochemicals in *E. dulcis*, which had an inhibitory effect on *M. aeruginosa*. Therefore, the exogenous compound eriodictyol (3',4',5,7-tetrahydroxyflavanone) which has a very similar chemical structure to this flavonoid, was selected to replace the identified flavonoid allelochemical for the following algal growth inhibition tests, to further verify that the flavonoid compound $C_{15}H_{12}O_6$ was one of the allelochemicals contained in *E. dulcis*.

**Effect of eriodictyol upon algal density:** As shown in Figure 5a, the growth of *M. aeruginosa* was significantly inhibited by eriodictyol, and the inhibitory effect of eriodictyol increased with increasing concentration [34-36]. Compared with the control group after seven days, eriodictyol treatment groups containing concentrations of 3 mg/L, 5 mg/L, 7 mg/L, and 9 mg/L eriodictyol, had algal cell mortalities of 30.51%, 79.01%, 88.13%, and 89.33%, respectively. In terms of the effect of algal inhibition, the algal cells were generally killed in 7 mg/L and 9 mg/L eriodictyol treatment groups after seven days of culture, and no significant differences were found in algal inhibition between both groups. Therefore, 7 mg/L was used as an experimental group to study the inhibition mechanism of eriodictyol on the growth of *M. aeruginosa*.

**Effect of eriodictyol upon chlorophyll:** It can be seen from Figure 5b that the chlorophyll a content of the high concentration eriodictyol treatment group (9 mg/L and 7 mg/L) fluctuated around 1 μg/L, and showed no significant change in the whole culture cycle. When the amount of added eriodictyol was less than 5 mg/L, the lower the concentration of eriodictyol in the culture medium was, the higher the chlorophyll a content of *M. aeruginosa* became. At the same time, the concentration of chlorophyll a was related to the duration of contact between eriodictyol and algae. Between Day 1 and Day 2 of the culture, *M. aeruginosa* adapted to the new growth environment and no significant changes were observed in the chlorophyll a concentration of algal cells in all groups. From Day 3, the growth of algal cells in the 5 mg/L eriodictyol treatment group was significantly inhibited, and the chlorophyll a concentration increased slightly. After two days of adaptation, due to a relatively weak inhibitory effect of low-concentration eriodictyol upon algal cells, the chlorophyll a content in the 3 mg/L eriodictyol treatment group still showed a continuous increase in the presence of suppression, and there was a faster increase at lower concentrations.

**Effect of eriodictyol upon the antioxidant enzyme systems:** The

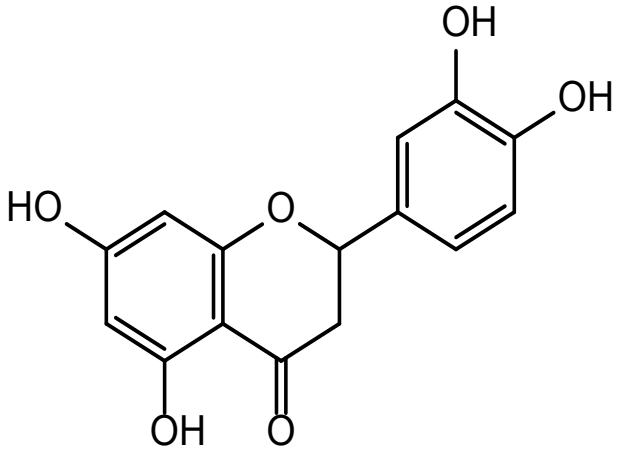


**Figure 4:** Chemical Structure of $C_{15}H_{12}O_6$.

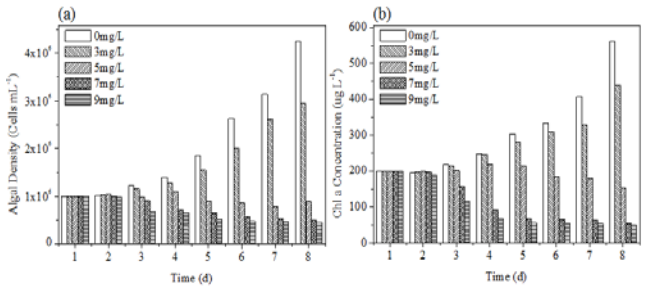


**Figure 5:** Effect of Different Concentrations of Eriodictyol upon the Growth of *M. aeruginosa* (a) Growth Curve, (b) Chl a Concentration Curve.

| Time | SOD (U mg$^{-1}$ prot$^{-1}$) | CAT (U mg$^{-1}$ prot$^{-1}$) | MDA (μg protein$^{-1}$) | GSH (μg·mL$^{-1}$) |
|---|---|---|---|---|
| 1 h | 632 | 281 | 5.01 | 46 |
| 8 h | 564 | 244 | 6.28 | 64 |
| 16 h | 517 | 206 | 7.84 | 77 |
| 24 h | 415 | 185 | 8.32 | 112 |
| 48 h | 389 | 153 | 10.12 | 135 |
| 96 h | 173 | 142 | 11.26 | 108 |

**Table 2:** Effect of eriodictyol upon the SOD, CAT, MDA and GSH concentrations in *M. aeruginosa*.

activity of superoxide dismutase (SOD) is shown in Table 2. When the algal cells were exposed to 7 mg/L eriodictyol, the activity of SOD decreased continuously with time. After 96 h of culture, the activity of SOD in algal cells decreased to 173 U/mg·prot, which was only 28.57% of the initial activity of SOD. SOD can specifically catalyse $O_2$ that decomposes into oxygen and hydrogen peroxide, so SOD is the first line of defence that affects the potential toxicity of reactive oxygen species (ROS) [37]. The results showed that SOD activity was seriously reduced after *M. aeruginosa* was exposed to eriodictyol. This suggests that the capacity to detoxify $O_2$ was reduced, leading to damage in the algal cells' first line of defence. The disruption of SOD activity was thus considered to be a key to the inhibition of the growth of *M. aeruginosa*.

The activity of catalase (CAT) is shown in Table 2. CAT activity continuously decreased with increasing culture time. In the presence of 7 mg/L eriodictyol, the activity of CAT in *M. aeruginosa* algal cells was stimulated, in order to eliminate the external stress caused by eriodictyol. However, with prolonged culture time, the amount of eriodictyol entering the cells accumulated, such that the stress of this compound on *M. aeruginosa* was more and more intense.

This resulted in a continuous decrease in the resistance of the algal cells to this external stress with time. After 96 h of culture, the CAT activity decreased significantly to 142 U/mg·prot.

**Effect of eriodictyol upon the content of malondialdehyde:** Membrane lipid peroxidation is positively correlated with membrane damage [35]. The malondialdehyde (MDA) level in cells is often used to assess lipid peroxidation level in cell membranes [36]. In Table 2, the increase in the amount of eriodictyol entering the cells resulted in an increase in the MDA content of algal cells. After 7 mg/L eriodictyol was added to the medium, the growth of *M. aeruginosa* was significantly affected. At the same time, 7 mg/L eriodictyol exceeded the tolerance range of *M. aeruginosa*, and the algal cell antioxidant systems were significantly damaged. The algal immune system was unable to cope with the interference of foreign substances and was destroyed. Thus, the algal cells underwent lipid peroxidation, suffered considerable damage, and gradually died.

**Effect of eriodictyol upon reduced glutathione:** Reduced glutathione (GSH) is an important non-enzymatic substance that protects algal cells by removing free radicals and exerting antioxidant functions [37]. The concentration of GSH reached a peak value of 135 μg/mL at 48 h of culture, which indicated that GSH molecules were scavenging free radicals to protect the algal cells from external environmental stress. With increasing culture time, the relative concentration of eriodictyol entering the algal cells also increased, so that the activity of free radical scavenging in algal cells [38-40] was inhibited, and the GSH activity of algal cells was affected and showed a downward trend in Table 2.

## Conclusions

Interfering organics in the extract of *E. dulcis* peel were removed by repeated separation and purification steps, and four types of organic compounds, including sterols, flavonoids, organic acids and esters, were identified in the purified material by GC-MS. Algal growth inhibition tests showed that the flavonoid $C_{15}H_{12}O_6$ was one of the allelochemicals contained in *E. dulcis*, and its corresponding exogenous compound, eriodictyol, could inhibit the growth of *M. aeruginosa*. The results of physiological indexes of *M. aeruginosa* revealed that the damage of eriodictyol to the antioxidant enzymes of algal cells might be due to the allelopathic effect of eriodictyol, which could be the main reason for the decrease in the number of algal cells and even cell death in algae. Other allelochemicals that could inhibit the growth of *M. aeruginosa* and the allelochemicals or compounds with synergistic effects in *E. dulcis* need to be further verified. The extract of *E. dulcis* peel could be a potential and effective strategy for controlling the growth of *M. aeruginosa*.

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