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Identification and antialgal properties of *o*- coumaric acid isolated from *Eupatorium fortune* Turcz

Nhận dạng và nghiên cứu tính chất diệt tảo của axit o-Coumaric phân lập từ cây Mần tưới Eupatorium fortunei Turcz

Research article

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In our pervious study, the ethanol extracts from *Eupatorium fortunei* Turcz at concentrations of 200 ÷ 500 mg L^{-1} significantly inhibited the growth of *Microcystis aeruginosa*, which is the most common species, responsible for toxic cyanobacteria blooming in fresh water. *o*-Coumaric acid (or 2-hydroxy-cinnamic acid) isolated from *E. fortunei* was tested its growth-inhibitory effect on *M. aeruginosa* and *Chlorella vulgaris* at the concentrations of 1.0, 10.0 and 100.0 mg L^{-1} in the 96-hour experiment by the optical density and the analytical method of chlorophyll *a* concentration. Results indicated that the compound strongly affected towards *M. aeruginosa* at the concentration of 100.0 mg L^{-1} with the inhibition efficiency (IE) values of 76.76 % and 84.66 %, respectively while those for *C. vulgaris* were lower just of 60.59%, and 74.53 %, respectively. The obtained data demonstrated that two methods were highly consistent and *o*- coumaric acid was more toxic to *M. aeruginosa* than *C. vulgaris* at all tested concentrations ($p<0.05$). The images of *M. aeruginosa* and *C. vulgaris* cells under the light microscope clearly showed the damage of these cells under the attck of *o*-coumaric acid. Although *o*-coumaric compound was widely demonstrated antibacterial properties in previous reports, to the best of our knowledge, our study was the first report about effect of *o*- coumaric acid on the growth of *M. aeruginosa* and *C. vulgaris*.

Những nghiên cứu trước đây đã chỉ ra rằng cao chiết etanol từ cây Mần tưới Eupatorium fortune Turcz tại dải nồng độ 200 ÷ 500 µg mL^{-1} ức chế mạnh sinh trưởng của Microcystis aeruginosa, -loài phổ biến nhất gây nên sự bùng nổ tảo độc trong hệ sinh thái nước ngọt. o-Coumaric axit (hay 2-hydroxy-cinnamic axit) phân lập từ E. fortune được tiến hành đánh giá ảnh hưởng lên sinh trưởng của hai loài M. aeruginosa và Chlorella vulgaris tại ba nồng độ là 1.0, 10.0 and 100.0 mg L^{-1} trong thời gian 96 giờ thực nghiệm theo phương pháp đo mật độ quang và phân tích hàm lượng chlorophyll a. Kết quả nghiên cứu cho thấy sau 96 giờ phơi nhiễm tại nồng độ 100 mg L^{-1} hoạt chất ức chế mạnh tới M. aeruginosa với giá trị ức chế sinh trưởng (IE) tương ứng là 76.76 và 84.66%. Giá trị IE đối với C.vulgaris ghi nhận thấp hơn chỉ là 60.65 và 74.53%, tương ứng. Hai phương pháp phân tích trên có tính nhất quán cao và o-coumaric ức chế sinh trưởng lên loài M.aeruginosa mạnh hơn so với loài C.vulgaris tại tất cả các nồng độ nghiên cứu ($p<0.05$). Ảnh chụp các tế bào M.aeruginosa và C.vulgaris dưới kính hiển vi điện tử đã chứng minh những tổn thương của tế bào dưới tác động của o-coumaric axit. Mặc dù o- coumaric cho thấy đặc tính chống khuẩn cao trong các công bố trước đây, nhưng theo hiểu biết của chúng tôi đây là nghiên cứu đầu tiên công bố về ảnh hưởng của hoạt chất o-coumaric axit lên sinh trưởng của M. aeruginosa và C. vulgaris.

Keywords: *Eupatorium fortune* Turcz, *o*-coumaric acid, *Microcystis aeruginosa*, *Chlorella vulgaris*, bloom water, inhibition efficiency

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1. Introduction

Recently, there has been significant interest for the growth inhibition of cyanobacteria by allelochemicals isolated from plant materials [1, 2]. Among all allelochemicals, phenolic substances have been studied most widely and it has been demonstrated that many phenolic compounds strongly against cyanobacteria [3, 4, 5]. Some of these bioactive substances have been extracted from plant materials and purified successfully as well as evaluated high antialgal properties such as benzaldehyde, 2-phenylphenol, *p*-cresol and benzoic acid (*Hordeum vulgare*) [6] or *p*-hydroxybenzoic, ferulic, vanillic, salicylic acids (*Oryza sativa L.*) [3], pyrogallol, protocatechuic acids, catechin; ellagic, gallic acids (*Myriophyllum spicatum)* [4, 5]. Application of appropriate allelochemicals was a potential method to the control of cyanobacteria blooms, causing deterioration of water quality and biodiversity, becoming a human health hazard in recent decades.

Among all sorts of algae, *Microcystis aeruginosa* is the most common species, responsible for the water blooming. Many strains of *Microcystis* are known to produce cyanobacterial microcystins such as hepatotoxins and neurotoxins leading to headache, fever, abdominal pain, nausea, vomiting and even cancer [2]. The traditional approaches such as physical (ultrasonication, sonication, microwave irradiation, pH, temperature), chemical ($CuSO_4$, $KMnO_4$, H_2O_2) and biological methods (*Bacillus* sp**,** *Aeromonas sp.)* showed several negative impact to the environment. Although chemical and physical treatments can effectively and rapidly remove algal bloom, they can cause secondary pollution of aquatic environment or persistence in the environment and their inhibitory effects do not selectively target harmful cyanobacteria; leading to the collapse of aquatic ecosystems. The efficiency of biological method is depending on many biotic and abiotic factors in the environment leading to preventing large-scale application [2].

Over last two decades, extracts of some plants have been reports to selective inhibition the growth of *M. aruginosa* among to others organisms in aquatic ecosystem. For examples, extracts from rice straw or extracts from the family *Papaveraceae* indicated more toxic to *M. aeruginosa* than other species like green alga (*Ankistrodesmus convolutus* and *Scenedesmus quadricauda)*, duckweeds (*Lemna minor*), freshwater cladoceran (*Daphnia magna*), phytoplankton [7, 8]. Our previous results [9] revealed that the plant extracts from *Cyperus rotundus, Chromolaena odorata*, *Callisia fragrans* and *Eupatorium fortunei* with their concentrations from 4 to 500 μg mL^{-1} effectively inhibited the growth of *M.aeruginosa*. Among of them, *Eupatorium fortunei* showed the selective anti-cyanobacteria properties which was higher toxic to *M. aeruginosa* (IC_{50} of 119.3 μg mL^{-1}) than to *Chlorella vulgaris* (IC_{50} of 315.1 μg mL^{-1}). The ethyl acetate extract from *Eupatorium fortunei* also selectively inhibited the growth of *Microcystis* population (IE value of 34.5%) among to the phytoplankton community (IE of 16.3%) collected from the Hoan Kiem lake [10]. We also tested the toxicity of the extracts from *E. fortunei* to duckweeds (*Lemna minor* and *Spirodella polyrhiza)* as representatives of sensitive non-target aquatic organisms to evaluate environmental safety [11]. In comparison with significant growth inhibition of the extract on *M.aeruginosa*, *L.minor* was slightly affected by the extracts at 500 μg mL^{-1} with IE of 25%, while *S. polyrhiza* was stimulated to about 5 % through fresh weight determinations. The analysis of photosynthetic pigments revealed that pigment contents in both duckweeds exposed to the extracts were not significant changes compared with the untreated controls. The obtained results demonstrated that the extracts from *Eupatorium fortunei* was a promosing algicide for controlling harmful cyanobacterial blooms. This study aimed to isolate the active compound from *E. fortunei* and evaluate its effect on the growth of toxic cyanobacteria *M. aeruginosa* and the green alga *C.vulgaris*.

2. Materials and methods

2.1. Plant materials

A culture of *M. aeruginosa* was isolated from Nui Coc Lake, Thai Nguyen province, Vietnam, using the Shirai method [12], which was available at the Environmental Hydrobiology Department, Institute of Environmental Technology. The green algae *Chlorella vulgaris* was offered by Institute of Environmental Technology, Vietnam, grown in C_B that contains Ca $(NO_3)_2.4H_2O$ (150 mg mL^{-1}); KNO_3 (100 mg mL^{-1}); $MgSO_4.7H_2O$ (40 mg mL^{-1}); 1-disodium glycerol phosphate (50 mg mL^{-1}); bicine (500 mg mL^{-1}); biotin (0.0001 mg mL^{-1}); vitamin B_{12} (0.0001 mg mL^{-1}); thiamine hydrochloride (0.01 mg mL^{-1}) and 3 mL PIV, maintained at 25^0C with a 12-hour light/12-hour dark cycle in a shaking incubator [9].

Figure 1. *E. fortunei*

The aerial parts (leaves and stems) of *E. fortunei* Turcz were collected in January 2016 from Soc Son district, HaNoi, Vietnam, and identified by Dr Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

A voucher specimen (No Ef.28032017) has been deposited in the Department of Environment and Technology, Faculty of Chemistry, Hanoi National University of Education. The cleaned fresh material (38.92kg) was dried on trays at room temperature to constant weight, cut into small pieces and then ground to powder. Then, the powdered material (5.19 kg) was immersed in 96 % ethanol solvent (5L x 3 times) and subsequently macerated for two days at room temperature. Ethanol was chosen in this experiment because its crude extract from *E. fortune* showed more effective inhibition on the growth of *M.aeruginosa* than those from other solvents such as methanol or water at the concentration of 200 and 500 μg mL^{-1} [13].

The combined extracts were concentrated under vacuum to obtain ethanol crude residue (476.37 g) which was resuspended in water (2 L) and successively partitioned in hexane (1 L × 3 times) and ethyl acetate (1 L × 3 times). The organic layers were concentrated to give 90.51 g and 76.70 g of *n*-hexane and ethyl acetate residues, respectively**.** The

fractionation of the ethyl acetate residue on a silica gel column eluted by a gradient of 0-100% methanol in dichloromethane afforded six fractions F1-F6. Fraction F3 was fractionated on a silica gel column eluted with dichloromethane-methanol (10:1 v/v) to give compound **EfD 1.8** (0.115 g) – white powder.

2.2. Experimental procedure

After identifying the chemical structure, the compound **EfD 1.8** was tested the growth inhibition on *M. aeruginosa and C.vulgaris* at the concentration of 1, 10 and 100 mg L^{-1}. The test was conducted in 100-ml Erlenmeyer flasks covered with plastic foil to avoid evaporation and placed on a sterile at room temperature (25^0C) with 1000 lux light intensity under a 12-h light :12-h dark cycle. The glassware used in the test was sterilized with steam for 30 minutes at 120 ^{0}C in advance and the flasks were shaken twice a day during the experiment. The control with no addition of any extract as well as chemicals was set. The results were recorded after 0, 24, 48 and 96 hours. Experiments were conducted in triplicate under the same environmental conditions

2.3. Data analysis

Growth of *M. aeruginosa* and *C. vulgaris* was observed and determined at 0, 24, 48 and 96 hours by optical density (OD) at 680 nm wavelength using UV-Vis spectrophotometer (Shimadzu). The efficiency of growth inhibition was calculated using the following equation: [8]

$$\textbf{Inhibition efficiency (IE)(\%)} = \frac{(\textbf{Control}-\textbf{Treatment})}{\textbf{Control}} \times 100$$

Chlorophyll *a* content of *M. aeruginosa* and *C.vulgaris* was determined according to Lorenzen (1967). 10 ml of the sample collected at 0, 24, 48 and 96 hours of the incubation was filtered through a Whatman GF/C glass paper filter (47 mm diameter) and followed by extracted with 10 mL of 90% acetone at 4 $^{\circ}$C for 24 hours. Chlorophyll *a* determined spectrophotometrically at 665 and 750 nm. The spectrophotometrically absorbance of the sample was measured at 750 nm and 650 nm by an UV-VIS V-630 (JASCO, Japan), before and after acidification, and the concentration of chlorophyll *a* was determined according to the equations of Lorenzen (1967).

The data was expressed as the mean value ± SE of triplicate experiments. The data was analyzed and drawn by the softwre Graph Pad Prism 6 (one – way ANOVA). The validity of investigation was expressed as probability value of $p<0.05$.

3. Results and discussion

3.1. Identification of the compound EfD 1.8 isolated from the *Eupatorium fortunei* extracts

Compound EfD 1.8 **^{1}H NMR** (500 MHz, CD_3OD): δ_H 7.99 (1H, d, J = 16.5 Hz), 6.83 (1H, br d, overlapped with H-3), 6.86 (1H, d, J = 7.5 Hz), 7.21 (1H, br d, *J =1.5, 9.0* Hz), 7.49 (1H, br d, *J =1.5, 8.5* Hz), 6.57 (1H, d, J = 16.5 Hz); **^{13}C NMR** (125 MHz, CD_3OD): δ_C 122.6 (C-1), 158.1 (C-2), 118.6 (C-3), 132.5 (C-4), 120.7 (C-5), 129.9 (C-6), 142.4 (C-7), 116.9 (C-8), 171.3 (C-9).

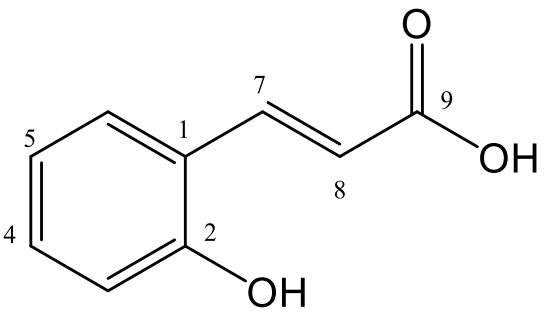

Figure 2. Structure of *o*-coumaric acid.

EfD1.8 was obtained in the form of white powder. In ^{1}H NRM, on the weak field, there appeared 6 olefin proton signals, including 4 proton signals of the aromatic ring at 6.83 (1H, br d, overlapped with H-3, H-5), 6.86 (1H, d, J = 7.5 Hz, H-3), 7.21 (1H, br d, *J =1.5, 9.0* Hz, H-4) and 7.49 (1H, br d, *J =1.5, 8.5* Hz, H-6). Besides, there are two proton signals of the double bond at δ_H 7.99 (1H, d, J = 16.5 Hz, H-8), 6.57 (1H, d, J = 16.5 Hz, H-7).

Table 1. ^{13}C NMR spectra of *o*- coumaric acid and reference

Proton Number	Chemical shift (σ_{ppm})		
	Theoretical [15]	Experiment [**DMSO-D6**]	Experiment **[CD_3OD]**
1	126.79	120.97	122.66
2	165.17	156.64	158.16
3	121.59	118.30	118.63
4	141.22	131.47	132.53
5	127.09	119.45	120.76
6	134.14	128.72	129.98
7	175.50	168.13	142.49
8	117.72	116.18	116.99
9	150.54	139.65	171.30

Table 2. ^{1}H NMR spectra of *o*- coumaric acid and reference

Proton Number	Chemical shift (σ_{ppm})		
	Theoretical [15]	Experiment [**DMSO-D6**]	Experiment **[CD_3OD]**
1	6.03	-	-
2	4.94	-	-
3	7.00	6.90	6.86
4	7.58	7.21	7.21
5	7.20	6.82	6.83
6	8.01	7.56	7.49
7	6.76	6.51	6.57
8	8.82	7.82	7.99

The ^{13}C NMR spectral data combined with the DEPT spectral data showed that the compound EfD 1.8 contained 9 signals of carbon atom groups including six signals of aromatic ring at 122.66 (C-1), 158.16 (C-2), 118.63 (C-3), 132.53 (C-4), 120.76 (C-5) and 129.98 (C-6) which were in a good agreement with the signals observed in the ^{1}H NMR spectrum; Two signals of double bond at δ_C 142.4 (C-7) and 116.9 (C-8), that are conjugated with the carboxylic group at δ_C 171.30 (C-9). At double bonds, the two protons have relatively high interacting constants (J =

16.5 Hz). It reveals that this double bond has a *trans* geometric configuration. These data established the structure of EfD 1.8 as *o* -coumaric acid or *o*-hydroxyl cinnamic acid ($C_9H_8O_3$) [Table 1 and 2] [14, 15]. A number of phenolic compounds were isolated from the aerial parts of *E. fortune* [16, 17]. In our study, *o*-coumaric acid was isolated with the yield approximately of 0.03 g powder per 10 kg of fresh plant material. The yield was much lower than that of this compound isolated from *Eupatorium adenophorum* [18]. *o*-Coumaric acid is also found in many plant products, such as, *Mikania laevigata* Sch.Bip. ex Baker (Compositae), *Mikania glomerata Spreng*, *Medicago sativa* L. (Leguminosae), *Caucalis platycarpos* L. (Apiaceae), and *Urtica urens, Mikania laevigata* [19, 20, 21, 22, 23, 24]. Previous studies had already demonstrated that *o*-coumaric has different biological activities, such as antibacterial, antilipidemic, antioxidant, and anticarcinogenic activities [25, 26] as well as strongly inhibited seed germination [18].

3.2. Effect of *o*- coumaric acid on the growth of *M. aeruginosa* and *C. vulgaris.*

The influence of *o*-coumaric acid at the concentratrion of 0÷100 mg L^{-1} on the growth of *M. aeruginosa and C. vulgaris* during 96 – hours treatment was tested. The results by optical density method (λ= 680nm) were shown in Figure 3 and 4.

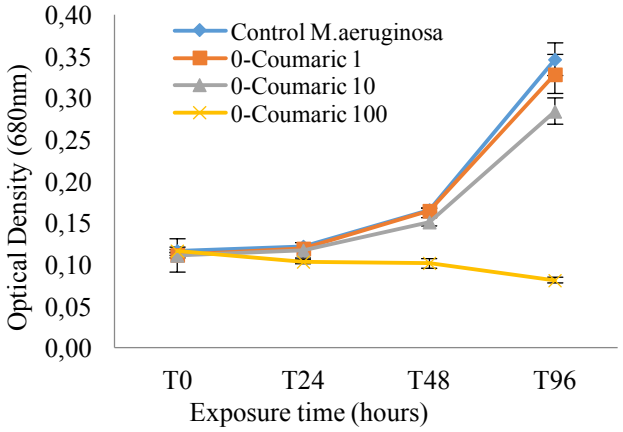

Figure 3. Effect of *o*-coumaric acid on the growth of *M. aeruginosa* (data are mean ± standard deviation, n=3)

As clearly seen from the Figure 3, the optical density value of the sample exposed to *o*-coumaric acid at the concentration of 1.0 mg. L^{-1} was similar to that of the control, increased from 0.112 ± 0.01 at the beginning (T0) to about 0.346 ± 0.025 at the end of experiment (T96) ($p< 0.05$). However, at higher concentrations, for example, *o*-coumaric acid at 10.0 mg L^{-1} had already shown a slight toxicity to *M. aeruginosa* with OD value of 0.284 ± 0.02 at the end (IE of 18.00 %). The highest inhibition was observed at the concentration of 100-mg L^{-1} *o*-coumaric acid with IE of 76.76% ($p< 0.05$). This compound had similar effects on the *C. vulgaris* growth during 96 hours experiment (Figure 4). However, its toxicity on *C. vulgaris* was lower than that on *M. aeruginosa* (Figure 5). Obviously, *o*-coumaric acid at 1.0 and 10.0 mg L^{-1} inhibited on *C.vulgaris* growth with the OD value increasing from 0.116 ± 0.001 at the beginning to about 0.351± 0.04 and 0.321 ± 0.015, corresponding to IE value just of 3.02 and 8.45 %. The IE of *o*-coumaric acid at 100.0 mg L^{-1} to *C.vulgaris* was increased (60.59%) but lower than that of *M. aeruginosa* ($p< 0.05$).

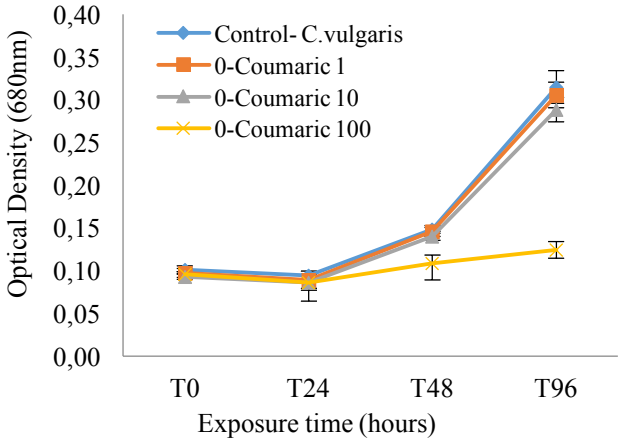

Figure 4. Effect of *o*-coumaric acid on the growth of *C. vulgaris* (data are mean ± standard deviation, n=3)

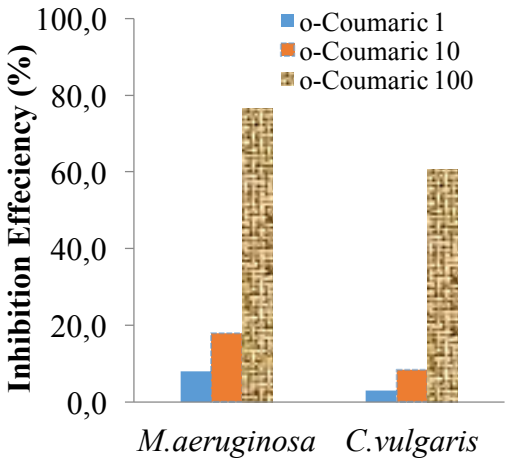

Figure 5. Inhibition Effeciency of *o-coumaric* acid on the growth of *M. aeruginosa* and *C.vulgaris*

According to previous studies [15, 18], *o*-coumaric acid was reported to have strong antibacterial and antioxidant properties, which showed a considerable growth inhibition of *Bacillus subtilis*, *Proteus vulgaris* and *Staphylococcus aureus* after 24 h and 48 h of treatment at the concentration of 1%. To the best of our knowledge, no previous study was conducted to evaluate the effect of this compound on the growth of *M. aeruginosa* and *C. vulgaris*. However, *p*-coumaric compound was reported to play a crucial role in the inhibition of *M. aeruginosa* [27]. In contrast, the study of Park [3] and Nakai [28] showed that this compound indicated no antialgal effect at the concentrations in the range of 0.01 ÷ 10 mg L^{-1}. At the higher conentrations from 16.40 to 114 mg L^{-1}, ρ-coumaric acid strongly inhibited the growth of *M. aeruginosa* with EC_{50} of 42.65 mg L^{-1}. The IE value was 100% observed in the sample exposed to 114.82 mg L^{-1} after 8 day of the experiment [29].

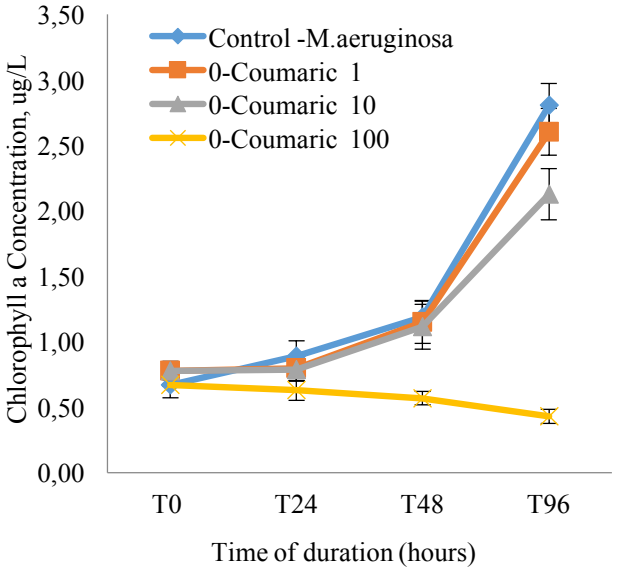

Figure 6. Effect of *o*-coumaric acid on the chlorophyll a concentration of *M.aeruginosa* cells (data are mean ± standard deviation, n=3)

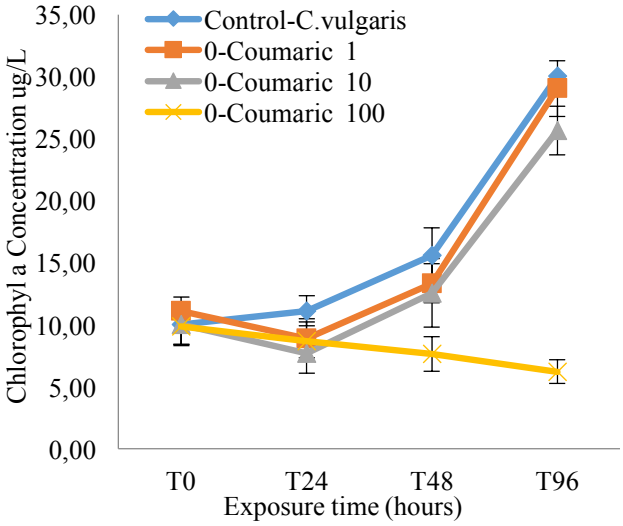

Figure 7. Effect of *o*-coumaric acid on the chlorophyll a concentration of *C.vulgaris* cells (data are mean ± standard deviation, n=3)

The effect of *o*-coumaric compound on chlorophyll *a* concentrations of *M. aeruginosa* and *C. vulgaris* was shown in Figure 6 and 7. The results were highly consistent with those obtained by optical density method. The control sample of *M. aeruginosa* increased rapidly through the experiment from 0.78 ± 0.01 to 2.80 ± 0.168 µg L^{-1} and that of C. *vulgaris* increased from 9.87 ± 0.97 to 30.04 ± 1.43 µg L^{-1} after 96- hour incubation. In general, *o*-coumaric acid was more toxic to *M .aeruginosa* than to *C. vulgaris* at all tested concentrations ($p<0.05$). At the concentrations of 1.0 and 10 $mg.L^{-1}$ this compound showed slight inhibited effect on the growth of *M. aeruginosa* (IE of 7.17 and 24.18 %, respectively) and of *C. vulgaris* (IE of 3.33. and 14.55%, respectively). At the concentration of 100 mg L^{-1} its inhibitory effect to *M. aeruginosa* and *C. vulgaris* was also shown by the gradually decrease of chlorophyll a contents to 0.43 ± 0.05 µg L^{-1} with IE of 84.66 % and to 7.65 ± 0.94 µg $L^{-1}$with IE of 74.53 %, respectively at the end of the experiment, compared with the controls ($p<0.05$). The obtained results based on the optical density and the analytical method of chlorophyll *a* concentration indicated that two methods were high consistent and *M. aeruginosa* was more sensitive to *o*- coumaric compound than *C. vulgaris* ($p <0.05$). The different impact of this substance to two species could be explained by the differences in their cell wall structures. The major constituents of the cyanobacterial cell wall are peptidoglycan (synonymous with murein), glycopeptides, and mucopeptide, whereas the green algal cell wall generally has cellulose as the main structural polysaccharide. That was why *o*-coumaric acid could easily penetrate through thinner cyanobacterial cell walls, such as those of *Microcystis* species. In addition, the compound possibly caused increases in cell membrane permeability leading to *M. aeruginosa* death [30].

3.3. Effects of the extracts on *M. aeruginosa and C.vulgaris* morphological appearance

The changes of morphological appearance of *M. aeruginosa* and *C. vulgaris* cells under light microscope BX 51 were shown in Figure 8. Obviously, the *M. aeruginosa* control cells maintained the typical shape of prokaryote, which commonly occurs as large colonial morph under natural conditions, but disaggregates and exists as single cells in laboratory cultures [31]. Under the treatment of *o*-coumaric at the concentration of 100 mg L^{-1} after 96 hours, the cells were broken leading to destroy partly or whole cells structure, which were comparable with previous reports that ρ- hydroxybenzoic acid inhibited the growth of *M. aeruginosa* by destroying the cell wall structure [31].

Figure 8D demonstrated the toxicity of *o* -coumaric acid on *C. vulgaris* microalgae, in which cells were also damaged, changing from oval shape cell to injured structure. The phenolic compound effectively inhibited the growth of the green alga *C. vulgaris*, due to the enhanced respiration of phenolics, including the uptake of oxygen [32]. There are three isomers of coumaric acid, *i.e. o*-, *m*- and *p*-coumaric acid, that differ in the position of the hydroxyl group substitution on the phenyl group. In nature, the most widespread is the *para*-isomer [33], which showed stronger antibacterial properties than *o*-coumaric and *m*-coumaric acid. [34]. The inhibitory effects induced by the polyphenols depend not only on the carbon strain, but also on the number and positions in which phenolic hydroxy groups were substituted. The ‘‘ortho’’ and/or‘‘para’’ to another phenolic hydroxy group are stronger than those induced by polyphenols in which phenolic hydroxy groups are at only meta-positions [28].

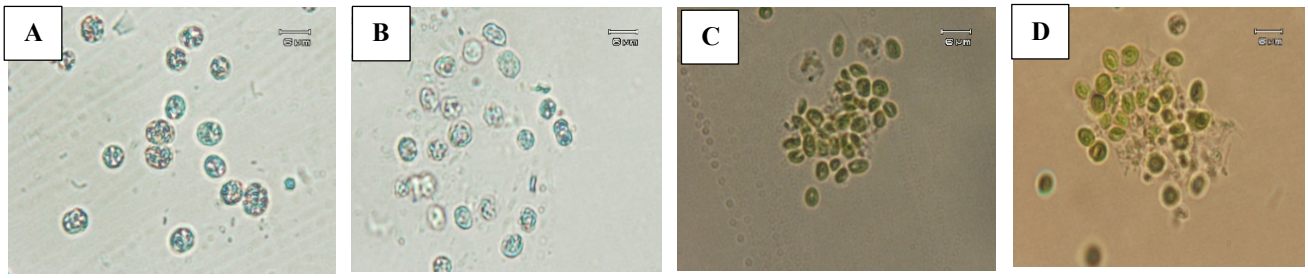

Figure 8. Morphological appearance of *M. aeruginosa* and *C. vulgaris* colonize under light microscope BX 51: A. *M.aeruginosa cells (control); B. M.aeruginosa cells exposed to o*-coumaric acid at 100 mg L^{-1} after 96 hours; *C.vulgaris* cells (Control); D. *C.vulgaris* cells *cells exposed to o*-coumaric acid at 100 mg L^{-1} after 96 hours

4. Conclusion

o-Coumaric or 2-hydroxy-cinnamic acid isolated from *E. fortunei* was identified and tested its influence on the growth of *M. aeruginosa* and *C. vulgaris* at the three concentrations of 1.0, 10.0 and 100.0 mg L^{-1} during 96 hours of exposure. The obtained results showed that o-coumaric was more toxic to *M. aeruginosa* than *C. vulgaris* at all tested concentrations. At lower concentrations of 1.0 and 10.0 mg L-1 L, o- coumaric acid indicated slight inhibited effect on two species (IE in the range from 3 ÷25%). At higher concentration of 100.0 mg L^{-1}, this compound strongly affected to *M. aeruginosa* and *C. vulgaris* (IE values of 76.76% and 60.59%, respectively, based on the optical density method and were 84.66 and 74.53 %, respectively, by chlorophyll a concentration). The images of *M. aeruginosa* and *C. vulgaris* cells under light microscope demonstrated the damage of these cells under the o-coumaric acid impact.

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