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Original Research

Investigation of Onion Peel Waste as a Valuable Resource in Controlling the Potentially Toxic Cyanobacterium *Microcystis aeruginosa* Growth

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Abstract

Onion peel waste was tested to explore its anti-cyanobacterial activity on the growth of *Microcystis aeruginosa*. First, the onion peel was extracted by maceration using three different solvents: aqueous water, ethanol, and ethyl acetate. The efficacy of the aqueous, ethanol, and ethyl acetate extracts of onion peel against *M. aeruginosa* was assessed using both microdilution and paper disc diffusion techniques. Secondly, in a batch bioassay, the anti-cyanobacterial effect of the ethyl acetate extract, as the most active extract, on *M. aeruginosa* growth and pigment production was evaluated. To identify the potential allelochemicals, total phenolic and total flavonoid contents were analyzed in the different extracts. The obtained results demonstrated the ability of the ethyl acetate extract of onion peel to inhibit *M. aeruginosa* growth. This effect was dose-dependent. The highest inhibitory rate (99%) was achieved on day 8 under the highest concentration (100 mg/L). Consequently, all treatment groups exhibited a significant reduction in chlorophyll-a and carotenoid contents compared to the control group. The preliminary results demonstrated the anti-cyanobacterial effect of the ethyl acetate extract of onion peel on *Microcystis* growth and suggest it as a potential eco-friendly solution for controlling *Microcystis* blooms in eutrophic water bodies.

Keywords: onion peel, *Microcystis aeruginosa*, blooms, anti-cyanobacterial activity, allelochemicals, growth inhibition

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Introduction

Harmful cyanobacterial blooms (CyanoHABs) emerging as a result of climate change and eutrophication have been considered as a serious problem worldwide [1, 2]. *Microcystis spp.* are the most common cyanobacterium species implicated in CyanoHABs, which can produce cyanotoxins [3]. Toxic *Microcystis* blooms contaminate aquatic ecosystems and generate negative impacts on the health of diverse living organisms [4]. To confront this situation, various methods have been used to control CyanoHABs, such as thermal destratification, artificial mixing [5], UV irradiation, ultrasound, coagulation, and flocculation techniques [6, 7]. However, these methods are expensive and generate secondary pollution that has an impact on the ecosystem [8].

New ecological approaches have emerged around the world, which involve searching for non-toxic plant-based allelochemical compounds capable of inhibiting the growth of harmful microalgae [9]. The allelopathy and algaecide potential of various macrophytes [10-14], terrestrial plants [15-19], seaweeds [20], batangas mandarin skin, dwarf banana peel [21], and pomegranate peel [22] have been regarded as alternative ecological solutions.

Several polyphenolics were identified through the phytochemical analysis of the plant extracts and considered as the most prevalent allelochemicals in the regulation of CyanoHABs [23]. They are mainly ρ-coumaric and vanillic acids [24], pyrogallic, gallic, and ellagic acids, (+)-catechin [25], epigallocatechin-3-gallate [26], luteolin [22], and quercetin [23]. Moreover, recent studies have demonstrated the use of allelopathic potentialities of the invasive plants to control CyanoHABs. This approach offers two innovative solutions: firstly, it addresses the problem of invasive plant biomass, thereby reducing the proliferation of toxic cyanobacteria through the use of a natural agent [18, 19, 27].

Onion (*Allium cepa* L.) is one of the main cultivated plants, utilized worldwide as both a vegetable and a flavouring in various types of foods [28]. Growing demand and production of onions generate significant byproducts such as onion peel. Onion peel waste is a serious threat to environmental health and the food industry. Effective technologies for valorizing onion peel are required to generate value-added products that are more environmentally friendly and cost-effective [29]. Several researchers have found that onion peel is rich in phenolic acids such as p-coumaric and protocatechuic acids, as well as flavonoids like isorhamnetin and kaempferol, and various quercetin glucosides. Quercetin and quercetin glucosides are the primary flavonols present in onion [30]. These biomolecules serve as an excellent source of diverse bioactive compounds [31]. Onion peel compounds are documented for their antifungal, antibacterial, and antioxidant activities [32, 33]. Their anti-cyanobacterial potential in the biocontrol of hazardous algae has not been described in the literature.

The present study hypothesizes that allelochemicals from onion peel extracts possess the ability to control CyanoHABs and may inhibit the growth of *M. aeruginosa*. To assess this hypothesis, this study aims to investigate the anti-cyanobacterial effects of aqueous and organic extracts of onion peel on *M. aeruginosa* in an experimental bioassay by measuring the growth of algal cells, analyzing photosynthetic pigments, and characterizing potential allelochemicals. The reuse and valorization of onion peel could be an economic and eco-friendly alternative agent to treat waters contaminated by *M. aeruginosa* blooms and minimize onion peel waste.

Materials and Methods

Biological Materials and Culture Conditions

Samples of *M. aeruginosa* were collected from Lalla Takerkoust reservoir, Morocco (31°21’36”N, 8°7’48”W) in August 2020. *M. aeruginosa* was isolated and inoculated as a monoalgal strain. The culture was maintained in flasks containing sterile BG-11 medium under aseptic laboratory conditions (25ºC±1ºC, an illumination intensity of 70 µE/m^2·S, and a light/dark cycle of 15/9 h).

The onion peel used consisted of red onion collected in May 2024 from the local market in the region of Béni Mellal, Khénifra-Morocco. The onion peels were rinsed, using distilled water, to eliminate debris, and then dried in the air to avoid microbial degradation during storage. Once dried, biomass was crushed and preserved.

Preparation of Onion Peel Extracts

To prepare the aqueous and organic extracts of onion peel, two methods were employed. The aqueous extract of onion peel was prepared according to the method adopted by Chen et al. [34], with certain modifications. Ten grams of onion peel were mixed with 100 mL of distilled water and kept under agitation for 24 h at 37ºC. Wattman No. 3 paper was used to filter the macerate. The dry extracts obtained after freeze-drying the filtrate were stored at 4ºC in a refrigerator. For the organic extraction, ethyl acetate and ethanol/water (70/30: v/v) were used separately to extract the bioactive compounds from the onion peel [35]. Briefly, ten grams of onion peel were extracted at room temperature by maceration for 24 h with 100 mL of each solvent separately under magnetic stirring. Wattman No. 3 paper was used to filter the macerates. The filtrates were dried completely by evaporation under reduced pressure with a rotary evaporator. The dry extracts were stored in the refrigerator at 4ºC.

Phytochemical Characterization

The Total Phenolic Content (TPC) was determined spectrophotometrically according to the Folin-Ciocalteu method [36]. A volume of 125 µL of the extract was added to 0.5 mL of distilled water. Then, 125 µL of Folin-Ciocalteu reagent was added, and the mixture was incubated in the dark at room temperature for 3 min before adding 1.25 mL of a 7% (w/v) sodium carbonate solution. After that, distilled water was added to a final volume of 3 mL. After 90 min in the dark at room temperature, the absorbance of the mixture was measured at 760 nm. The results are expressed in milligrams of Gallic Acid Equivalent per gram of dry extract (mg GAE/g of dry extract). The measurements were performed in triplicate.

The Total Flavonoids Content (TFC) was determined according to the method described by Ghedadba et al. [37]. 1 mL of the extract was mixed with 1 mL of a 2% aluminum chloride $AlCl_3$ (dissolved in methanol). After 10 min, the absorbance was measured at 430 nm. The results are expressed in milligram Quercetin Equivalent per gram of dry extract (mg QE/g of dry extract). The measurements were performed in triplicate.

Assessment of the Anti-Cyanobacterial Activity of the Different Extracts

Minimum Inhibitory Concentration (MIC) and Minimum Algicidal Concentration (MAC) Determination: The MIC for the Aqueous (AE), Ethanol (70%) (EE), and Ethyl Acetate (EAE) Extracts of onion peel was determined by the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) using 96-well microplates [38]. In each 96-well plate, a volume of 100 µL of *M. aeruginosa* in its exponential growth phase (~5×10^5 cells/mL) was added. Then, 100 µL of each extract dissolved in dimethyl sulfoxide (DMSO 1%) was added to obtain a concentration range from 12.5 to 400 mg/L. Quercetin, copper sulfate, and 1% DMSO were used as positive and negative controls, respectively. Plates were incubated under the same conditions detailed in the section "Biological Materials and Culture Conditions" for 5 days. Subsequently, 100 µL of medium from each well showing no visible cyanobacterial growth was inoculated in BG-11 medium to determine the MIC. The MAC was determined by subculturing on microplates under the same controlled conditions as the clear wells that did not show any visible growth after incubation during the test. Each test was conducted in triplicate.

Disc Diffusion Assay: Sterile filter paper discs (diameter = 9 mm) were infused with 20 µL of AE, EE, and EAE of the onion peel extracts and placed on the surface of the BG-11 culture medium, which had previously been inoculated with *M. aeruginosa* [39]. To allow the bioactive substances to diffuse, the treated Petri dishes were stored at 4ºC for a minimum of 2 h. Quercetin, copper sulfate, and DMSO (1%) were used as positive and negative controls. Anti-cyanobacterial activity was determined by measuring the inhibition zones (in mm) around the discs with the applied extracts. Each test was performed in triplicate.

Preliminary Assessment of the Anti-Cyanobacterial Activity of Organic Extracts in Liquid Medium: A preliminary experimental bioassay was carried out in 24-well plates containing 3 mL of BG-11 liquid medium. Each well was inoculated with *M. aeruginosa*, with an initial density of ~5×10^5 cells/mL. The *M. aeruginosa* cultures were exposed to the different treatments at concentrations (0 mg/L [control], 6.25 mg/L [MIC/16], 12.5 mg/L [MIC/8], 25 mg/L [MIC/4], 50 mg/L [MIC/2], and 100 mg/L [MIC]) of EE and EAE extracts of onion peel dissolved in DMSO 1% (v/v). DMSO (1%) was used as a negative control. The well plates were incubated in a controlled room under the same conditions described in the section "Biological Materials and Culture Conditions" for 10 days. All groups were conducted in triplicate.

Allelopathic Effect of the Most Active EAE on *M. aeruginosa* Growth and Pigment Production in a Batch Bioassay

Experimental Design: Five groups of 250 mL Erlenmeyer flasks, each containing 250 mL BG-11 medium, were utilized to test four concentrations (0 [control], 25 mg/L [MIC/4], 50 mg/L [MIC/2], and 100 mg/L [MIC]) of the onion peel EAE dissolved in 1% DMSO (v/v). DMSO (1%) was used as a negative control. A volume of *M. aeruginosa*, in exponential growth phase, was introduced to each flask to create an initial density (~5×10^5 cells/mL). The cultures were incubated in a controlled room under the same conditions described in the section "Biological Materials and Culture Conditions" for 10 days. Each test was performed in triplicate.

Growth Density: The number of *Microcystis* cells was estimated every 2 days by observation under a light microscope using a Malassez hemocytometer. The Inhibitory Rate (IR) was determined using the following formula:

$$IR\ (\%) = [(N_0\text{-}N)/N_0] \times 100$$

Where: N_0 is the control cell density (cells/mL), and N is the treatment cell density (cells/mL).

Pigment Quantification: Chlorophyll-a (Chl-a) and carotenoids were extracted with 96% ethanol at 4ºC for 48 h. The extracts were then examined at 470, 649, and 665 nm using a spectrophotometer (TOMOS V-1100) and calculated according to the method described by Lichtenthaler and Wellburn [40].

The concentrations (µg/mL) of Chl-a and carotenoids were calculated using the following formulas:

$$\text{Chl-a } (\mu g/mL) = (13.95 \times DO665) - (6.88 \times DO649)$$

$$\text{Carotenoids } (\mu g/mL) = [(1.000 \times DO470) - (2.05 \times \text{Chl-a})]/229$$

Statistical Analysis

SPSS V26 was used for statistical analysis. The results are presented as mean ± standard deviation. A one-way ANOVA followed by the Tukey test was employed to evaluate growth and photosynthetic pigments. The comparison of result significance was carried out at $\alpha<0.05$. Each test was performed in triplicate.

Results and Discussion

Phytochemical Characterization

The quantification of both TPC and TFC in onion peel extracts showed a high content in the ethyl acetate fraction (TPC = 296±4.5 mg GAE/g, TFC = 140±1.7 mg QE/g), and ethanol/water fraction (70/30: v/v) (TPC = 248±7.8 mg GAE/g, TFC = 116±1.2 mg QE/g). In comparison, the water fraction yielded significantly lower values (TPC = 96±1.3 mg GAE/g, TFC = 25±1.6 mg QE/g).

These findings indicate that the contents of TPC and TFC vary significantly depending on the solvent used for extraction (Table 1). These results align with previous research, which also showed that the TPC and TFC contents in ethyl acetate extracts were higher than those in ethanol/water (70/30: v/v) and water extracts of onion peel [35].

Anti-Cyanobacterial Activity Assessment

The anti-cyanobacterial activity of aqueous and organic extracts of onion peel was tested using both microdilution and disc diffusion techniques. The results showed that among the three onion peel extracts, only the organic extracts (EE and EAE) exhibited anti-cyanobacterial activity.

The results presented in Table 2 and Fig. 1 revealed that the MIC and MAC values for both EE and EAE were equal at concentrations of 100 mg/L and 200 mg/L, respectively. However, on the solid medium, using the disc diffusion method, the EAE demonstrated the highest activity against *M. aeruginosa,* with an inhibition zone of 20±2.3 mm, followed by the EE with 19±1 mm. The AE and DMSO (1%), which were used as negative controls, did not exhibit any antialgal activity. In contrast, copper sulfate and quercetin, serving as positive controls, demonstrated strong inhibitory effects against *M. aeruginosa* (Table 2).

The two extracts, EE and EAE, having the same MIC (100 mg/L) and MAC (200 mg/L), were tested on 24-well plates to determine the most active extract on *M. aeruginosa*. Consequently, the results of the bioassay were presented as a percentage of inhibition. IC_{50} and IC_{90} values were determined on the last day (day 10) of the experimentation. EAE showed an IC_{50} (43.21 mg/L) and IC_{90} (84.88 mg/L), which are lower than those of EE, whose IC_{50} was 45.70 mg/L) and IC_{90} was 90.64 mg/L. This suggests that the EAE shows the highest activity in comparison to the EE.

According to the results of TPC and TFC quantification, the extract containing the highest polyphenol content (EAE) showed the strongest anti-cyanobacterial activity. These results are consistent with previous studies showing that polyphenols and flavonoids extracted from plants exhibit anti-cyanobacterial activity against *M. aeruginosa* [9]. Most of these bioactive compounds are common allelochemicals and are recognized for their antioxidant and antimicrobial properties, as well as their potential in controlling CyanoHABs [27].

Effect of EAE of Onion Peel on the *M. aeruginosa* Growth, Morphological Evolution, and Pigment Production in a Batch Bioassay

An experimental bioassay under batch conditions was performed using EAE, which demonstrated a stronger inhibitory effect. Fig. 2 shows that EAE may inhibit the growth of *M. aeruginosa*. Compared to the control group, the cell densities of *M. aeruginosa* were significantly reduced ($p<0.05$) during the 10 days of exposure to the various tested concentrations.

The IR is shown in Table 3, which demonstrates that *M. aeruginosa* growth was clearly inhibited by EAE, and the IR was observed to be dose-dependent. After only 2 days, the IR exceeded 69±1.7% and 71±0.8% at

Table 1. The phytochemical characterization of the three extracts of onion peel.

Extraction solvent	Total phenolics content TPC([a])	Total flavonoid content TFC([b])
Water	96±1.3	25±1.6
Ethyl acetate	296±4.5	140±1.7
Ethanol/Water (70/30: v/v)	248±7.8	116±1.2

Note: ([a]): mg GAE/g dry extract, ([b]): mg QE/g dry extract.

Table 2. The anti-cyanobacterial activity against *Microcystis aeruginosa* using the microdilution and disc diffusion methods (zone of inhibition in a disc of 9 mm).

Treatments	MIC (mg/L)	MAC (mg/L)	Zone of inhibition (mm)
DMSO (1%)	NA	NA	0±0
Onion peel AE	NA	NA	0±0
Onion peel EAE	100	200	20±2.3
Onion peel EE	100	200	19±1
Copper sulphate	3.125	3.125	48±2.4
Quercetin	50	100	22±1.6

Note: NA: Not Active.

the two highest concentrations tested (50 and 100 mg/L), respectively. On day 4, the IRs reached 49±2.3%, 89±0.3%, and 93±1.5% for concentrations of 25, 50, and 100 mg/L, respectively. The highest IR (99±0.2%) was obtained at the highest concentration (100 mg/L) after 8 days. These results are generally consistent with those reported in previous studies of EAE of Moroccan medicinal plants *T. maroccanus* and *O. compactum* (97.41% and 97.16%, respectively) at (100 mg/L) after 8 days [17].

Microscopic and visual analyses of the *M. aeruginosa* cultures indicated that *M. aeruginosa* cells had a clear, rounded shape in the control groups. However, at the highest concentration (100 mg/L), the *M. aeruginosa* cells lost their typical regular shape and formed sediment aggregates with a damaged morphology (Fig. 3).

The measurement of the two photosynthetic pigments (Chl-a and carotenoids) was conducted to evaluate physiological modifications. During the 10 days of the experimental period, the treatment groups (50 and 100 mg/L) showed a significant reduction ($p<0.05$) in Chl-a and carotenoid contents in comparison to the control group (Fig. 2). The inhibition of growth was accompanied by a reduction in photosynthetic pigments, which are considered as markers of physiological disturbance under stressful conditions [10, 16]. In addition, some research has indicated that the extracts negatively influence the content of Chl-a and carotenoids. The reduction signifies a disturbance in

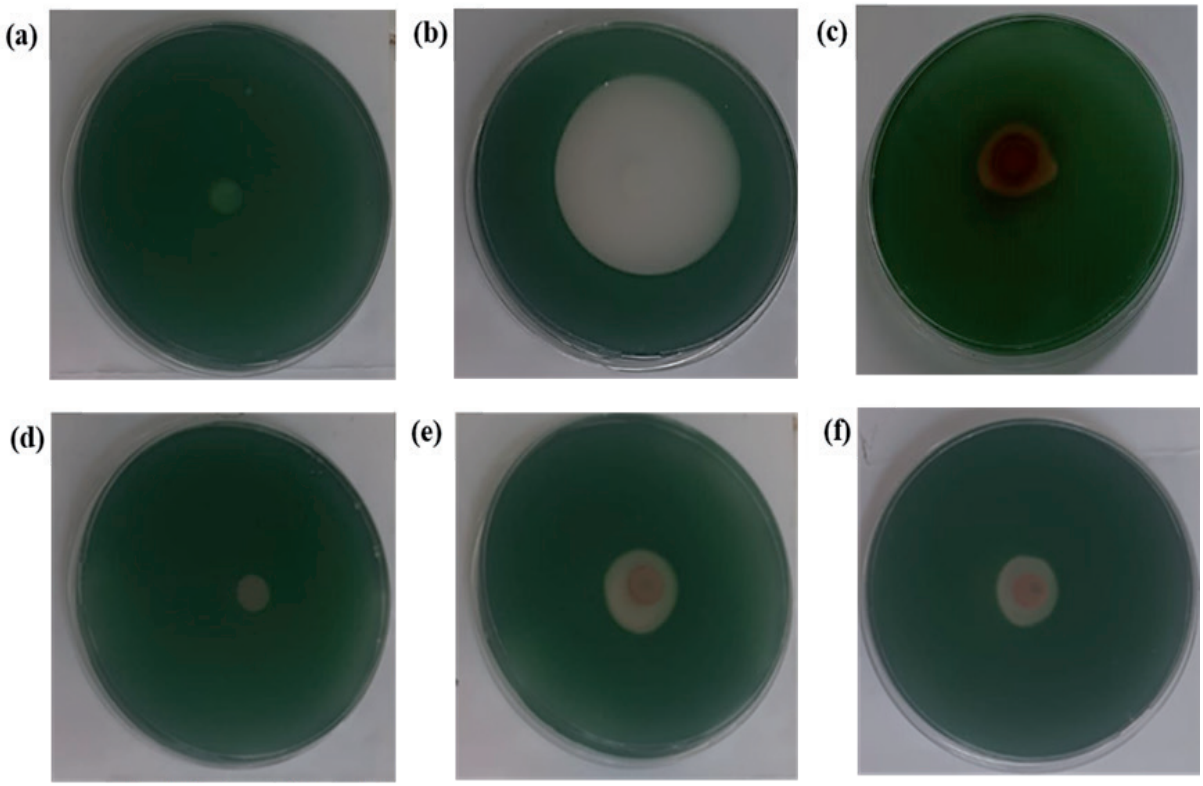

Fig. 1. Anti-cyanobacterium activity: a) DMSO (1%), b) copper sulfate, c) quercetin, d) AE, e) EAE, and f) EE against *M. aeruginosa*.

photosynthesis, influencing the growth and reproduction of *M. aeruginosa* [13, 18, 19].

Quercetin is the major flavonoid present in onion [41]. The research conducted by Zhao et al. [23] demonstrated that quercetin significantly inhibited the growth of *M. aeruginosa*, and the inhibitory effect was concentration-dependent. The IR of 40 mg/L of quercetin on algal density reached 90.79% after 4 days. Furthermore, quercetin affects photosynthesis and causes damage to the cell membrane as well as to respiratory and enzyme systems. According to Gigova and Ivanova [42], allelochemical substances are known

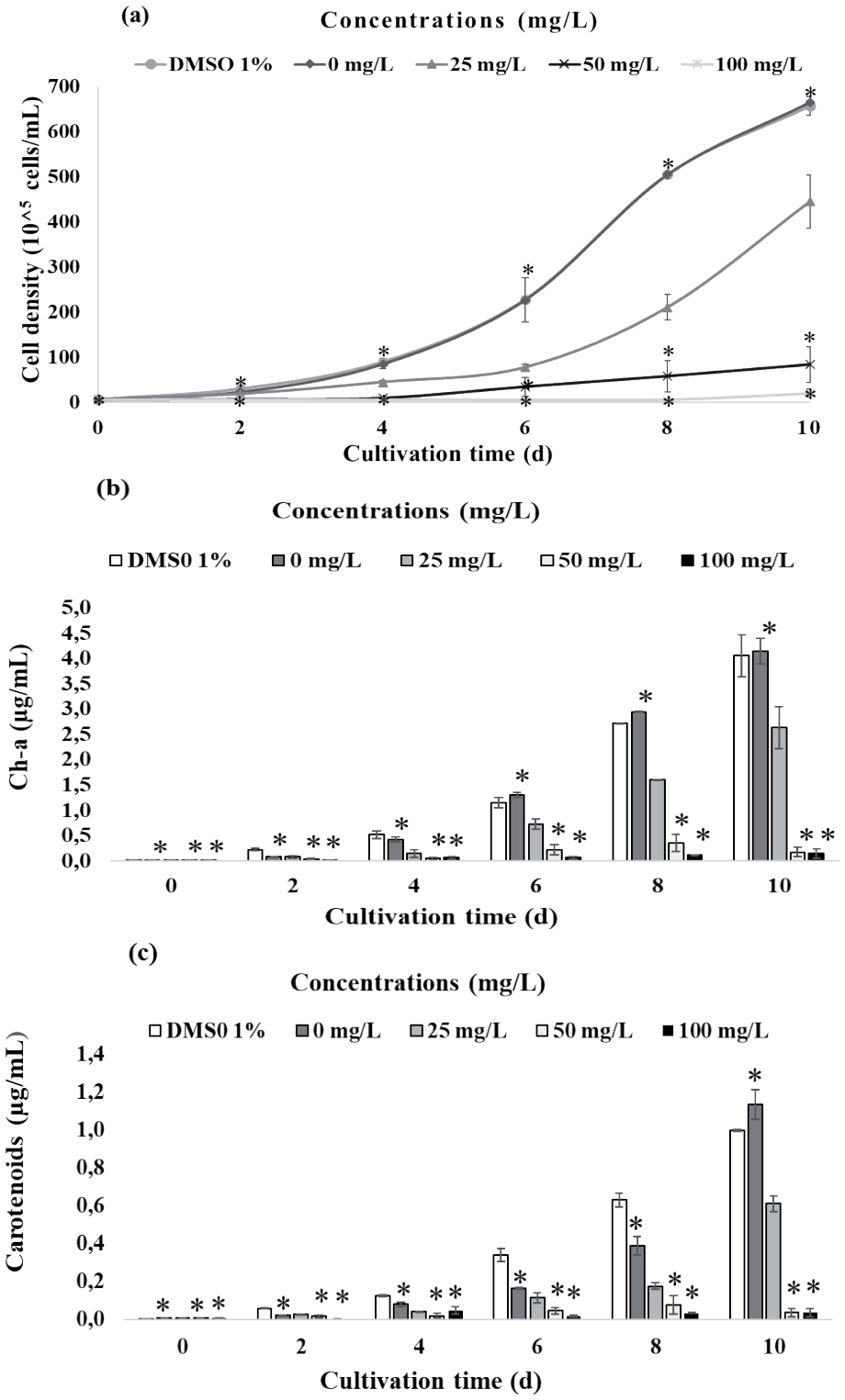

Fig. 2. Effects of various onion peel concentrations on *M. aeruginosa*. a) growth, b) Chl-a, and c) carotenoids in *M. aeruginosa* cultures, respectively. Error bars represent the mean±standard deviation (n = 3). $p<0.05$ indicates significant differences in comparison to the untreated culture (one-way ANOVA).

Table 3. Inhibition rate IR (%) of onion peel EAE on *M. aeruginosa* on different days.

Treatments (mg/L)	Day 2	Day 4	Day 6	Day 8	Day 10
25	17±10	49±2.3	67±1.9	56±3.2	29±5.1
50	69±1.7	89±0.3	85±9.2	88±6.9	87±5.9
100	71±0.8	93±1.5	98±0.2	99±0.2	97±1.4

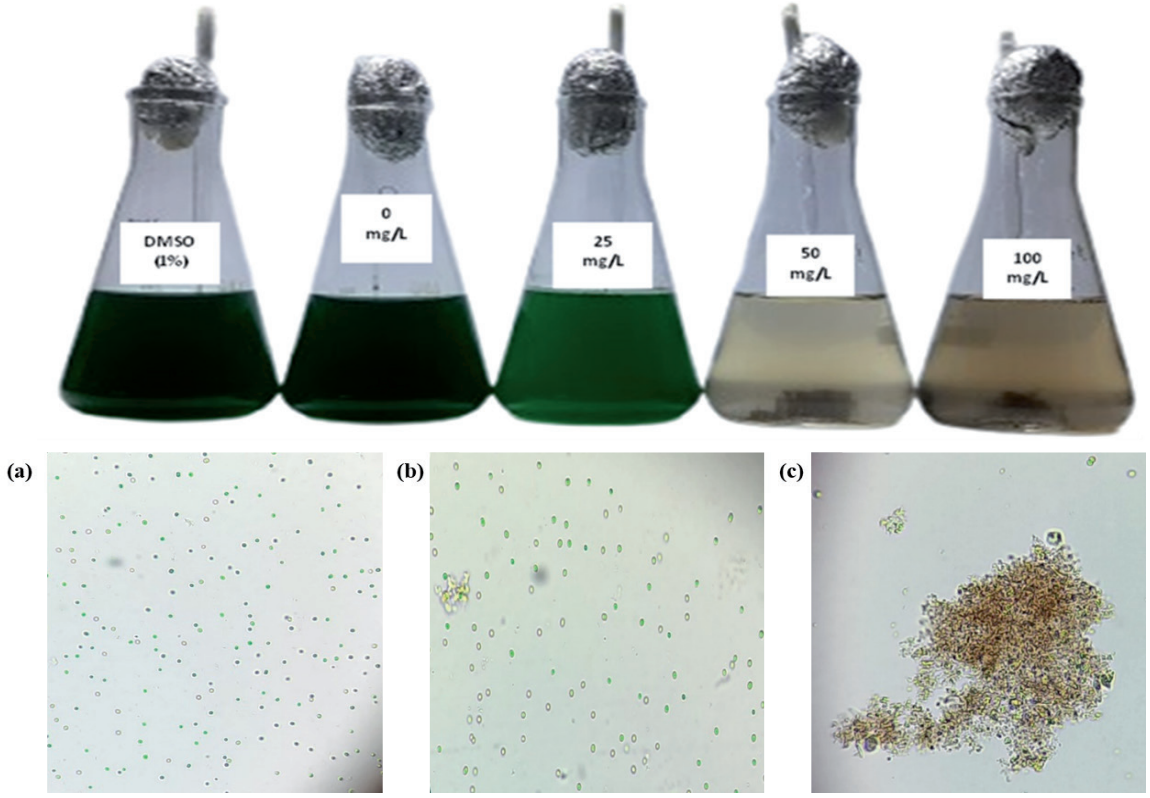

Fig. 3. Visual and microscopic observations of *M. aeruginosa*. a) control, b) negative control DMSO (1%), c) under the highest concentration (100 mg/L) of onion peel EAE after 8 days (Gr. x 40).

to inhibit cell growth by disrupting the physiological regulation and cellular structure. Because they are amphiphilic and lipophilic, phenolic acids increase cell permeability [43]. According to Wang et al. [44], the cell membrane integrity of *M. aeruginosa* was compromised by p-ferulic and coumaric acids. Under stressful conditions, reactive oxygen species (ROS) break down unsaturated phospholipids, increasing the permeability of cell membranes [13]. Therefore, by interfering with the components of photosystem II (PS II), disturbances in the antioxidant defense system hinder photosynthesis and oxygen evolution, ultimately leading to cell death [15].

Conclusions

Controlling CyanoHABs is crucial for the protection of aquatic environments. To develop a cost-effective, environmentally friendly, and practical alternative for controlling microalgae proliferation, the present results demonstrate the ability of onion peel extract to inhibit the growth of *M. aeruginosa*. This effect was dose-dependent. During the 10-day experimental period, all three treatment groups showed a significant reduction in Chl-a and carotenoid contents compared to the control group. The characterized TPC and TFC could be the primary allelochemicals responsible for reducing *Microcystis* growth. Therefore, onion peel can be recommended as a green anti-cyanobacterial agent to control *Microcystis* proliferation. Further research is needed to identify the dominant and specific allelochemicals and to examine their potential impacts on aquatic ecosystems.

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Conflict of Interest

The authors declare no conflict of interest.

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