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Screening of Fruit and Vegetable Waste Extracts as an Eco-Friendly Strategy to Control the Proliferation of the Toxic Cyanobacterium *Microcystis aeruginosa*

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Abstract

Fruits and vegetables are among the most widely consumed products; however, their processing by both industry and households generates substantial peel waste, leading to significant environmental concerns. This study aimed to assess fruit and vegetable peels as an eco-friendly source of natural cyanbactericidal compounds for controlling toxic algal blooms. Aqueous Extract (AE), Ethyl Acetate Extract (EAE), and Ethanol Extract (EE) of five fruit and vegetable peels (Avocado, tomato, mango, eggplant, and potato) were screened for their inhibitory effects on *Microcystis aeruginosa* using both paper disc diffusion and microdilution techniques. Therefore, the extracts of avocado peel, the most bioactive of the five peels, were selected for further testing in a batch bioassay. The inhibitory effect appeared to be dose-dependent. The maximum inhibition rate (98%) was recorded following an 8-day exposure period at the dose (MIC = 0.8 mg/mL) for both the AE and EAE. Thus, the EE showed significant potential, particularly at the highest concentration (MIC = 0.2 mg/mL), where inhibition peaked at 92% on day 8. Accordingly, all treated groups demonstrated a significant decrease in primary photosynthetic pigments and phycobiliproteins in comparison to the control groups. In conclusion, avocado peel extracts show the most potent inhibition in batch bioassay. These extracts could constitute a promising strategy to valorize avocado peel waste as a sustainable cyanobactericidal agent to suppress the proliferation of *M. aeruginosa* blooms in eutrophic aquatic ecosystems.

Keywords: fruits; vegetables; peels; wastes; *Microcystis aeruginosa*; anti-cyanobacterial activity; eco-friendly algaecide

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1. Introduction

Water contamination by toxic cyanobacteria is a global problem, exacerbated by both eutrophication and climate change [1]. Cyanobacteria possess the capacity not only to proliferate forming blooms but also to synthesize various cyanotoxins, which present substantial risks to human health and the aquatic environment [2,3]. Microcystins represent a class of potent, cyclic heptapeptide cyanotoxins produced by multiple genera of cyanobacteria, including *Microcystis*, *Oscillatoria*, *Anabaena*, *Nostoc*, etc. [4].

To control *Microcystis* blooms in water bodies and microcystins in drinking-water treatment plants various physical and chemical strategies-including thermal destratification, artificial mixing, ultrasound treatment, UV irradiation, and flocculation-coagulation-were adopted [5–7]. However, the application of these elimination techniques generates secondary pollution, high costs and adverse effects on the ecosystem [8].

In recent years, researchers have shown interest in the extraction of allelochemical compounds from various non-toxic plants, which show significant potential as algaecides to suppress the proliferation of cyanobacteria [2,9–11]. Analysis of the plant extracts identified several polyphenolics as potent antialgal agents [12], such as ρ-coumaric, vanillic [13], pyrogallic, gallic, and ellagic acids, (+)-catechin [14], epigallocatechin-3-gallate [15], luteolin [16], and quercetin [12].

The utilization of aquatic or terrestrial plant biomass in the search for allelochemicals faces constraints regarding the conservation and protection of the resource. Consequently, the recourse to biomasses deemed useless or abandoned proves to be both highly justified and ambitious—serving simultaneously to remove it from the environment and to utilize it as value-added by-product. From this perspective, fruit and vegetable waste could constitute a green and economical alternative. Fruits and vegetables play a crucial role in our diet and lives. Consequently, the demand for these essential foodstuffs has increased significantly due to global population growth and changing eating habits. The food industry discards huge amounts of fruit and vegetable waste, including peels, seeds and other residues [17,18].

However, fruit and vegetable peels are also an excellent source of carbohydrates, fibre, protein, and phytochemicals, including phenolic compounds with high antioxidant properties. Polyphenols are a large group of secondary metabolites commonly found in fruits and vegetables that play a major role in nutrition and human health [19,20]. Various studies have extracted numerous components with antimicrobial, antioxidant, and anti-inflammatory properties from various peels [19–22]. This approach provides an environmentally friendly and economical solution for both recycling waste and creating added value, which can then be applied in other sectors, including pathogen biocontrol [23].

The main phenolic compounds present in various fruit and vegetable peels (Avocado, tomato, mango, eggplant, and potato) include phenolic acids such as caffeic, chlorogenic, gallic, protocatechuic, ρ-coumaric, syringic, ferulic, and sinapinic acids, as well as flavonoids like kaempferol, quercetin, and quercetin glucosides [20,24,25].

We hypothesized that the reuse and valorization of fruit and vegetable peels, potentially rich in phenolic compounds, could be a cost-effective and sustainable source of cyanobactericides for mitigating *M. aeruginosa* proliferations. Specifically, this study aims to characterize the inhibitory effect of both organic (Ethyl Acetate and 70% Ethanol) and Aqueous Extracts of five different fruits and vegetables peels on *M. aeruginosa*. Through in vitro assays, by measuring both the inhibitory effect on *Microcystis* growth, and the responses in the production of phycobiliproteins and photosynthetic pigments. These hypotheses were tested through experiments using a cultivated strain of *M. aeruginosa*.

2. Materials and Methods

2.1. Biological Materials and Culture Conditions

M. aeruginosa was sampled from Morocco's Lalla Takerkoust reservoir (31°21′36″ N, 8°7′48″ W) during August 2020. Following their isolation into a monoalgal strain, the cells were grown in vitro using sterile liquid medium (BG-11) under aseptic conditions. The laboratory environment was regulated to maintain a constant temperature of 25 °C (±1 °C) and a 15/9 h light/dark photoperiod, with a specified light intensity of 70 μE/m^2·s.

Three fruits and two vegetables were purchased from a local marketplace situated in Morocco's Béni Mellal-Khénifra region. These fruit and vegetable were avocado (*Persea americana* Mill.), tomato (*Solanum lycopersicum* L.), mango (*Mangifera indica* L.), eggplant (*Solanum melongena* L.), and potato (*Solanum tuberosum* L.). After rinsing with distilled water to eliminate contaminants, the fruits and vegetables were peeled. These peels were then air-dried and subsequently processed into a fine powder for storage at 4 °C.

2.2. Extraction

Bioactive compounds from avocado, tomato, mango, eggplant, and potato peels were extracted using three distinct solvents: distilled water, Ethyl Acetate, and a 70:30 (*v*/*v*) Ethanol/water mixture. A mass of 10 g of each powdered peel was macerated separately with 100 mL of each solvent under agitation for 24 h at room temperature. The macerate was filtered using Whatman No. 3 filter paper. The Aqueous Extract was freeze-dried (Martin Christ, Alpha 1–4 LSbasic, Osterode am Harz, Germany), and the organic extracts (Ethyl Acetate and 70% Ethanol) were concentrated by evaporating the solvents using a rotary evaporator (Scilogex RE100-Pro, Beijing, China). The dry extracts were stored at 4 °C until further use [26].

2.3. Phytochemical Assessment

Total phenolics content were measured spectrophotometrically using gallic acid as a standard, following the Folin–Ciocalteu method [27]. Results were expressed as milligram Gallic Acid Equivalent per gram of dry extract (mg GAE/g). The total flavonoids content was determined according to the method described by Ghedadba et al. [28] using quercetin as the standard. Results were expressed as milligram Quercetin Equivalent per gram of dry extract (mg QE/g).

2.4. In Vitro Screening of the Anticyanobacterial Activities

The Aqueous (AE), Ethyl Acetate (EAE), and 70% Ethanol (EE) extracts of the five fruit and vegetable peels were preliminary screened in vitro against *M. aeruginosa* growth.

2.4.1. Microdilution Assay

The Minimum Inhibitory Concentration (MIC) and Minimum Algicidal Concentration (MAC) values of AE, EAE, and EEs of five fruit and vegetable peels were determined following the microdilution method described by Joshi et al. [29]. Each extract, dissolved in 1% dimethyl sulfoxide (DMSO) at doses between 0.003 and 6.4 mg/mL, was added in 100 µL aliquots to an equivalent volume of *M. aeruginosa* culture (~5×10^5 cells/mL). Copper sulfate, gallic acid, catechin, and quercetin served as the positive controls, while a 1% solution of DMSO was used as the negative control. The 96-well microplates were maintained under the identical cultivation conditions described in Section 2.1 for 5 days [3,11]. The MAC was identified by sub-culturing the contents of wells where no growth was observed.

2.4.2. Disc Diffusion Assay

AE, EAE, and EEs of each fruit and vegetable peels were impregnated using sterile discs (9 mm, 20 µL per disc), and then placed on Petri dishes containing solid BG-11 medium inoculated with *M. aeruginosa*, following a 2 h pre-diffusion period at 4 °C [30]. Copper sulfate, gallic acid, catechin, quercetin, and a 1% solution of DMSO was used as controls. The Petri dishes were maintained using the identical cultivation conditions described in Section 2.1. The inhibition zone diameters (mm) were determined after 7 days of incubation [31].

2.5. Assessment of the Anticyanobacterial Activities in Batch Bioassay

The selected active extracts were applicated on *M. aeruginosa* in a batch bioassay.

2.5.1. Experimental Design

The bioassay was conducted in sterilized 500 mL flasks. Triplicate cultures (300 mL) of *M. aeruginosa* (~5×10^5 cells/mL), maintained in BG-11 medium, were exposed to both the MIC and sub-MIC (MIC/2) concentrations of AE, EAE, and EE of the most potent peel. AE was directly applied, while EAE and EE were prepared using a 1% DMSO (v/v) solution. A 1% DMSO was employed as a negative control. In a controlled room, the cultures were incubated under the identical cultivation conditions described in Section 2.1 for 10 days. Samples were collected at two-day intervals to measure cell density, pH, photosynthetic pigments, and phycobiliproteins.

2.5.2. Growth Density Evaluation

A Malassez hemocytometer was employed to monitor cell density. The inhibition rate (IR, %) was subsequently determined according to Equation (1):

$$\text{IR}\ (\%) = \frac{\text{N}_0 - \text{N}}{\text{N}_0} \times 100 \quad (1)$$

Cell densities (Cells/mL) for control and treated samples are represented by N_0 and N, respectively.

2.5.3. Photosynthetic Pigment Quantification

Following the methodology of Lichtenthaler and Wellburn [32], 96% Ethanol was utilized to extract carotenoids and chlorophyll-a over a 48 h period at 4 °C. Subsequently, a TOMOS V-1100 spectrophotometer (Macylab 2013, Beijing, China) was employed to record absorbances at 470, 649, and 665 nm, with concentrations determined using the following Equations (2) and (3):

$$\text{Carotenoids}\ (\mu\text{g/mL}) = \frac{(1.000 \times \text{A470}) - (2.05 \times \text{Chl} - \text{a})}{229} \quad (2)$$

$$\text{Chlorophyll-a}\ (\mu\text{g/mL}) = (13.95 \times \text{A}_{665}) - (6.88 \times \text{A}_{649}) \quad (3)$$

2.5.4. Phycobiliprotein Quantification

Following the protocol established by Li et al. [33], phycobiliprotein contents specifically phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE) were quantified. An amount of 5 mL of *M. aeruginosa* culture was first isolated via centrifugation at 4 °C (Eppendorf, Centrifuge 5804R, Hamburg, Germany). After removing the supernatant, the pellet was resuspended in 5 mL of phosphate buffer (50 mM, pH 7.28). The mixture underwent 3 to 5 cycles of freezing and thawing in the dark. The final extract was centrifuged again, and the supernatant's absorbance was recorded at 565, 620, and 650 nm. Finally, we applied Equations (4)–(6) to calculate the respective phycobiliprotein contents (PC, APC, and PE).

$$\text{PC}\ (\text{mg/mL}) = \frac{\text{A620} - 0.7 \times \text{A650}}{7.38} \quad (4)$$

$$\text{APC}\ (\text{mg/mL}) = \frac{A650 - 0.19 \times \text{A620}}{5.65} \quad (5)$$

$$\text{PE}\ (\text{mg/mL}) = \frac{A565 - 2.8 \times \text{PC} - 1.34 \times \text{APC}}{1.27} \quad (6)$$

2.6. Statistical Analysis

Statistical analysis was executed using SPSS software (Version 26), applying ANOVA (one or two-way) and Tukey's test for comparisons ($\alpha < 0.05$). All findings are reported as the mean ± standard deviation (SD) of triplicate assays. Each test was performed in triplicate.

3. Results

3.1. Phytochemical Assessment

To evaluate the phytochemical composition of the extracts, the total phenolic and total flavonoid contents were determined. Table 1 summarizes the total phenolic and total flavonoid contents in the AE, EAE, and EE of the five selected fruit and vegetable peels. It appeared that the total phenolic and total flavonoid content values varied considerably depending on the extraction solvent. Overall, avocado extracts showed the highest values for both total phenolic and total flavonoid content. The avocado EE showed the highest total phenolic content value (212.31 mg GAE/g). The total flavonoid content value in avocado was 1.5–1.7 times higher in the EAE than in the AE and EE.

Table 1. Phytochemical screening results for the five fruit and vegetable peels extracts.

Extracts	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
Avocado: AE	104.02 ± 1.81^{b}	15.70 ± 1.13^{b}
Avocado: EE	212.31 ± 2.46^{a}	13.84 ± 0.06^{c}
Avocado: EAE	65.86 ± 0.65^{c}	23.72 ± 0.05^{a}
Tomato: AE	9.69 ± 2.02^{j}	3.21 ± 0.17^{g}
Tomato: EE	31.78 ± 8.04^{gh}	5.55 ± 0.10^{e}
Tomato: EAE	15.72 ± 0.14^{ij}	4.08 ± 0.33^{fg}
Mango: AE	26.19 ± 1.01^{ghi}	7.09 ± 0.17^{d}
Mango: EE	39.15 ± 2.60^{efg}	3.42 ± 0.27^{g}
Mango: EAE	47.49 ± 9.56^{def}	7.82 ± 0.06^{d}
Eggplant: AE	33.29 ± 7.97^{fgh}	2.82 ± 0.10^{g}
Eggplant: EE	33.56 ± 0.79^{fgh}	15.16 ± 0.38^{b}
Eggplant: EAE	27.88 ± 4.13^{ghi}	5.32 ± 0.05^{ef}
Potato: AE	21.67 ± 0.65^{hij}	4.94 ± 0.29^{ef}
Potato: EE	52.99 ± 0.57^{cde}	0.96 ± 0.05^{h}
Potato: EAE	60.01 ± 1.81^{cd}	0.17 ± 0.03^{h}

Values are presented as means ± SD for $n = 3$. Statistical significance ($p < 0.05$) was determined by a one-way ANOVA, with distinct lower-case letters representing significant differences. AE: Aqueous Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract.

3.2. Preliminary In Vitro Screening of the Inhibitory Potential of Different Extracts Against M. aeruginosa

The inhibitory effects of various extracts (AE, EAE, EE) from five fruit and vegetable peels on *M. aeruginosa* were tested using both paper disc diffusion and microdilution techniques. The values of MIC, MAC, and the inhibition zone for each tested fruit and vegetable peel extract against *M. aeruginosa* are summarized in Table 2.

The MIC values of avocado peel extracts against *M. aeruginosa* were ranged between 0.2 and 0.8 mg/mL, while mango, tomato, potato, and eggplant peel extracts showed higher MIC values than avocado peel extracts against *M. aeruginosa* (1.6 to 6.4 mg/mL).

The results show that the EE of avocado exhibited the lowest MIC (0.2 mg/mL) and MAC (0.4 mg/mL) against *M. aeruginosa*, followed by the AE and EAE of avocado which showed similar MIC (0.8 mg/mL) and MAC (1.6 mg/mL) values.

Table 2. Evaluation of *M. aeruginosa* inhibition using disc diffusion (9 mm) and microdilution assays.

Treatments	MIC (mg/mL)	MAC (mg/mL)	Zone of Inhibition (mm)
Avocado: AE	0.8	1.6	20 ± 2.45^{efg}
Avocado: EE	0.2	0.4	30.75 ± 0.20^{b}
Avocado: EAE	0.8	1.6	29 ± 0.82^{bc}
Tomato: AE	3.2	3.2	17.25 ± 0.20^{gh}
Tomato: EE	0.8	1.6	23.25 ± 1.84^{de}
Tomato: EAE	3.2	3.2	0 ± 0^{i}
Mango: AE	3.2	6.4	17 ± 0.82^{gh}
Mango: EE	1.6	3.2	17.50 ± 2.04^{fgh}
Mango: EAE	6.4	6.4	0 ± 0^{i}
Eggplant: AE	3.2	3.2	20.50 ± 0.41^{efg}
Eggplant: EE	3.2	3.2	14.50 ± 0.41^{h}
Eggplant: EAE	6.4	6.4	0 ± 0^{i}
Potato: AE	3.2	6.4	0 ± 0^{i}
Potato: EE	3.2	6.4	0 ± 0^{i}
Potato: EAE	3.2	3.2	0 ± 0^{i}
Copper sulphate	0.003	0.003	48 ± 2.4^{a}
Gallic acid	0.012	0.012	26.67 ± 2.49^{bcd}
Catechin	0.050	0.050	26 ± 0.82^{cd}
Quercetin	0.050	0.100	22 ± 1.6^{def}
DMSO (1%)	NA	NA	0 ± 0^{i}

NA indicates Not Active. Values are presented as means ± SD for $n = 3$. Statistical significance ($p < 0.05$) was determined by a one-way ANOVA, with distinct lower-case letters representing significant differences. AE: Aqueous Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract.

Moreover, the disc diffusion assay showed that the most inhibition zone were obtained for avocado peel extracts: EE (30.75 mm), and EAE (29 mm). While those of avocado (AE), mango (AE and EE), tomato (AE and EE), and eggplant (AE and EE) showed low and relatively average values. In contrast, the mango (EAE), tomato (EAE), eggplant (EAE) and potato (AE, EAE, and EE) showed no activity on solid media (Table 2).

The negative control, DMSO, remained inactive against *M. aeruginosa*; conversely, the phenolic compounds utilized as a positive control demonstrated significant anti-cyanobacterial activity (Table 2; Figure 1).

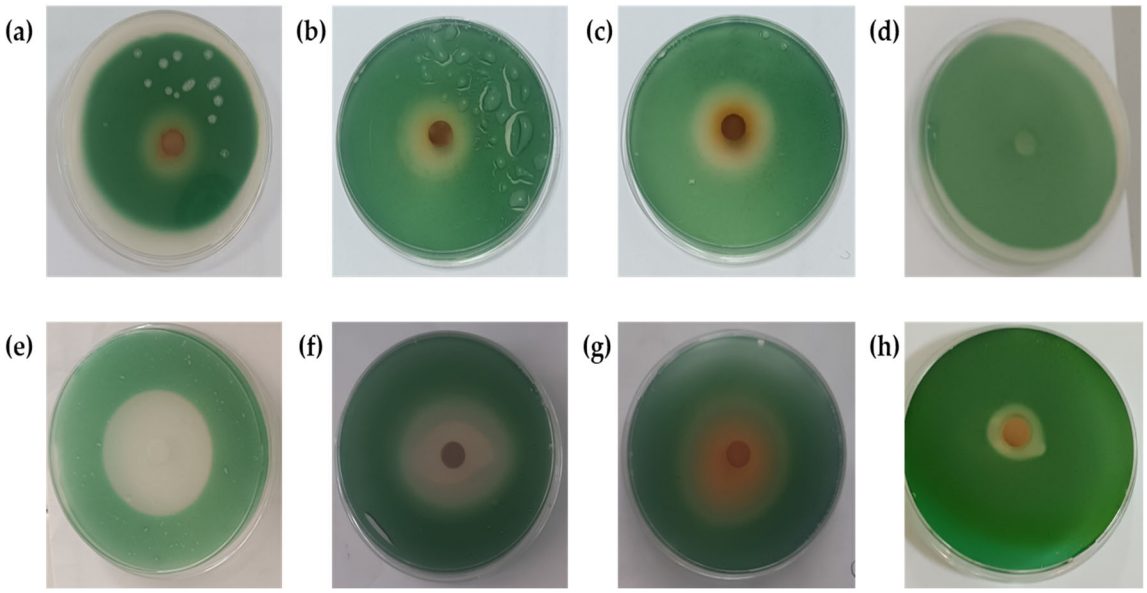


Figure 1. Anti-cyanobacterial activity of avocado peel extracts: (**a**) Aqueous Extract, (**b**) Ethyl Acetate Extract, (**c**) Ethanol Extract, (**d**) DMS0, (**e**) Copper sulphate, (**f**) gallic acid, (**g**) catechin, (**h**) quercetin.

3.3. Effects of the Most Bioactive Extracts on M. aeruginosa Culture in a Batch Culture Conditions

3.3.1. Effect on *M. aeruginosa* Growth

An experimental bioassay was conducted in batch conditions using the MIC and MIC/2 of AE, EAE, and EE of avocado peel, preliminarily screened and demonstrated as the most bioactive extracts, with stronger anticyanobacterial activities compared with other fruit and vegetable peels.

Figure 2a shows that the three extracts of avocado inhibit *M. aeruginosa* growth. Exposure to the selected concentrations significantly inhibited the growth of *M. aeruginosa* over 10 days, with cell densities remaining consistently below control levels ($p < 0.05$).

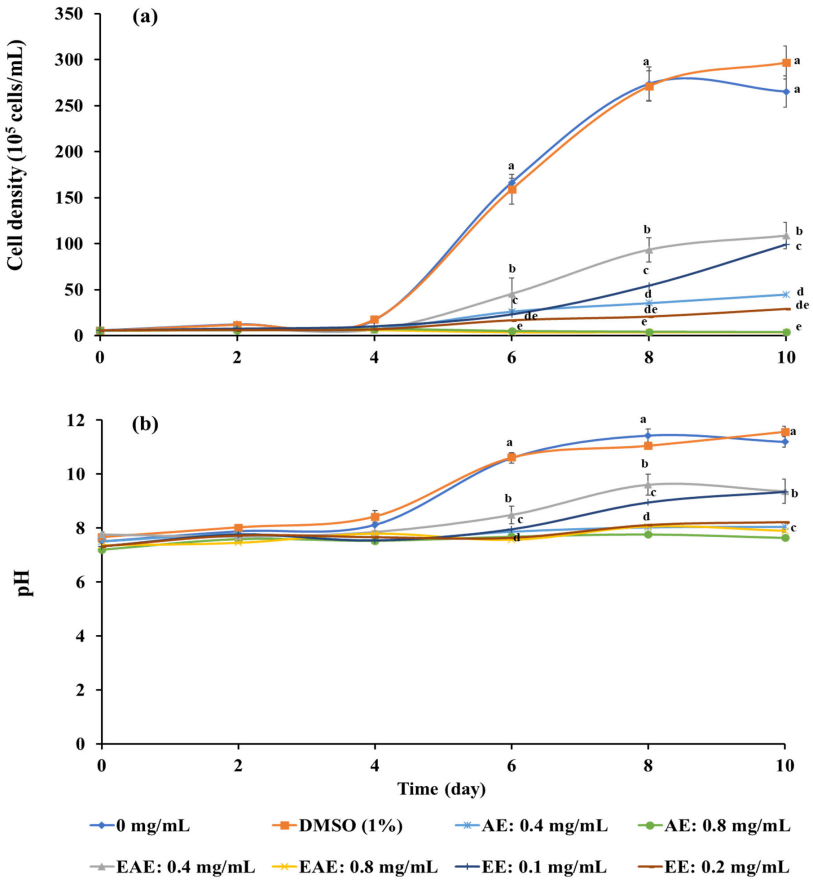


Figure 2. Effects of avocado peel extracts on *M. aeruginosa*. (**a**) growth and (**b**) pH in *M. aeruginosa* cultures, respectively. The error bars indicate the SD of three replicates. Significant differences (Two-way ANOVA, $p < 0.05$) are indicated by different lower-case letters. AE: Aqueous Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract.

The evolution of the pH of the culture is illustrated in Figure 2b. In the control group, the pH increased, reaching a maximum value (11.19) on day 10. The pH of the culture medium in the treated groups with AE, EAE, and EE of avocado peel, showed a general trend towards stability for MIC dose. However, it followed an increase for MIC/2 dose. On day 6, the pH increased slowly, reaching 9.35 for EAE and EE MIC/2. On day 10, it reached 8.05 for AE MIC/2.

The evaluation of the anti-cyanobacterial activity, in batch bioassays, of AE, EAE, and EE from avocado revealed an impact on growth, translated by an important and significant IR values (Table 3). The AE of avocado peel demonstrated remarkable efficacy, and reaching its maximum at the high concentration (0.8 mg/mL). The IR increased from 53% on day 2 to 97% on day 6. After, it maintained 98% until day 10. Even at 0.4 mg/mL, the inhibition increased significantly, reaching 87% on day 8. The EE of avocado peel also

showed significant potential, particularly at 0.2 mg/mL concentration, where inhibition peaked at 92% on day 8. Finally, the EAE of avocado peel showed similar results under the high-concentration (0.8 mg/mL) in comparison to the AE of avocado peel, achieving 97% inhibition on day 6 and 98% on days 8 and 10. At 0.4 mg/mL concentration, although inhibition reached 72% on day 6, it subsequently declined to stabilize at 59% on day 10. Overall, the highest concentrations of 0.8 mg/mL for both the AE and the EAE of avocado peel induced the strongest anti-cyanobacterial activity beyond day 6.

Table 3. Inhibition rate (%) of *M. aeruginosa* following treatment with MIC and MIC/2 concentrations of AE, EAE, and EE of avocado peel extracts.

Treatments (mg/mL)	Day 2	Day 4	Day 6	Day 8	Day 10
AE: 0.4	44.13 ± 4.86^{b}	56.50 ± 7.35^{b}	84.19 ± 0.40^{b}	87.03 ± 0.18^{b}	83.07 ± 0.03^{b}
AE: 0.8	53.06 ± 1.35^{a}	57.75 ± 3.37^{a}	97.04 ± 0.06^{a}	98.50 ± 0.07^{a}	98.60 ± 0.08^{a}
EE: 0.1	39.34 ± 2.29^{c}	41.14 ± 16.99^{c}	86.23 ± 7.34^{c}	80.24 ± 3.75^{c}	62.69 ± 6.79^{c}
EE: 0.2	51.40 ± 2.97^{ab}	60.64 ± 14.08^{ab}	89.96 ± 3.33^{ab}	92.41 ± 3.80^{ab}	88.95 ± 6.42^{ab}
EAE: 0.4	36.69 ± 0.67^{c}	61.08 ± 1.76^{c}	72.76 ± 10.44^{c}	65.93 ± 4.86^{c}	59.08 ± 5.43^{c}
EAE: 0.8	48.46 ± 8.24^{a}	66.35 ± 5.63^{a}	97.76 ± 0.03^{a}	98.59 ± 0.11^{a}	98.56 ± 0.10^{a}

Values expressed as means ± SD for $n = 3$. Statistical significance ($p < 0.05$) was assessed by a two-way ANOVA, with distinct lower-case letters indicating significant variations between groups. AE: Aqueous Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract.

3.3.2. Effect on Photosynthetic Pigments

Physiological assessment was performed by quantifying photosynthetic pigments, specifically carotenoids and chlorophyll-a. Over the 10-day experiment, the AE, EAE, and EE treatment groups exhibited a statistically significant reduction ($p < 0.05$) in both carotenoids and chlorophyll-a concentrations relative to the control (Figure 3a,b).

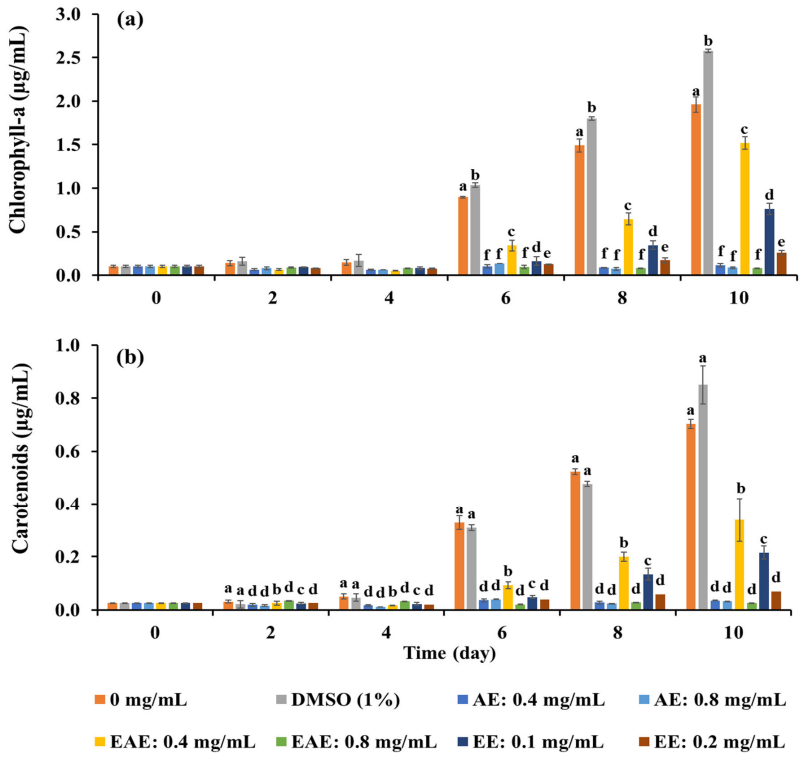


Figure 3. Changes in chlorophyll-a (**a**) and carotenoid (**b**) contents of *M. aeruginosa* cultures treated with avocado peel extracts. The error bars indicate the SD of three replicates. Significant differences (two-way ANOVA, $p < 0.05$) are indicated by different lower-case letters. AE: Aqueous Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract.

Increasing the concentration of the extracts appears to strongly inhibit pigment contents. After 6 days of bioassay, the levels of carotenoids and chlorophyll-a, at the highest concentration (0.8 mg/mL), for the AE and EAE, showed significant inhibition.

3.3.3. Effect on Phycobiliproteins

The evolution of the phycobiliprotein contents of *M. aeruginosa* under the application of AE, EAE, and EE of avocado peel is illustrated in Figure 4a–c. The PC, APC, and PE contents of the traeted groups decreased significantly during the 10 days of the experiment. The PC, APC, and PE contents of the three extracts appear lower than that of the control. Remarkably, the AE of avocado peel, under the high-concentration (0.8 mg/mL), appeared very powerful in reducing PC, APC, and PE contents after the 6th day.

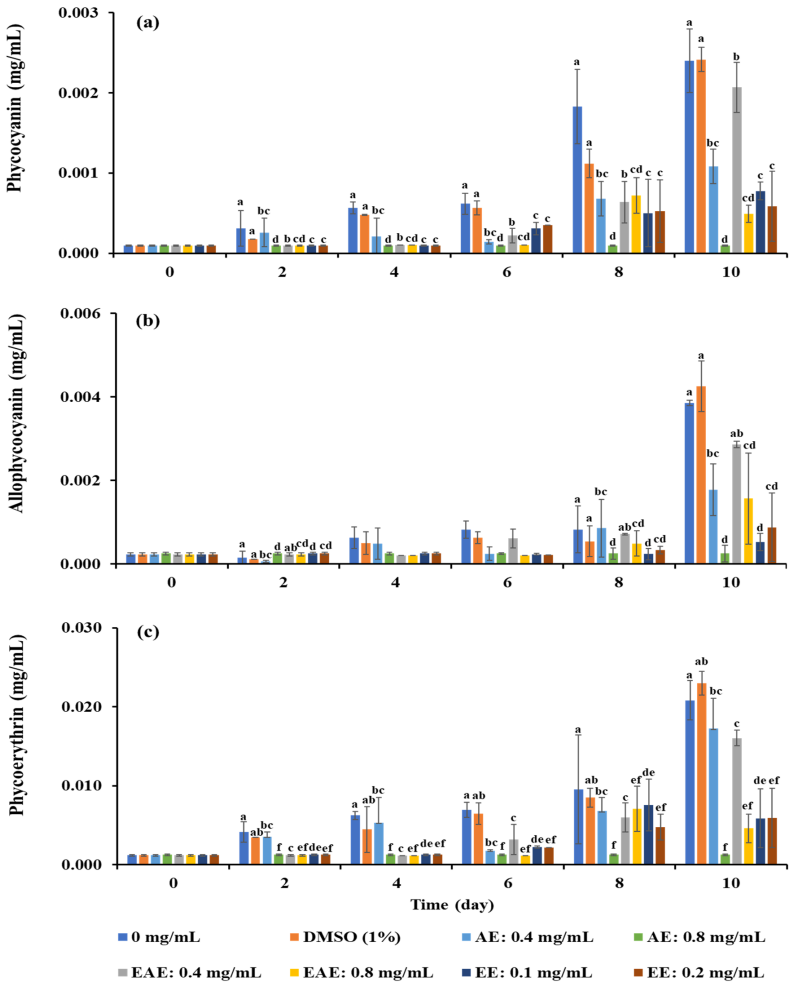


Figure 4. Changes in phycocyanin (**a**), allophycocyanin (**b**), and phycoerythrin (**c**) contents of *M. aeruginosa* cultures treated with AE, EAE, and EE of avocado peel. The error bars indicate the SD of three replicates. Significant differences (two-way ANOVA, $p < 0.05$) are indicated by different lower-case letters. AE: Aqueous Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract.

4. Discussion

The mitigation of harmful cyanobacteria remains a major and global environmental challenge in water ecosystems worldwide [34]. The secondary metabolites found in fruit and vegetable peels demonstrate significant inhibitory effects against fungal, bacterial, and cyanobacterial pathogens, as established by previous studies [16,19,21,35].

In this context, the present research assesses the anti-cyanobacterial effects of AE, EAE, and EE of five fruits and vegetables (Avocado, tomato, mango, eggplant, and potato)

peels, in vitro and in batch bioassay, using solid and liquid mediums, for qualitative and quantitative analysis. The anti-cyanobacterial potential of the selected fruits and vegetables peels has not been described in the literature.

All fruit and vegetable peel extracts (AE, EAE, and EE) tested could be recommended as an anti-cyanobacterial agent, the most significant anticyanobacterial activity was observed in avocado peel extracts.

The EE of avocado peel exhibited the lowest MIC (0.2 mg/mL) and MAC (0.4 mg/mL) against *M. aeruginosa*, followed by the AE and the EAE of avocado peel that showed similar MIC of 0.8 mg/mL and MAC of 1.6 mg/mL (Table 2). The inhibition zone using the disc assay showed the potent activity of EE avocado peel extract (30.75 mm) compared to the other extracts: EAE (29 mm) and AE (20 mm) (Table 2). This powerful activity of the EE of avocado peel, tested and demonstrated in vitro by both microdilution and diffusion disc approaches, raises its potentially effective interest in the extraction of biomolecules with cyanobactericidal effect.

The growth of *M. aeruginosa* was significantly suppressed during batch bioassays testing various extracts (AE, EAE, and EE) of avocado peel. At both MIC and MIC/2 concentrations, the avocado peel-derived compounds exhibited a clear inhibitory capacity.

The obtained results demonstrated that the AE had a remarkable efficacy. The highest IR (98%) was obtained in 8 days at the highest concentration (0.8 mg/mL). Furthermore, the EE of avocado peel showed significant potential particularly at 0.2 mg/mL, where inhibition of *M. aeruginosa* peaked at 92% on day 8. Finally, the EAE of avocado peel showed similar results to the high-concentration (0.8 mg/mL) of AE, achieving 98% inhibition on day 8 (Table 3).

These results are in accordance with previous research on AE of the invasive weed *Verbesina encelioides* (91–92%) after 8 day at (0.75–1 mg/mL) [10], and EE of the invasive plant species *Ailanthus altissima* (91.8%) after 5 day at (0.2 mg/mL) [36].

The growth suppression of *M. aeruginosa* coincided with the reductions in the photosynthetic pigments, signalling a significant physiological disturbance in response to stress [34,37]. Furthermore, several studies have indicated that the extracts from aquatic and terrestrial plants exert a negative impact on pigment content, specifically carotenoids and chlorophyll-a by compromising photosynthetic efficiency, influencing the growth and reproduction of *M. aeruginosa* [10,38,39].

The phycobiliproteins of *M. aeruginosa* (PC, APC, and PE) form the primary functional components of the cyanobacteria's photosynthetic light-harvesting antenna [40,41]. These proteins are essential, as they transfer absorbed energy to the algal cells, enabling the algae's survival and growth in biomass [33,42].

The avocado extracts investigated here inhibited phycobiliprotein synthesis, subsequently impairing photosynthesis in *M. aeruginosa*. This mechanism parallels previous research showing that phenolic acid disrupts light harvesting by suppressing chlorophyll-a and other pigments, thereby altering the composition of all phycobiliprotein components. The final result is the inhibition of cyanobacteria growth, potentially leading to cell mortality [43].

The fluctuation of pH levels was directly correlated with the concentration of dissolved carbon dioxide CO_2 in the water. As CO_2 dissolves, it forms carbonates, and the subsequent ionization, which releases hydrogen ions H^+, leads to a decrease in pH. Notably, in the control group, the algae demonstrated remarkable adaptability by regulating the water's pH during their growth phase, consequently causing a relative alkalinization of the medium [43,44].

According to the results of the phytochemical characterization (total phenolic and total flavonoid content) (Table 1), it was noted that the avocado extracts, containing the

highest content of polyphenols, presented the highest anticyanobacterial activity. These results are aligned with previous research demonstrating the algaecide effects of plant-derived polyphenols and flavonoids against *M. aeruginosa* [2]. These bioactive substances are among the common allelochemicals and are valued for their antimicrobial and antioxidant properties, and their potential in mitigating HABs [45]. In fact, avocado peel extract was known as a significant reservoir of phenolic constituents [46]. The primary phenolics present in avocado peel extract are flavonoids such as catechins, quercetins, and procyanidins, and derivates of chlorogenic acid such as coumaroylquinic and caffeoylquinic acids [47–49]. These compounds underpin the extract's potent antioxidant, anti-inflammatory, antimicrobial, antifungal, and anticancer activities [47,50,51].

Gigova and Ivanova demonstrate that allelochemicals inhibit cell growth by destabilizing their physiological regulation and their cellular structure. Because phenolic acids possess both lipophilic and amphiphilic properties, they directly affect the permeability of the cell [52,53]. Research by Wang et al. [54], indicates that p-coumaric and ferulic acids damage the cellular integrity of *M. aeruginosa*. Under these stressful conditions, the production of reactive oxygen species (ROS) causes unsaturated phospholipid breakdown, resulting in increased cellular membrane permeability. Moreover, the degradation of unsaturated phospholipids by ROS during periods of stress enhances membrane permeability [3]. This disruption of the antioxidant mechanisms subsequently impairs photosynthesis and oxygen production by damaging the PS II complex, which eventually results in cellular death [35]. Thus, the obtained results contribute to the growing evidence that fruit and vegetable peels waste can serve as sustainable strategies for managing toxic microalgal blooms in waterbodies.

5. Conclusions

Managing CyanoHABs is crucial for safeguarding aquatic ecosystems. To establish a green and viable method for mitigating microalgae growth, this study reveals that fruit and vegetable peels waste successfully inhibits the proliferation of *M. aeruginosa*. The avocado peel extracts show the most potent inhibition in vitro and in batch bioassays. During the 10-day experiment, all tested extracts (AE, EAE, and EE) of avocado peel induced a significant, dose-dependent decrease in phycobiliprotein (PC, APC, and PE) and photosynthetic pigment (Carotenoids and chlorophyll-a) concentrations compared to the control group. This inhibitory effect is potentially driven by the total phenolic and total flavonoid contents, which appear to function as the main allelochemicals. Consequently, avocado peel extracts, especially AE, can be recommended as a promising and eco-friendly biological agent to mitigate cyanobacterial proliferation. Optimizing this potential strategy for industrial application will require further essays under mesocosm and natural conditions, as well as a clearer understanding of the underlying active components.

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