fermentation

MDPI

Article

Juglone and Other Biogenic Quinones Differentially Inhibit Cyanobacterial Growth and Could Be Used to Help Maintain Monospecificity of Microalgae Cultures

Giuseppe Forlani

Department of Life Science and Biotechnology, University of Ferrara, Via L. Borsari 46, I-44121 Ferrara, Italy; flg@unife.it

Abstract

Raceway ponds would allow the sustainable production of algal biomass because of their lower cost. However, for successful cultivation, the target organism needs to prevail despite unavoidable contamination by environmental strains. The development of efficient methods to control cyanobacterial proliferation is thus highly desirable. With the aim to identify new cyanobactericidal substances, a set of natural compounds was screened for the ability to inhibit the growth of a model cyanobacterial strain, *Synechococcus elongatus* PCC 6301. Three compounds, namely hydroquinone, juglone and plumbagin, were found to be active in the 10^{-6} to 10^{-4} M range. Activity was confirmed on a panel of 10 other cyanobacteria that showed different sensitivity, with concentrations causing 50% growth inhibition varying up to 2 orders of magnitude. Co-cultivation experiments showed that the growth of *Microcystis aeruginosa* PCC 7941 was almost completely suppressed at quinone concentrations at which that of *Tolypothrix* PCC 7601 was substantially unaffected. Juglone and plumbagin in the micromolar range also exerted toxic effects on eukaryotic microalgae, bacteria and yeast, whereas the growth of higher plants was affected only at higher concentrations. In the case of juglone, activity was lost with time after being dissolved, allowing water discharge/recycling. The results point at the aromatic 1.4-quinone/diol ring as a lead moiety for the development of chemicals to help maintaining monospecificity of microalgae cultures.

Keywords: cyanobacterial strains; growth inhibitors; plant natural compounds; monospecific microalgae cultures; quinones

Academic Editor: Diomi Mamma

Received: 19 September 2025
Revised: 21 October 2025
Accepted: 24 October 2025
Published: 25 October 2025

Citation: Forlani, G. Juglone and Other Biogenic Quinones Differentially Inhibit Cyanobacterial Growth and Could Be Used to Help Maintain Monospecificity of Microalgae Cultures. *Fermentation* **2025**, *11*, 608. https://doi.org/10.3390/fermentation11110608

1. Introduction

As the only prokaryotes able to couple oxygenic photosynthesis with biological N_2 fixation, cyanobacteria play a pivotal role in carbon and nitrogen global cycling. Because of their notable tolerance to even harsh environmental conditions [1], they are broadly distributed in almost every water habitat on Earth, from caves to glaciers. Under certain conditions, such as sunny weather, warm temperature and increased anthropogenic nutrient loading, several cyanobacterial strains are capable of sudden, fast and massive proliferation [2]. These blooms form a dense surface scum in coastal waters and freshwater bodies, thereby causing the establishment of anoxic conditions and subsequent ecosystem decline [3]. Most cyanobacteria synthesize a remarkable variety of potentially lethal inhibitors targeting the nervous system, the gastrointestinal apparatus or the liver, thus

cyanobacterial proliferation may also cause the release of dangerous toxins into the environment [4]. During recent years, environmental pollution by human activities and global warming have appeared to cause a progressive increase in bloom frequency [5].

Because of this widespread presence and the ability to grow under strikingly diverse conditions, cyanobacteria are susceptible to contaminating microalgae cultures. In the last three decades, prokaryotic and eukaryotic microalgae have been widely exploited as cell factories of bio-based products [6]. With the aim of contributing to solving the problems of sustainability and energy shortage, the biotechnological potential of many photosynthetic microorganisms as sources of renewable energy and/or valuable industrial and agri-food products has been investigated [7]. Large-scale production of algal biomass can proceed either in open ponds or in photobioreactors ranging from simple, low-cost designs like conventional stirred tank systems to highly sophisticated, high-tech versions such as flat-panel and hybrid membrane reactors [8]. Because of lower cost, raceway ponds would allow the sustainable production of bulk volumes of microalgal biomass [9]. However, under such conditions, a microalgal culture is not a monoculture, as in conventional microbial fermentation, but a complex system of microalgae, zooplankton and bacteria [10]. For a successful cultivation, the target microalga needs to prevail despite the unavoidable contamination by environmental strains [11,12]. Because of their generally low doubling time and superior ability to adapt to any environmental condition, contamination by cyanobacteria can significantly reduce the growth of a target microalga by resource competition and allelopathy. Even in the case of limited growth, the contaminants can affect the quality of the final biomass, hindering its utilization. For these reasons, open pond cultivation has been successfully obtained only for some extremophiles to date [13], such as the halophilic green alga *Dunaliella salina* [14] and the alkalophilic cyanobacterium *Spirulina platensis* [15].

In order to improve the feasibility of microalgae growth in open systems, the development of efficient methods to control cyanobacterial proliferation is therefore highly desirable. Physical and chemical strategies have been successfully exploited at a pilot scale [16–19]. This notwithstanding, the former are generally too expensive to be applied in the field, whereas the latter are susceptible to causing pollution. In nature, cyanobacterial blooms seem to be limited by some bacteria through the production and secretion of algicidal compounds [20,21]. Isolation, characterization, and facile chemical synthesis of these natural substances would allow a biological approach to the problem, providing an effective strategy for bloom control. However, chemical synthesis of the algicidal compounds identified so far does not seem trivial, and no active principle has been commercialized to date with this aim. Moreover, the activity spectrum of these substances has been poorly investigated. To be effective, algicidal compounds should exert differential effects on target and non-target organisms.

Bioactive natural products can be isolated from agricultural wastes and unprocessed residues [22]. By processing raw materials, the agri-based industry produces several billion tons of residues in non-edible portions each year. The management of these residues is sometimes a serious problem, as they can cause pollution, and the cost of their disposal may significantly reduce the economic sustainability of the supply chain [23]. The use of agricultural by-products to yield valuable bioactive compounds may couple the low-cost production of active principles with cleaner production and increased sustainability of industrial processes [24]. Vascular plants produce a myriad of secondary metabolites endowed with biological activity [25], representing a promising source of new phytochemicals to be used in crop protection [26], human therapy [27] or food fortification [28].

In this work, the possibility of identifying plant secondary metabolites that can be obtained from agricultural by-products and show cyanobactericidal activity was investigated. With this aim, a panel of plant secondary metabolites was screened for the ability to

inhibit cyanobacterial growth. Three quinones were found to be active in the micromolar range, at concentrations at which plant growth was less affected. Interestingly, they showed differential effectiveness against various prokaryotic and eukaryotic algal strains. The results point at these compounds as a promising lead for the development of control agents to limit contamination of microalgae cultures in open ponds.

2. Materials and Methods

2.1. Reagents

Unless specified otherwise (Supplementary Table S1), the chemicals used were purchased from Sigma-Aldrich-Merck (Darmstadt, Germany) and were of analytical grade.

2.2. Cyanobacterial and Eukaryotic Algal Growth

Cyanobacterial strains (Table 1) and eukaryotic algae were grown at 24 ± 1 °C under 14 h: 10 h day (100 µmol m^{-2} s^{-1} photosynthetically active radiation [PAR]): night in Bg11 mineral medium, as previously described [29]. *Chlorella protothecoides* ATCC 30411 was grown in Bg11 medium supplemented with 1 g L^{-1} proteose peptone. *Chlorella vulgaris* SAG 211-11p and *Chlamydomonas reinhardtii* T222 were grown mixotrophically in TAP medium, as described [30]. Growth was followed by measuring chlorophyll concentration. Culture aliquots (0.5−1.0 mL) were centrifuged for 3 min at 14,000× *g*. Pellets were solubilised with 1.0 mL of either methanol or DMSO for 30 min in the dark, vortexing the samples every 5 min. Samples were then centrifuged as above, and the chlorophyll concentration in the supernatant was measured at 660 nm.

Table 1. Cyanobacterial strains used in this study.

Strain	Subsection
Anabaena sp. PCC 7120	IV
Calothrix sp. PCC 7507	IV
Chroococcidiopsis thermalis PCC 7203	II
Gloeothece sp. PCC 6909	I
Leptolyngbya boryana PCC 6306	III
Lyngbya sp. PCC 7419	III
Microcystis aeruginosa PCC 7941	I
Nostoc sp. PCC 7524	IV
Synechococcus elongatus PCC 6301	I
Synechocystis sp. PCC 6803	I
Tolypothrix sp. PCC 7601	IV

2.3. Evaluation of the Inhibitory Activity on Algal Growth

Cells in the late exponential phase of growth were pelleted by centrifugation 5 min at 4000× *g*, and used to inoculate 96-well plates, 0.2 mL per well, to an initial density of about 0.2 mg L^{-1} chlorophyll. The compounds were dissolved in DMSO to obtain 10 mM solutions. Aliquots (2 µL) of these solutions and of their 1:2 serial dilutions were added to each well, obtaining final concentrations ranging from 1000 to 0.1 µM. For each concentration, 4 replicates were carried out, and 12 for controls treated with the same volume of DMSO. Growth in each well was measured as the increase in absorbance at 660 nm up to one week following the inoculum using a Ledetect 96 plate reader (Labexim, Lengau, Austria). Turbidity at 750 nm was also measured. Data were linearized by logarithmic transformation, allowing calculation of growth constants that were expressed as a percentage of the mean value obtained with controls.

In the case of competition experiments, growth was followed as the increase in phycobiliprotein (PBP) content. Each cell suspension (1.5 mL) was centrifuged and the supernatant was discarded, while the pellet was resuspended with 150 µL of glycerol and stored

for 24 h at −20 °C. Samples then had 1.35 mL distilled water added, and were vortexed and further centrifuged as above. The obtained supernatants were read at 562, 615 and 652 nm, and the concentrations of PBP were calculated according to the method of Bennett and Bogorad, as previously described [31], using the following equations:

$$\text{Phycocyanin (PC) [mg mL}^{-1}\text{]} = [A_{615} - (0.472 \cdot A_{652})]/5.34$$

$$\text{Allophycocyanin (APC) [mg mL}^{-1}\text{]} = [A_{652} - (0.208 \cdot A_{615})]/5.09$$

$$\text{Phycoerhythrin (PE) [mg mL}^{-1}\text{]} = [A_{562} - (2.41 \cdot [\text{PC}] - 0.849 \cdot [\text{APC}]/9.62)$$

2.4. Bacterial and Yeast Growth

Escherichia coli strain BL21(DE3)pLysS was grown at 37 °C in the dark on a rotary shaker at 150 rpm in 250 mL Erlenmeyer flasks containing 50 mL of either standard Luria Broth or Davis and Mingioli medium. Baker yeast Ura3^{-} strain S23344C was grown at 30 °C in either Yeast Carbon Base supplemented with 50 mg L^{-1} uracil and 1.0 g L^{-1} ammonium sulphate or Yeast Extract Peptone Dextrose medium.

2.5. Evaluation of the Inhibitory Activity on Bacterial and Yeast Growth

The effect of quinones on the growth of the above-mentioned bacterial and yeast strains was assessed as described for cyanobacteria, but by measuring the absorbance at 600 nm. The inoculum was made to an initial density of about 0.1 optical units, and growth was followed for up to 10 h by reading the absorbance at 30 or 60 min intervals.

2.6. Plant Cell Culture and Seedling Growth

Seedlings and suspension-cultured cells of rice (*Oryza sativa* L. cv. Gigante Vercelli) and rapeseed (*Brassica napus* L., cv. Zeruca) were grown as described [32,33]. Briefly, undifferentiated cells were cultured in dim light at 24 °C on a rotary shaker (100 rpm; Lauda, Königshofen, Germany) in 500 mL Erlenmeyer flasks containing 125 mL of MS medium [34] that had been supplemented with 30 g L^{-1} sucrose and 2 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) for rice, or 0.5 mg L^{-1} of both 2,4-D and 6-benzylaminopurine for rapeseed. For seedling growth, seeds of the same cultivars were surface sterilized by treatments with ethanol (for 1 to 3 min, depending on the species) and bleach (3% NaClO in the presence of 0.04% Triton X100 for 2 to 5 min). Following extensive washing with sterile distilled water, seeds were sown in Magenta GA7 vessels (6 ⇨ × 6 ⇗ × 20⇧ cm) containing 50 mL of half-strength MS medium and allowed to germinate and grow at 24 ± 1 °C under 14 h: 10 h day (250 µmol m^{-2} s^{-1} PAR): night. In the case of rice, seeds were covered with 50 mL of sterile water.

2.7. Evaluation of the Inhibitory Activity on Plant Cultured Cells and Seedlings

To measure the effect of quinones on the growth of cultured cells, cultures in the early stationary phase of growth were harvested and used to inoculate 100 mL culture flasks containing 25 mL of fresh medium, so as to obtain an initial density of about 1.0 mg mL^{-1} (dry weight). After 8 days of incubation, when untreated controls approached the stationary phase of growth, cells were harvested on paper filters, and the dry weight increase was determined for each sample after drying at 80 °C for 48 h. At least four replicates were carried out for each treatment. The results for treated samples were expressed as a percentage of mean values obtained for untreated controls. In the case of seedling growth, seeds were directly sown in medium either containing the test compound or not, with 16 seeds per vessel. A randomized complete block design with three replications was used. Destructive harvest was carried out 8 to 15 days after sowing, when controls had reached the three-leaf stage. Each plantlet was weighed individually ($40 \leq n \leq 48$, depending on

germination rate) before and after treatment in the oven (J.P.Selecta, Abrera, Spain) at 80 °C for 48 h.

2.8. Statistical Analysis

The results were analyzed using Prism for Windows, version 6.07 (GraphPad Software, La Jolla, CA, USA). The concentrations able to inhibit growth by 50% (IC_{50}) and their confidence limits were calculated by nonlinear regression analysis of growth rates. To evaluate the effect of quinones on plant cell and seedling growth, the results were analyzed by multiple *t* tests, corrected by multiple comparisons using the Holm–Sidak method. Where differences are reported, they are at the $\alpha = 0.05$ level.

3. Results

3.1. Among a Panel of 22 Phytochemicals, Three Quinones Showed the Ability of Inhibiting Cyanobacterial Growth in the Micromolar Range

A set of 22 plant secondary metabolites (Supplementary Table S1) was screened for the ability to interfere with the growth of the cyanobacterial model strain *Synechococcus elongatus* PCC 6301. When added to the culture medium at concentrations ranging from 10^{-7} to 10^{-3} M, five of them were found to be completely ineffective. Most compounds exerted mild inhibitory effects at levels exceeding 10^{-4} M. Three of them, namely hydroquinone, juglone and plumbagin, were more effective, with IC_{50} values lower than 50 µM (Table 2).

Table 2. Effect of selected phytochemicals on the growth of the cyanobacterial model strain *Synechococcus elongatus* PCC 6301, expressed as the concentration causing 50% inhibition of cell growth (IC_{50}). Confidence limits are also shown.

Compound	IC_{50}	Lower Limit	Upper Limit
Allyl isothiocyanate	185 µM	157 µM	219 µM
Amygdalin	N [1]	-	-
Anisaldehyde	941 µM	823 µM	1076 µM
α-asarone	173 µM	158 µM	188 µM
β-asarone	157 µM	121 µM	204 µM
Betanin	N [1]	-	-
Caffeine	N [1]	-	-
Chlorogenic acid	5760 µM	3400 µM	9770 µM
Cinnamaldehyde	149 µM	138 µM	161 µM
Eugenol	365 µM	328 µM	406 µM
Gallic acid	132 µM	111 µM	157 µM
Hydroquinone	46.0 µM	42.9 µM	49.2 µM
Juglone	8.5 µM	8.1 µM	9.0 µM
Linalool	1164 µM	1031 µM	1315 µM
Methoxysalicylaldehyde	213 µM	178 µM	255 µM
Phloridzin	N [1]	-	-
Plumbagin	22.5 µM	20.8 µM	24.5 µM
Rutin	473 µM	195 µM	1144 µM
Scopoletin	N [1]	-	-
Thymol	157 µM	145 µM	170 µM
Tomatine	799 µM	720 µM	886 µM
Vanillin	308 µM	274 µM	345 µM

[1] N, not inhibitory in the range (from 0.1 to 1000 µM) tested.

Interestingly, these substances have a similar structure, since the two most active compounds show a naphthoquinone core with different substituents, whereas hydroquinone is a benzene-1,4-diol deriving from benzoquinone reduction.

3.2. Hydroquinone, Juglone and Plumbagin Exerted Differential Effects on the Growth of Various Cyanobacterial Strains, and Altered Population Dynamics in Mixed Cultures

The inhibitory potential of these compounds was then assessed on a set of ten other cyanobacteria, as listed in Table 1. With the only exception of *Chroococcidiopsis thermalis* PCC 7203, which showed low sensitivity, the growth of all these strains was reduced by 50% at concentrations ranging from 0.6 to 70 μM (Table 3). Juglone and plumbagin were approximately equipotent, whereas hydroquinone was less effective. Interestingly, differential effects were evident among strains, with the bloom-forming cyanobacteria *Microcystis aeruginosa* and *Synechocystis* sp. showing the highest susceptibility. No relationship was evident between strain sensitivity and their phylogenetic clades, with the resistant *Chroococcidiopsis thermalis* being the only analyzed species belonging to subsection II (*Pleurocapsales*; Table 1).

Table 3. Concentrations of hydroquinone, juglone and plumbagin able to inhibit by 50% the growth of different cyanobacterial strains.

Strain	IC_{50}		
	Hydroquinone	**Juglone**	**Plumbagin**
Anabaena sp. PCC 7120	59 ± 4 μM	8.1 ± 0.5 μM	18 ± 1 μM
Calothrix sp. PCC 7507	65 ± 34 μM	38 ± 19 μM	22 ± 9 μM
Chroococcidiopsis thermalis PCC 7203	183 ± 5 μM	>200 μM	>200 μM
Gloeothece sp. PCC 6909	70 ± 15 μM	28 ± 7 μM	34 ± 4 μM
Leptolyngbya boryana PCC 6306	29 ± 9 μM	20 ± 4 μM	11 ± 3 μM
Lyngbya sp. PCC 7419	6.5 ± 1.1 μM	5.1 ± 0.7 μM	4.9 ± 0.7 μM
Microcystis aeruginosa PCC 7941	1.1 ± 0.2 μM	1.5 ± 0.2 μM	0.6 ± 0.1 μM
Nostoc sp. PCC 7524	71 ± 7 μM	18 ± 2 μM	8.7 ± 1.3 μM
Synechococcus elongatus PCC 6301	46 ± 3 μM	9 ± 1 μM	23 ± 2 μM
Synechocystis sp. PCC 6803	26 ± 5 μM	3.2 ± 0.4 μM	1.2 ± 0.1 μM
Tolypothrix sp. PCC 7601	59 ± 9 μM	18 ± 1 μM	8.8 ± 0.2 μM

As a consequence of the differential sensitivity, the presence of these compounds may be susceptible to altering natural equilibria among species. To verify this possibility, co-cultivation experiments were carried out with *Microcystis aeruginosa* PCC 7941 and *Tolypothrix* sp. PCC 7601. The two strains were chosen not only for their different susceptibility to hydroquinone but also because of a distinguishable morphology, being coccal and filamentous, respectively. Moreover, their growth was easily distinguishable because of the absence and the presence of PE. The mixed culture was treated with increasing concentrations of hydroquinone in the range 1 to 10 μM, levels higher than the IC_{50} value for *Microcystis* in pure culture, but lower than that for *Tolypothrix*. Indeed, the treatments did not substantially affect the growth of the latter, whereas that of the former was progressively reduced (Figure 1a,b). In the absence of hydroquinone, *Microcystis* growth was comparable with that of *Tolypothrix* (Figure 1c). At the highest dose tested, on the contrary, an almost monospecific culture of *Tolypothrix* was obtained after three weeks of incubation (Figure 1d). Similar results were obtained with mixed cultures of *Calothrix* sp. PCC 7507 and *Synechocystis* sp. PCC 6803 treated with 10 μM juglone (Supplementary Figure S1), and *Chroococcidiopsis thermalis* PCC 7203 and *Synechococcus elongatus* PCC 6301 treated with 30 μM plumbagin (Supplementary Figure S2).

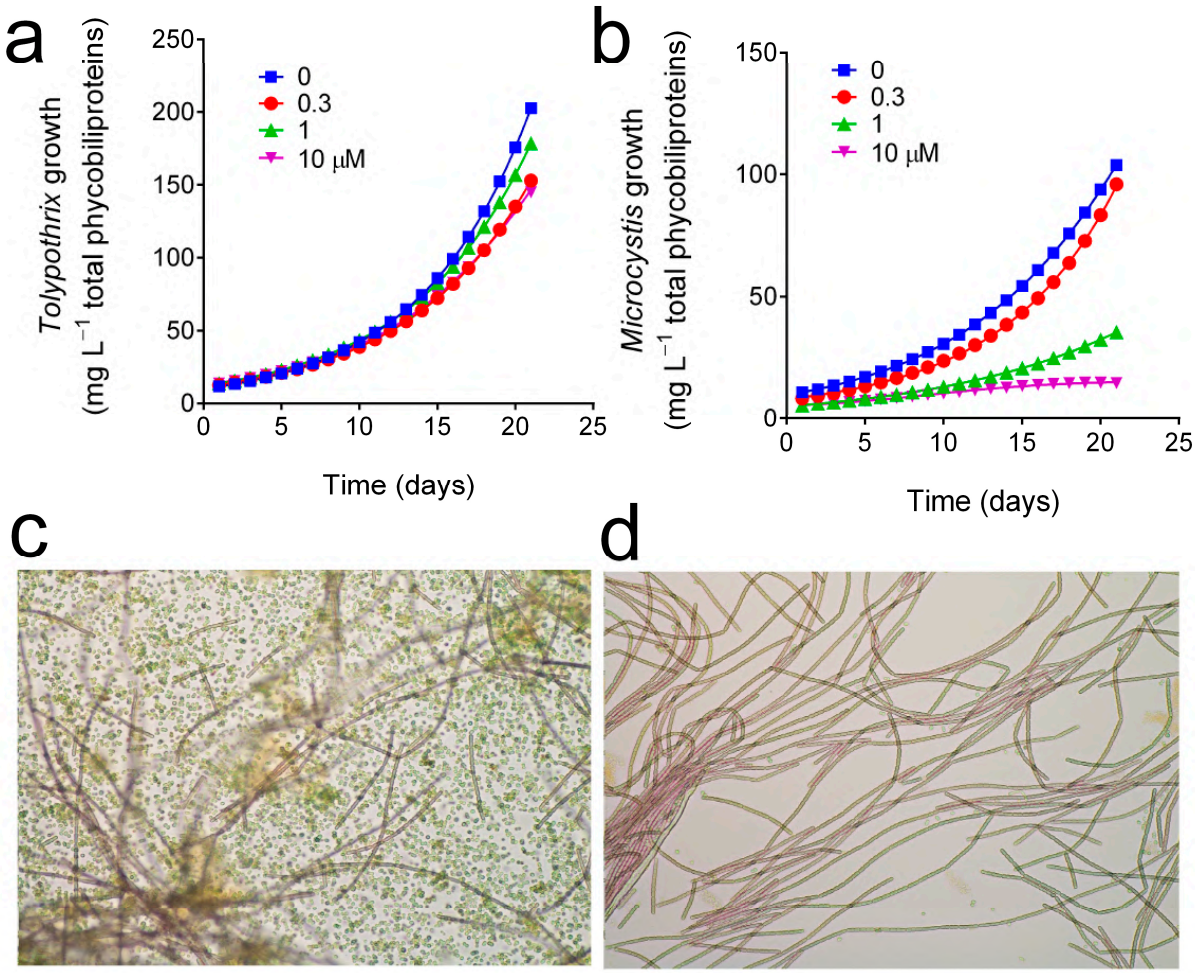

Figure 1. Influence of hydroquinone on cyanobacterial growth in co-cultivation experiments. *Microcystis aeruginosa* PCC 7941 and *Tolypothrix* sp. PCC 7601 were inoculated at a 1:1 ratio (based on phycobiliprotein content, with an initial concentration of 10 mg L^{-1}) in the presence of increasing levels of hydroquinone, as indicated. (**a**,**b**) Growth was followed as the increase in PBP content, taking advantage of the fact that *M. aeruginosa* does not contain PE. Results are mean over 3 replicates. (**c**,**d**) Optical microscopy imaging at the end of the incubation period of an untreated mixed culture and of a culture treated with 10 μM hydroquinone, respectively. Images were acquired in bright-field mode using an eyepiece camera (Amscope, Irvine, CA, USA) mounted on the optical microscope. Image capture, processing and scaling were performed with the USB Microscope Camera Measure Software (version x64 4.11; HAYEAR, Shenzhen, China).

3.3. Juglone and Plumbagin in the 1 to 50 μM Range Also Differentially Inhibited the Growth of Bacteria, Yeast, Eukaryotic Algae and Plants, Whereas Hydroquinone Was Much Less Effective

Because, as discussed below, cytotoxic effects of aromatic quinones have been reported in other biological systems, the effects of these compounds against some microorganisms were also evaluated to see whether cyanobacteria are less, equally or more susceptible. The results, summarized in Table 4, showed that juglone exerts a strong inhibition in all cases, being able to reduce the growth of *E. coli*, yeast and eukaryotic algae by 50% at 1–20 μM. Plumbagin was slightly less effective, with IC_{50} values 2- to 15-fold higher. On the contrary, hydroquinone was substantially ineffective at concentrations lower than 100 μM, with *C. prototechoides* being the only exception (IC_{50} = 43 μM).

Table 4. Concentrations of hydroquinone, juglone and plumbagin able to inhibit by 50% the growth of some prokaryotic and eukaryotic microorganisms and eukaryotic algae.

Strain	Hydroquinone	IC_{50} Juglone	Plumbagin
Escherichia coli (mineral medium)	157 ± 8 μM	1.8 ± 0.1 μM	15 ± 2 μM
Escherichia coli (organic medium)	N [1]	34 ± 5 μM	156 ± 34 μM
Saccharomyces cerevisiae (mineral medium)	N [1]	1.6 ± 0.1 μM	25 ± 3 μM
Saccharomyces cerevisiae (organic medium)	N [1]	2.5 ± 0.2 μM	10 ± 2 μM
Chlorella protothecoides	43 ± 8 μM	5.6 ± 0.9 μM	39 ± 9 μM
Chlorella vulgaris	754 ± 50 μM	19 ± 1 μM	28 ± 2 μM
Chlamydomonas reinhardtii	355 ± 50 μM	1.9 ± 0.2 μM	3.1 ± 0.4 μM

[1] N, not inhibitory in the range (from 0.1 to 1000 μM) tested.

Similar investigations were also carried out with some higher plants, either at the seedling or at the undifferentiated tissue level. The results obtained with both a dicotyledonous and a monocotyledonous crop species are reported in Table 5. When added to the culture medium at 10 μM, quinones were almost completely ineffective, with a mild growth inhibition being evident only in the case of rapeseed. At higher concentrations, a significant effect was found on the contrary, which was proportional to the dose, and 100 μM juglone and plumbagin, in some cases, almost completely suppressed plant growth. Once again, hydroquinone exerted milder effects and was ineffective up to 30 μM. At both the plant and cell levels, rice growth was not reduced, even at 100 μM. Interestingly, growth inhibition of cultured cells was overall similar to that of seedlings, suggesting that quinone target(s) are also expressed in undifferentiated tissues.

Table 5. Effects of hydroquinone, juglone and plumbagin on the growth of higher plants at either the seedling or the undifferentiated tissue level, expressed as percentage inhibition (±SE) with respect to untreated controls.

Species	Stage	Compound	Concentration		
			10 μM	30 μM	100 μM
rapeseed	seedlings	hydroquinone	NS [1]	NS [1]	32.5 ± 4.9
		juglone	NS [1]	50.7 ± 4.3	73.3 ± 2.7
		plumbagin	24.6 ± 5.2	49.6 ± 6.0	85.0 ± 9.7
rapeseed	cultured cells	hydroquinone	NS [1]	NS [1]	18.1 ± 3.3
		juglone	16.5 ± 2.9	51.2 ± 3.5	100.0 ± 0.0
		plumbagin	NS [1]	77.1 ± 10.4	100.0 ± 0.0
rice	seedlings	hydroquinone	NS [1]	NS [1]	NS [1]
		juglone	NS [1]	NS [1]	43.7 ± 5.4
		plumbagin	NS [1]	27.7 ± 7.5	87.7 ± 0.6
rice	cultured cells	hydroquinone	NS [1]	NS [1]	NS [1]
		juglone	NS [1]	38.2 ± 1.1	100.0 ± 0.0
		plumbagin	NS [1]	NS [1]	50.8 ± 4.2

[1] NS, not statistically different from untreated controls.

3.4. Juglone Is Susceptible to Oxidation/Degradation Processes in Solution That Progressively Reduce Its Biological Activity, Allowing Water Discharge/Recycling

Following solubilisation, quinones may be subjected to oxidation/degradation processes. Mainly under acidic conditions, a protonation followed by ring cleavage might occur for juglone and plumbagin, whereas hydroquinone might be oxidized to benzoquinone. This seems particularly true for juglone, whose solutions indeed showed a rapid darkening, turning from yellow to dark black. To investigate the stability of the active principles, some of the experiments previously performed with freshly prepared solutions were repeated with solutions in DMSO that had been prepared and stored for 2 weeks at

8 °C. Comparable results were obtained for hydroquinone and plumbagin, whereas in the case of juglone, completely different patterns were evident (Figure 2). With aged solutions, IC_{50} values 30- to 50-fold higher were found, suggesting a substantial loss of the inhibitory activity due to juglone degradation over time. Interestingly, in the case of *Synechocystis* sp. PCC 6803, the addition of low concentrations of the aged compound resulted, on the contrary, in a significant growth enhancement.

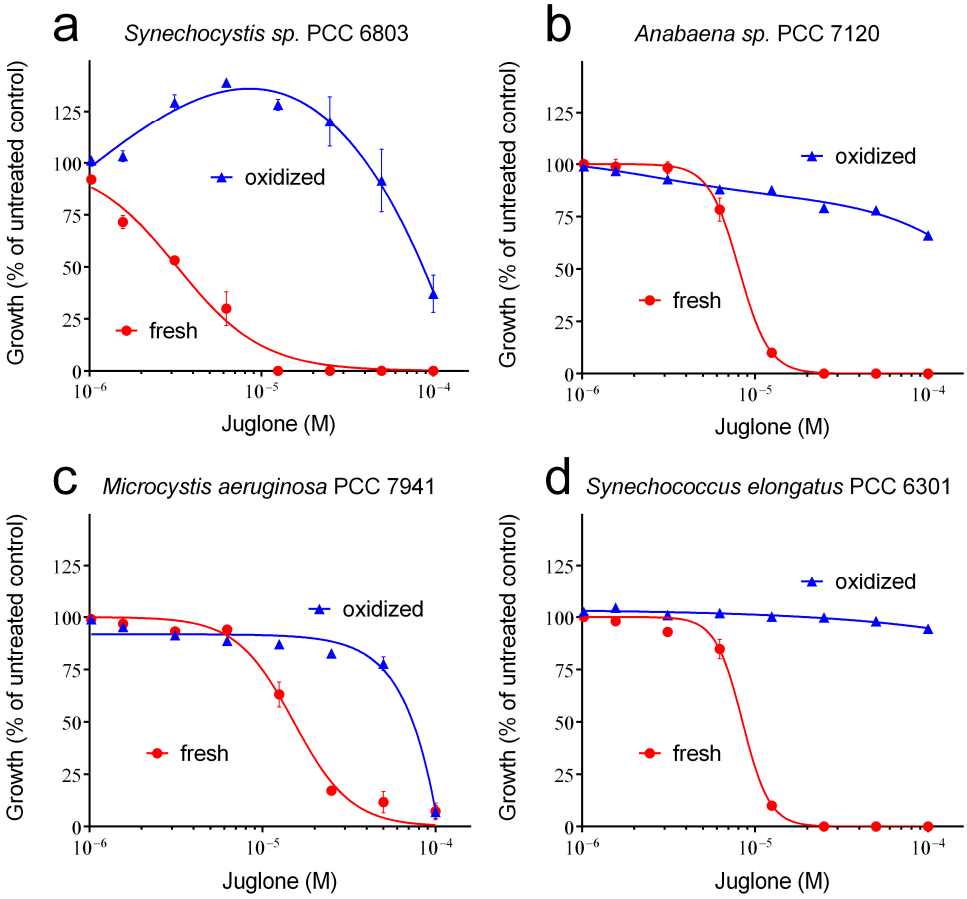

Figure 2. Influence of solution ageing of the inhibitory potential of juglone. Strains were grown in the presence of increasing levels of the quinone, which was added to the culture medium by dilution of either as a freshly prepared solution in DMSO (fresh) or a solution that had been stored for two weeks at 8 °C (oxidized). Growth constants were calculated in each case and expressed as a percentage of the mean value for controls treated with the same volume of DMSO. Results are mean ± SE over 4 replicates; (**a**) *Synechocystis* sp. PCC 6803; (**b**) *Anabaena* sp. PCC 7120; (**c**) *Microcystis aeruginosa* PCC 7941; (**d**) *Synechococcus elongatus* PCC 6301.

4. Discussion

In the present work, the possibility of using certain phytochemicals attainable from plant waste biomass as a means to selectively inhibit cyanobacterial growth was investigated. At this stage of the research, pure compounds were used to obtain unambiguous results. Because natural products are expected to be endowed with low recalcitrance and be rapidly mineralized by soilborne microorganisms, the use of plant-derived compounds to control cyanobacterial proliferation could have a lower environmental impact than that of synthetic inhibitors. On the other hand, the utilization of wastes from fruits and vegetables would greatly increase the economic sustainability of the supply chain.

Among the 22 plant compounds tested, only 3 were found to inhibit cyanobacterial growth at concentrations lower than 10^{-4} M. Remarkably, these three substances

show structural similarity, in that they possess aromatic 1.4-quinone/diol rings. Quinones are a wide class of naturally occurring compounds with a variety of biological properties, such as antibacterial, antifungal, and antineoplastic activity [35–37]. Some natural 1,4-benzoquinones bearing both an *N*-acetyl and an amino acid moiety, produced by a *Streptomyces* sp. named abenquines, were found to inhibit cyanobacterial growth in the 1 to 100 μM range. The synthesis of several chemical derivatives afforded a few analogs 25-fold more potent than the natural lead, with IC_{50} values ranging from 0.3 to 3 μM. Although the molecular target was not identified, these synthetic derivatives were substantially ineffective at micromolar levels against plants and yeast [38]. In a previous study on a group of thirteen synthetic 2,5-bis(alkyl/arylamino)-1,4-benzoquinones, two compounds, namely 2,5-bis(methylamino)- and 2,5-bis(propylamino)-1,4-benzoquinone, were found to inhibit the growth of *S. elongatus* PCC 6301 with a slightly lower effectiveness (IC_{50} values of 1.6 and 2.6 μM, respectively). In this case, plant growth was also much less affected, and the compounds were not toxic to insects [39]. The results herein reported and the previous literature data thus suggest that compounds with a 1,4-benzoquinone core have the potential to interfere, particularly even if not exclusively, with cyanobacterial growth. Analogously, some naphtoquinone derivatives have been reported as promising agents for the efficient mitigation of blooms of the freshwater diatom *Stephanodiscus hantzschii* [40].

Several early studies reported on the biological activity of hydroquinone, juglone and plumbagin. Plumbagin, which is found in plants belonging to the families *Plumbaginaceae*, *Droseraceae*, *Ancistrocladaceae* and *Dioncophyllaceae,* showed anti-inflammatory and anticancer properties [41], as well as significant antiprotozoal [42], anthelmintic [43], antimalarial [44] and antimicrobial [45,46] activity. However, in the last study, a minimum inhibitory concentration of about 30 μM was reported, well above those found in the present work for *Microcystis* and *Synechocystis* spp. (Table 3). Juglone, which is produced by walnut trees, is among the few compounds commonly recognized as true allelochemicals that are released into the soil and inhibit the growth of other plant species [47]. Besides some specific activity as a peptidyl-prolyl isomerase inhibitor [48], its action seems mediated by the formation of semiquinone radicals able to reduce O_2 to superoxide, which in turn causes oxidative damages to the cell [49]. As a consequence, some antibacterial [46,50], antifungal [51] and antineoplastic [52,53] properties have also been reported. Less is known about the biological activity of hydroquinone, which is a topical skin-lightening agent and the standard of care for patients with hyperpigmentation problems [54]. Nevertheless, a few studies showed a significant toxicity for aquatic organisms, such as *Pimephales promelas*, *Brachydanio rerio*, *Daphnia magna* and *Desmodesmus armatus*, being less harmful for bacteria and fungi [55]. To the best of our knowledge, only one previous study investigated the effect of hydroquinone on freshwater photoautotrophs [56]. In that case, three cyanobacterial strains showed a higher sensitivity than three green algae.

Most of the aforementioned data come from toxicity studies carried out with a single or a few model species. In this study, on the contrary, the results initially obtained with a single model strain were verified on a wider set of cyanobacteria belonging to different subsections. Besides confirming a general, high susceptibility of these photosynthetic prokaryotes, it indicates the occurrence of a differential sensitivity, with concentrations causing 50% inhibition of cell growth differing up to two orders of magnitude. This implies that these quinones can cause shifts in population dynamics among cyanobacteria, as shown by the results of the co-cultivation experiments (Figure 1). Interestingly, the highest susceptibility was evident for some of the species most commonly found in the environment that give rise to blooms, such as *Microcystis aeruginosa* [57] and *Synechocystis* sp. [58] (Table 3). Therefore, the addition of suitable quinone concentrations to raceway ponds could help in avoiding or limiting contamination of bulk cultures of eukaryotic

microalgae by cyanobacteria, or preserving cyanobacterial cultures from contamination by other, more sensitive cyanobacterial strains.

In most cases, hydroquinone showed a lower effectiveness than juglone and plumbagin, causing similar effects at concentrations 5- to 10-fold higher. However, the lower potency is counterbalanced by some potential advantages. Juglone and plumbagin inhibited cyanobacterial growth at concentrations at which they were found to be inhibitory against some heterotrophic microorganisms (Table 4). In the case of plumbagin, phytotoxic effects were also found (Table 5). On the contrary, hydroquinone was substantially ineffective against most non-algal species at concentrations at which the growth of some cyanobacterial strains was completely abolished (Table 3, Figure 1). Therefore, the treatment of water in open pond systems with micromolar concentrations of hydroquinone would limit cyanobacterial proliferation with only mild non-target effects on plants, yeasts and bacteria. This notwithstanding, the exhausted growth medium could not be discharged into the environment, given the significant toxicity of hydroquinone toward fish. In fact, aromatic quinones show a broad and remarkable acute toxicity against aquatic vertebrates. In the case of juglone, for instance, lethal concentrations 50% (LD_{50}) as low as 0.13–0.51 μM were found [59]. Concerning hydroquinone, although the LD_{50} against other non-mammalian terrestrial species is very high (4.5 mmol kg^{-1} for pigeon), that for freshwater fishes is in the micromolar range (1.7 μM for *Pimephales promelas* and >3.6 μM for *Brachydanio rerio*) [60]. In any case, its structure could be exploited for the synthesis of analogues and derivatives, showing lower toxicity to fish while retaining efficacy against cyanobacteria. Indeed, the monomethyl ether was reported to be 700-fold less toxic to goldfish than hydroquinone [61].

Based on present results, juglone seems to be the most suitable for use to control the growth of contaminating cyanobacteria in open pond systems. Juglone is quite abundant in walnut husks and shells, from which it can be purified in large amounts at relatively low costs [62]. Even if it would exert inherent toxicity against non-target organisms, such as plants and fish, juglone was herein found to lose most of its inhibitory activity in a few days following solubilisation (Figure 2), while hydroquinone and plumbagin showed a remarkable stability. This is consistent with previous studies showing that juglone is rapidly converted to less toxic components in solution [63] and is easily metabolized by the environmental microflora [64,65]. Therefore, the risk of residual toxicity associated with the release of juglone-treated water is low. Its utilization would be limited for cultivation in open pond systems of algal strains that are naturally resistant to this quinone. But a more extensive use could be obtained through its chemical modification, yielding analogues with a different activity spectrum. Because of its numerous biological activities, 1,4-naphthoquinone has been suggested as a privileged structure in medicinal chemistry, and the design, synthesis, and pharmacological evaluation of new juglone derivatives is in progress for the development of new prototypes with varied actions [66].

In summary, the body of evidence herein reported, together with the aforementioned literature data, point to the aromatic 1.4-quinone/diol ring as a lead structure to be exploited toward the development of specific inhibitors of cyanobacterial growth. Such inhibitors could find use to improve monospecificity in open systems for microalgae production. Work is underway to evaluate a set of commercially available structural analogues, as well as to perform pilot trials in the field.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fermentation11110608/s1, Table S1: Phytochemicals screened as potential inhibitors of cyanobacterial growth; Figure S1: Influence of juglone on cyanobacterial growth in co-cultivation experiments; Figure S2: Influence of plumbagin on cyanobacterial growth in co-cultivation experiments.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Some of the raw data supporting the conclusions of this article are presented in the Supplementary Materials; other raw data will be made available by the author upon reasonable request.

Conflicts of Interest: The author declares no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

APC	allophycocyanin
IC_{50}	concentration able to inhibit growth by 50%
LD_{50}	lethal concentrations by 50%
PAR	photosynthetically active radiation
PBP	phycobiliprotein
PC	phycocyanin
PE	phycoerhythrin

References

1. Rai, P.; Pathania, R.; Bhagat, N.; Bongirwar, R.; Shukla, P.; Srivastava, S. Current insights into molecular mechanisms of environmental stress tolerance in Cyanobacteria. *World J. Microbiol. Biotechnol.* **2025**, *41*, 53. [CrossRef] [PubMed]
2. Mohammed, V.; Arockiaraj, J. Unveiling the trifecta of cyanobacterial quorum sensing: LuxI, LuxR and LuxS as the intricate machinery for harmful algal bloom formation in freshwater ecosystems. *Sci. Total Environ.* **2024**, *924*, 171644. [CrossRef] [PubMed]
3. Gámez, T.E.; Manning, S.R. Blooming in the Anthropocene: Perspectives on the development of freshwater blooms, changing phytoplankton communities, and mitigation strategies. *Harmful Algae* **2025**, *147*, 102883. [CrossRef] [PubMed]
4. Villalobos, T.; Suárez-Isla, B.; Garcia, C. Health and environmental impacts of cyanobacteria and cyanotoxins from freshwater to seawater. *Toxins* **2025**, *17*, 126. [CrossRef]
5. Ma, J.; Wang, P. Effects of rising atmospheric CO_2 levels on physiological response of cyanobacteria and cyanobacterial bloom development: A review. *Sci. Total Environ.* **2021**, *754*, 141889. [CrossRef]
6. Einhaus, A.; Baier, T.; Kruse, O. Molecular design of microalgae as sustainable cell factories. *Trends Biotechnol.* **2024**, *42*, 728–738. [CrossRef]
7. Barbosa, M.J.; Janssen, M.; Südfeld, C.; D'Adamo, S.; Wijffels, R.H. Hypes, hopes, and the way forward for microalgal biotechnology. *Trends Biotechnol.* **2023**, *41*, 452–471. [CrossRef]
8. Soudagar, M.E.M.; Kiong, T.S.; Jathar, L.; Nik Ghazali, N.N.; Ramesh, S.; Awasarmol, U.; Ong, H.C. Perspectives on cultivation and harvesting technologies of microalgae, towards environmental sustainability and life cycle analysis. *Chemosphere* **2024**, *353*, 141540. [CrossRef]
9. Novoveská, L.; Nielsen, S.L.; Eroldoğan, O.T.; Haznedaroglu, B.Z.; Rinkevich, B.; Fazi, S.; Robbens, J.; Vasquez, M.; Einarsson, H. Overview and challenges of large-scale cultivation of photosynthetic microalgae and cyanobacteria. *Mar. Drugs* **2023**, *21*, 445. [CrossRef]
10. Molina, D.; de Carvalho, J.C.; Júnior, A.I.M.; Faulds, C.; Bertrand, E.; Soccol, C.R. Biological contamination and its chemical control in microalgal mass cultures. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 9345–9358. [CrossRef]
11. Barboza-Rodríguez, R.; Rodríguez-Jasso, R.M.; Rosero-Chasoy, G.; Rosales Aguado, M.L.; Ruiz, H.A. Photobioreactor configurations in cultivating microalgae biomass for biorefinery. *Bioresour. Technol.* **2024**, *394*, 130208. [CrossRef]
12. Wang, H.; Zhang, W.; Chen, L.; Wang, J.; Liu, T. The contamination and control of biological pollutants in mass cultivation of microalgae. *Bioresour. Technol.* **2013**, *128*, 745–750. [CrossRef]
13. Masojídek, J.; Lhotský, R.; Štěrbová, K.; Zittelli, G.C.; Torzillo, G. Solar bioreactors used for the industrial production of microalgae. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 6439–6458. [CrossRef]
14. de Souza Celente, G.; de Cassia de Souza Schneider, R.; Medianeira Rizzetti, T.; Lobo, E.A.; Sui, Y. Using wastewater as a cultivation alternative for microalga *Dunaliella salina*: Potentials and challenges. *Sci. Total Environ.* **2024**, *911*, 168812. [CrossRef] [PubMed]

15. AlFadhly, N.K.Z.; Alhelfi, N.; Altemimi, A.B.; Verma, D.K.; Cacciola, F. Tendencies affecting the growth and cultivation of genus spirulina: An investigative review on current trends. *Plants* **2022**, *11*, 3063. [CrossRef]
16. Jančula, D.; Maršálek, B. Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. *Chemosphere* **2011**, *85*, 1415–1422. [CrossRef] [PubMed]
17. Rajasekhar, P.; Fan, L.; Nguyen, T.; Roddick, F.A. A review of the use of sonication to control cyanobacterial blooms. *Water Res.* **2012**, *46*, 4319–4329. [CrossRef]
18. Zerrifi, S.E.A.; El Khalloufi, F.; Oudra, B.; Vasconcelos, V. Seaweed Bioactive compounds against pathogens and microalgae: Potential uses on pharmacology and harmful algae bloom control. *Mar. Drugs* **2018**, *16*, 55. [CrossRef] [PubMed]
19. Yang, X.; Wang, S.; Pi, K.; Ge, H.; Zhang, S.; Gerson, A.R. Coagulation as an effective method for cyanobacterial bloom control: A review. *Water Environ. Res.* **2024**, *96*, e11002. [CrossRef]
20. Yang, C.; Hou, X.; Wu, D.; Chang, W.; Zhang, X.; Dai, X.; Du, H.; Zhang, X.; Igarashi, Y.; Luo, F. The characteristics and algicidal mechanisms of cyanobactericidal bacteria, a review. *World J. Microbiol. Biotechnol.* **2020**, *36*, 188. [CrossRef]
21. Coyne, K.J.; Wang, Y.; Johnson, G. Algicidal bacteria: A review of current knowledge and applications to control harmful algal blooms. *Front. Microbiol.* **2022**, *13*, 871177. [CrossRef]
22. Sar, T.; Kiraz, P.; Braho, V.; Harirchi, S.; Akbas, M.Y. Novel perspectives on food-based natural antimicrobials: A review of recent findings published since 2020. *Microorganisms* **2023**, *11*, 2234. [CrossRef]
23. Babu, S.; Singh Rathore, S.; Singh, R.; Kumar, S.; Singh, V.K.; Yadav, S.K.; Yadav, V.; Raj, R.; Yadav, D.; Shekhawat, K.; et al. Exploring agricultural waste biomass for energy, food and feed production and pollution mitigation: A review. *Bioresour. Technol.* **2022**, *360*, 127566. [CrossRef] [PubMed]
24. Oyedeji, S.; Patel, N.; Krishnamurthy, R.; Fatoba, P.O. Agricultural wastes to value-added products: Economic and environmental perspectives for waste conversion. *Adv. Biochem. Eng. Biotechnol.* **2025**, *191*, 215–248. [CrossRef] [PubMed]
25. Elshafie, H.S.; Camele, I.; Mohamed, A.A. A Comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *Int. J. Mol. Sci.* **2023**, *24*, 3266. [CrossRef]
26. Divekar, P.A.; Narayana, S.; Divekar, B.A.; Kumar, R.; Gadratagi, B.G.; Ray, A.; Singh, A.K.; Rani, V.; Singh, V.; Singh, A.K.; et al. Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. *Int. J. Mol. Sci.* **2022**, *23*, 2690. [CrossRef] [PubMed]
27. Yeshi, K.; Crayn, D.; Ritmejerytė, E.; Wangchuk, P. Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules* **2022**, *27*, 313. [CrossRef]
28. Echegaray, N.; Munekata, P.E.S.; Gullón, P.; Dzuvor, C.K.O.; Gullón, B.; Kubi, F.; Lorenzo, J.M. Recent advances in food products fortification with anthocyanins. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 1553–1567. [CrossRef]
29. Forlani, G.; Bertazzini, M.; Barillaro, D.; Rippka, R. Divergent properties and phylogeny of cyanobacterial 5-enol-pyruvyl-shikimate-3-phosphate synthases: Evidence for horizontal gene transfer in the *Nostocales*. *New Phytol.* **2015**, *205*, 160–171. [CrossRef]
30. Barera, S.; Forlani, G. Osmo-induced proline accumulation in *Chlorella vulgaris* SAG 211-11p. *J. Appl. Phycol.* **2025**, *in press*. [CrossRef]
31. Drzyzga, D.; Forlani, G.; Vermander, J.; Kafarski, P.; Lipok, J. Biodegradation of the aminopolyphosphonate DTPMP by the cyanobacterium *Anabaena variabilis* proceeds via a C-P lyase-independent pathway. *Environ. Microbiol.* **2017**, *19*, 1065–1076. [CrossRef]
32. Forlani, G.; Bertazzini, M.; Zarattini, M.; Funck, D. Functional characterization and expression analysis of rice δ^1-pyrroline-5-carboxylate dehydrogenase provide new insight into the regulation of proline and arginine catabolism. *Front. Plant Sci.* **2015**, *6*, 591. [CrossRef] [PubMed]
33. Giberti, S.; Bertazzini, M.; Liboni, M.; Berlicki, L.; Kafarski, P.; Forlani, G. Phytotoxicity of aminobisphosphonates targeting both δ^1-pyrroline-5-carboxylate reductase and glutamine synthetase. *Pest. Manag. Sci.* **2017**, *73*, 435–443. [CrossRef] [PubMed]
34. Murashige, T.; Skoog, F. A Revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
35. Park, B.S.; Kim, J.R.; Lee, S.E.; Kim, K.S.; Takeoka, G.R.; Ahn, Y.J.; Kim, J.H. Selective growth-inhibiting effects of compounds identified in *Tabebuia impetiginosa* inner bark on human intestinal bacteria. *J. Agric. Food Chem.* **2005**, *53*, 1152–1157. [CrossRef] [PubMed]
36. Castro, M.; Gamito, A.M.; Tangarife-Castaño, V.; Zapata, B.; Miguel del Corral, J.M.; Mesa-Arango, A.C.; Betancur-Galvis, L.; San Feliciano, A. Synthesis and antifungal activity of terpenyl-1,4-naphthoquinone and 1,4-anthracenedione derivatives. *Eur. J. Med. Chem.* **2013**, *67*, 19–27. [CrossRef]

37. Kuete, V.; Omosa, L.K.; Tala, V.R.; Midiwo, J.O.; Mbaveng, A.T.; Swaleh, S.; Karaosmanoğlu, O.; Sivas, H. Cytotoxicity of plumbagin, rapanone and 12 other naturally occurring quinones from kenyan flora towards human carcinoma cells. *BMC Pharmacol. Toxicol.* **2016**, *17*, 60. [CrossRef]
38. Nain-Perez, A.; Barbosa, L.C.A.; Maltha, C.R.A.; Forlani, G. Natural abenquines and their synthetic analogues exert algicidal activity against bloom-forming cyanobacteria. *J. Nat. Prod.* **2017**, *80*, 813–818. [CrossRef]
39. Nain-Perez, A.; Barbosa, L.C.; Picanco, M.C.; Giberti, S.; Forlani, G. Amino-substituted para-benzoquinones as potential herbicides. *Chem. Biodivers.* **2016**, *13*, 1008–1017. [CrossRef]
40. Joo, J.-H.; Kang, Y.-H.; Park, B.S.; Park, C.-S.; Cho, H.; Han, M.-S. A field application feasibility assessment of naphthoquinone derivatives for the mitigation of freshwater diatom Stephanodiscus blooms. *J. Appl. Phycol.* **2016**, *28*, 1735–1746. [CrossRef]
41. Wang, S.H.; Lo, C.Y.; Gwo, Z.H.; Lin, H.J.; Chen, L.G.; Kuo, C.D.; Wu, J.Y. Synthesis and biological evaluation of lipophilic 1,4-naphthoquinone derivatives against human cancer cell lines. *Molecules* **2015**, *20*, 11994–12015. [CrossRef]
42. Sharma, N.; Shukla, A.K.; Das, M.; Dubey, V.K. Evaluation of plumbagin and its derivative as potential modulators of redox thiol metabolism of Leishmania parasite. *Parasitol. Res.* **2012**, *110*, 341–348. [CrossRef]
43. Saowakon, N.; Lorsuwannarat, N.; Changklungmoa, N.; Wanichanon, C.; Sobhon, P. *Paramphistomum cervi*: The in vitro effect of plumbagin on motility, survival and tegument structure. *Exp. Parasitol.* **2013**, *133*, 179–186. [CrossRef]
44. Sumsakul, W.; Plengsuriyakarn, T.; Chaijaroenkul, W.; Viyanant, V.; Karbwang, J.; Na-Bangchang, K. Antimalarial activity of plumbagin in vitro and in animal models. *BMC Complement. Altern. Med.* **2014**, *14*, 15. [CrossRef] [PubMed]
45. Nair, S.V.; Baranwal, G.; Chatterjee, M.; Sachu, A.; Vasudevan, A.K.; Bose, C.; Banerji, A.; Biswas, R. Antimicrobial activity of plumbagin, a naturally occurring naphthoquinone from *Plumbago rosea*, against *Staphylococcus aureus* and *Candida albicans*. *Int. J. Med. Microbiol.* **2016**, *306*, 237–248. [CrossRef]
46. Majdi, C.; Duvauchelle, V.; Meffre, P.; Benfodda, Z. An overview on the antibacterial properties of juglone, naphthazarin, plumbagin and lawsone derivatives and their metal complexes. *Biomed. Pharmacother.* **2023**, *162*, 114690. [CrossRef]
47. Ahmad, T.; Suzuki, Y.J. Juglone in oxidative stress and cell signaling. *Antioxidants* **2019**, *8*, 91. [CrossRef]
48. Fila, C.; Metz, C.; van der Sluijs, P. Juglone inactivates cysteine-rich proteins required for progression through mitosis. *J. Biol. Chem.* **2008**, *283*, 21714–21724. [CrossRef] [PubMed]
49. Nowicka, B.; Żądło, A.; Pluciński, B.; Kruk, J.; Kuczyńska, P. The oxidative stress in allelopathy: Participation of prenyllipid antioxidants in the response to juglone in *Chlamydomonas reinhardtii*. *Phytochemistry* **2017**, *144*, 171–179. [CrossRef]
50. Wang, J.; Cheng, Y.; Wu, R.; Jiang, D.; Bai, B.; Tan, D.; Yan, T.; Sun, X.; Zhang, Q.; Wu, Z. Antibacterial activity of juglone against *Staphylococcus aureus*: From apparent to proteomic. *Int. J. Mol. Sci.* **2016**, *17*, 965. [CrossRef] [PubMed]
51. Wianowska, D.; Garbaczewska, S.; Cieniecka-Roslonkiewicz, A.; Dawidowicz, A.L.; Jankowska, A. Comparison of antifungal activity of extracts from different *Juglans regia* cultivars and juglone. *Microb. Pathog.* **2016**, *100*, 263–267. [CrossRef]
52. Liu, X.; Chen, Y.; Zhang, Y.; Du, J.; Lv, Y.; Mo, S.; Liu, Y.; Ding, F.; Wu, J.; Li, J. Juglone potentiates TRAIL-induced apoptosis in human melanoma cells via activating the ROS-p38-p53 pathway. *Mol. Med. Rep.* **2017**, *16*, 9645–9651. [CrossRef]
53. Tang, Y.T.; Li, Y.; Chu, P.; Ma, X.D.; Tang, Z.Y.; Sun, Z.L. Molecular biological mechanism of action in cancer therapies: Juglone and its derivatives, the future of development. *Biomed. Pharmacother.* **2022**, *148*, 112785. [CrossRef]
54. Gonzalez, N.; Perez, M. Natural Cosmeceutical Ingredients for Hyperpigmentation. *J. Drugs Dermatol.* **2016**, *15*, 26–34.
55. Enguita, F.J.; Leitão, A.L. Hydroquinone: Environmental pollution, toxicity, and microbial answers. *Biomed. Res. Int.* **2013**, *2013*, 542168. [CrossRef]
56. Bährs, H.; Putschew, A.; Steinberg, C.E. Toxicity of hydroquinone to different freshwater phototrophs is influenced by time of exposure and pH. *Environ. Sci. Pollut. Res. Int.* **2013**, *20*, 146–154. [CrossRef] [PubMed]
57. Paerl, H.W.; Otten, T.G. Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microb. Ecol.* **2013**, *65*, 995–1010. [CrossRef] [PubMed]
58. Oudra, B.; El Andaloussi, M.; Franca, S.; Barros, P.; Martins, R.; Oufdou, K.; Sbiyyaa, B.; Loudlki, M.; Mezrioui, N.; Vasconcelos, V. Harmful cyanobacterial toxic blooms in waste stabilization ponds. *Water Sci. Technol.* **2000**, *42*, 179–186. [CrossRef]
59. Liu, S.; Cheng, S.; Jia, J.; Cui, J. Resource efficiency and environmental impact of juglone in Pericarpium Juglandis: A review. *Front. Environ. Sci.* **2022**, *10*, 999059. [CrossRef]
60. OECD SIDS. *Hydroquinone CAS N°: 123-31-9*. 2012. Available online: https://hpvchemicals.oecd.org/UI/handler.axd?id=7ca97271-99ed-4918-90e0-5c89d1ce200c (accessed on 23 October 2025).
61. Sollmann, T. Correlation of the aquarium goldfish toxicities of some phenols, quinones, and other benzene derivatives with their inhibition of autooxidative reactions. *J. Gen. Physiol.* **1949**, *32*, 671–679. [CrossRef]
62. Islam, A.K.M.M.; Widhalm, J. Agricultural uses of juglone: Opportunities and challenges. *Agronomy* **2020**, *10*, 1500. [CrossRef]
63. Marking, L.L. Juglone (5-hydroxy-l,4-naphthoquinone) as a fish toxicant. *Trans. Am. Fish. Soc.* **1970**, *99*, 510–514. [CrossRef]
64. von Kiparski, G.R.; Lee, L.S.; Gillespie, A.R. Occurrence and fate of the phytotoxin juglone in alley soils under black walnut trees. *J. Environ. Qual.* **2007**, *36*, 709–717. [CrossRef] [PubMed]

65. Wright, D.A.; Mitchelmore, C.L.; Dawson, R.; Cutler, H.G. The influence of water quality on the toxicity and degradation of juglone (5-hydroxy 1,4-naphthoquinone). *Environ. Technol.* **2007**, *28*, 1091–1101. [CrossRef] [PubMed]
66. Dos, S.M.C.; Santos, T.B.; Freitas, R.; Pacheco, P.A.F.; da Rocha, D.R. Juglone: A versatile natural platform for obtaining new bioactive compounds. *Curr. Top. Med. Chem.* **2021**, *21*, 2018–2045. [CrossRef]