Heliyon 10 (2024) e37851

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Colombian cyanobacteria with cytotoxic activity in cancer cell lines

Mohamed T. Darwich-Cedeño [a,*], Luis Carlos Montenegro-Ruiz [a], Mariana Reis [b], Vitor Vasconcelos [b]

[a] Universidad Nacional de Colombia, PO Box, 111311, Bogotá, Colombia
[b] Universidade do Porto and CIIMAR, PO Box 4450, Matosinhos, Portugal

ARTICLE INFO

Keywords:
Cyanobacteria
Secondary metabolism
Anticancer activity
Osteosarcoma (MG063)
Colon cancer (HCT116)
Fibroblast (3T3L1)
Brain endothelial cells (HCMEC)

ABSTRACT

In the last decade, cyanobacteria have emerged as significant reservoirs of bioactive molecule for the pharmaceutical, cosmetic, and alimentary industries, given the wide spectrum of new possibilities of different organic pigments, proteins, carbohydrates, lipids, and powerful antioxidant sources for specific and stronger cancer treatments, autoimmune syndromes, obesity, and inflammatory diseases. A bioactivity screening was executed for 12 strains of Colombian cyanobacteria (10 marine and 2 freshwater) representative of the orders present in the LAUN culture collection, performing methanol extracts per sample, which was fractionated by reverse phase HPLC protocol, obtaining 8 fractions for each crude extract. All these crude extracts were tested for antimicrobial activities through disk diffusion methodology, and fractions were tested for cytotoxic activity against cancer cell lines by cell viability detection with MTT. Cyanobacteria's extracts showed considerable cytotoxic activity for osteosarcoma (MG063) and colon cancer (HCT116) cell lines (61 and 66 % of reduced viability compared to untreated controls, respectively) and a surprising cell growth promotion for the control group fibroblast (3T3L1) and brain endothelial (HCMEC) cell lines (121 and 127 % growth, respectively), no bioactivity was observed in the antimicrobial tests. These findings underscore the expansive cytotoxic potential of Colombian cyanobacteria against cancer cell lines and, notably, their growth-promoting effects on healthy cell lines. This positions them as promising sources of bioactive compounds for future pharmaceutical developments.

1. Introduction

Nature serves as a primary reservoir for new drugs and active ingredients, with over half of all cancer drugs deriving from chemical structures isolated from plants, marine invertebrates, and microbes [1,2]. The microorganisms, such as cyanobacteria, lacking physical protection such as hard shells or spines, have developed these chemical weapons for defense or competition purposes, and some of them are little-known bioactive substances with pharmacological potential such as anti-cancer, anti-inflammatory, antibiotic agents, etc. [3]. Research on the secondary metabolism of cyanobacteria has intensified, as it has been demonstrated that these natural products have broad potential in biotechnological applications [4,5]. During the course of cyanobacteria evolution, it had to adapt to

* Corresponding author.
E-mail addresses: mdarwich@unal.edu.co (M.T. Darwich-Cedeño), lcmontenegor@unal.edu.co (L.C. Montenegro-Ruiz), mreis@ciimar.up.pt (M. Reis), vmvascon@fc.up.pt (V. Vasconcelos).

https://doi.org/10.1016/j.heliyon.2024.e37851
Received 19 June 2023; Received in revised form 9 September 2024; Accepted 11 September 2024
Available online 14 September 2024

many different environments and develop the ability to produce secondary metabolites to overcome exogenous challenges [6].

Cyanobacterial natural products are a promising and valuable source of new pharmacological compounds, with more than 1600 molecules exclusively produced by these microorganisms, and the majority are polyketide (PK) and non-ribosomal peptide (NRP) families, or hybrids of both, belonging to the secondary metabolism of those organisms [7–9]. A major part of these molecules has been reported to possess antibacterial, antitumor, antifungal, anti-inflammatory, anticancer, antiviral, immunosuppressive, and cytotoxic bioactivity. One example of such a molecule is Dolastatin, which has been extensively studied for its potent cytotoxic activity in inhibiting the polymerization of tubulin protein [1,7,10].

In Colombia, some studies have been carried out to establish antifungal and antimicrobial activity [7]; in addition, cyanobacteria consortiums with larvicide and herbicide activity have been reported [11–13]. Positive results have also been observed as cytotoxic against carcinogenic cell lines [7,14]. As a result of the above, there is a need to expand the information on the biotechnological potential presented by the cyanobacteria of the Colombian territory as sources of new compounds for the agricultural and pharmaceutical industries. In this sense, a biotechnological approach was conducted for antimicrobial and anticancer purposes to evaluate the activity of crude extracts and fractions derived from cyanobacteria collected from the LAUN algae collection in coastal and inland waters.

2. Materials and methods

2.1. Selection of cyanobacteria strains

Was used strains identified and registered by the Algal and Cyanobacteria Collection (LAUN) of the Algal Culture Laboratory of the National University of Colombia. The cyanobacteria strains come from: San Andrés and Providencia Islands, Rosario Islands, and the Colombian Andean region. All marine strains were cultivated in ASNIII medium [15], and freshwater strains were cultivated in BG11 culture medium [15], under light conditions at 100 μmol photons/m2/sec, photoperiod 12:12, 25 °C, and 45 days of growth. Twelve representative genera of LAUN strains were selected, identified in morphological and molecular methods: *Synechococcus* sp. (LAUN 29), *Baaleninema* sp. (LAUN 33, LAUN 45, LAUN 47), *Pleurocapsa* sp. (LAUN 34), *Leptolyngbya* sp. (LAUN 55, LAUN 69), *Phormidium* sp. (LAUN 74, LAUN 75, LAUN 81), *Hyellla* sp. (LAUN 78), *Staniera* sp. (LAUN 79), as shown in Table 1. The cyanobacteria were harvested by filtration, rinsed with distilled water to remove salt residues from culture media, and frozen at −80 °C for later lyophilization and grinding with liquid nitrogen.

2.1.1. Selection of bacterial strains for antimicrobial activity

The potential antimicrobial effect of LAUN strains extracts was performed using two Gram-negative strains (*Escherichia coli.*, *Bacillus subtilis)*, two Gram-positive strains (*Salmonella typhi.*, *Staphylococcus aureus*), and a fungus strain (*Candida albicans*), all acquired from the American Type Culturing Collection (ATCC) under the codes ATCC 33694, ATCC 6051, ATCC 6539, ATCC 12600, ATCC 18804 respectively.

2.2. Preparation of extracts and fractions

2.2.1. Methanol extracts

For methanol extracts, was followed the protocol implemented by Ferreira [16]. 2g of lyophilized biomass was placed in to a flask with 50 mL of pure methanol, and sonicated it for 5 min in a Fisher Brand FB15053 sonic bath at 30 °C. Subsequently, the supernatant was filtered, and the extraction protocol was repeated twice more, each time with 25 mL of pure methanol and the same biomass.

The extract was dried in a BUCHI R-210 rotary evaporator at 30 °C and a pressure of 210 mbar until all the solvent had evaporated. To recover the extract, it was necessary to resuspend the dried layer with methanol, performing multiple washes with 3 mL of methanol once at a time to reach a final volume of 12 mL, recovering all the extract in a 20 mL flask previously weighed, and then evaporating the solvent of the final extract again under the same conditions already mentioned.

Table 1
LAUN's strains and collect locations.

Codes	Location	Final Identification (Based on Phylo Tree and P-distance)
LAUN 29	San Andres Island (Marine)	*Synechococcus* sp. LAUN 29
LAUN 33	San Andres Island (Marine)	*Baaleninema* sp. LAUN 33
LAUN 34	San Andres Island (Marine)	*Pleurocapsa* sp. LAUN 34
LAUN 45	San Andres Island (Marine)	*Baaleninema* sp. LAUN 45
LAUN 47	San Andres Island (Marine)	*Baaleninema* sp. LAUN 47
LAUN 55	La Regadera Reservoir (Freshwater)	*Synechococcales cyanobacterium* LAUN 55 (possible new genera or species)
LAUN 69	La Regadera Reservoir (Freshwater)	*Leptolyngbya* sp. LAUN 69
LAUN 74	Rosario Islands (Marine)	*Synechococcales cyanobacterium* LAUN 74 (possible new genera or species)
LAUN 75	Rosario's Islands (Marine)	*Synechococcales cyanobacterium* LAUN 75 (possible new genera or specie)
LAUN 78	Rosario's Islands (Marine)	*Hyella* sp. LAUN 78
LAUN 79	Rosario's Islands (Marine)	*Stanieria* sp. LAUN 79
LAUN 81	Rosario's Islands (Marine)	*Synechococcales cyanobacterium* LAUN 81 (possible new genera or specie)

To determine the yield of each extract, each vial was reweighed, and was determined the net weight of one. It was taken 40 mg of each extract and resuspended in an HPLC vial with 1 mL of ultrapure methanol (LC-MS grade), filtering each solution through 0.2 μm pore filters if it was necessary.

2.2.2. Fractionation of the extracts

The fractionation of extracts was carried out as indicated by Ferreira [16], starting from 40 mg mL^{-1} extracts, which were injected into an HPLC Alliance Waters e2695 with an ACE 10C8 50 × 10 mm column coupled to a guard cartridge of the same chemical characteristics. Chromatography was run under a final gradient of 10 % Acetonitrile (MeCN) and 90 % ultrapure H2O and ultrapure methanol as blank at an injection rate of 3 mL/min. The equipment automatically fractionated each extract into 8 fractions labeled "A, B, C, D, E, F, G, H" respectively, with intervals of 1.3min in a 48-well plate (Table 2). The final volume of each fraction is 4 mL, with a concentration of 1.25 mg mL^{-1}.

2.3. Anticancer analysis

2.3.1. Cell culture

The protocol proposed by Ferreira [16] was followed for cell culture. Two cancer cell lines were used, HCT116 derived of human colon carcinoma (cultured in McCoy medium) and MG063 derived of human osteosarcoma (cultured in DMEM medium). Additionally, two healthy cell lines were employed: 3T3L1, derived of fibroblasts (cultured in DMEM medium) and HCMEC, derived of endothelial cells (cultured in DMEM medium). Both media were supplemented with 10 % of fetal bovine serum (Biochrom, Berlin, Germany), 1 % of penicillin/streptomycin (Biochrom, Berlin, Germany), and 0.1 % of amphotericin (GE Healthcare, Little Chafont, Buckinghamshire, UK). All cell lines were grown at 37 °C with 5 % CO2 atmosphere.

For culture maintenance, 200 mL translucent sterile acrylic flasks containing 5 mL of culture medium corresponding to each cell line were used. To initiate culture, 25 μL of old cell culture resuspended in 1 mL of new sterile culture medium was added to each flask. The old culture medium was aspirated and rinsed twice with 1 mL of phosphate saline buffer (PSB). Then, 1 mL of TrypLE was added to the cell culture and incubated at 37 °C for 5min. After cell detachment from the container walls and bottom, enzymatic action of TrypLE was neutralized with 5 mL of fresh medium. The suspension was transfer to a sterile 15 mL falcon tube, centrifuge at 1200RPM for 5min, the supernatant was discarded, and the cell pellet resuspended in 1 mL of a sterile medium.

From the resulting cell suspension, 25 μL were added to 5 mL of sterile medium in a new container, where they were allowed to grow until reaching between 70 and 80 % of the visual field in the microscope at 400x (approximately one week), This process was repeated for subculturing.

For cells counting, 20 μL of the suspension was mixed in equal volume of Trypan blue dye and placed in a Neubauer Cell Counting Chamber. The cell concentration was adjusted to $3.3x10^4$ cells/mL with viability greater than 80 %. Subsequently, the cells were seeded in 96-well plates at a final volume of 100 μL for cell viability assays using MTT.

2.3.2. Cytotoxicity

To test the LAUN's cyanobacteria strain extracts, cell lines were cultured in triplicate for 24 h in 96-well plates. After this period, the medium was aspirated and replaced with 100 μL of fresh medium containing the corresponding LAUN strains extract fraction in each well at a final concentration of 0.625 μg/mL (0.5 %) of the extract, for 48 h at 37 °C. After incubation, 20 μL of MTT (1 mg MTT 1 mL^{-1} PSB) was added and incubated for 4H at 37 °C. Confirming the intracellular formation of violet crystals (Formazan), the supernatant was aspirated and 100 μL of DMSO were added to dissolve the formazan crystals and subsequently read at 570 nm on a Biochrom EZ Read 800 Plus plate reader. The baseline was established with the negative controls, which were taken as 100 % survival, the behavior of the absorbance maintains a linear relationship with the subsequent survival of each extract.

Table 2
HPLC chromatographic and collection program.

Time (min)	Flow (mL·min−1)	MeCN (%)	H2O (%)	Collection Time (min)	Fraction
0	3	10	90	1.00–2.30	A
2	3	80	20	2.30–3.60	B
3	3	80	20	3.60–4.90	C
4	3	100	0	4.90–6.20	D
8.9	3	100	0	6.20–7.50	E
9.2	3.5	100	0	7.50–8.80	F
12	3.5	100	0	8.80–10.36	G
12.3	3	100	0	10.36–11.50	H
14	3	100	0		
15	3	10	90		
18	3	10	90		

The resulting plates were desiccated in SpeedVac and each extract was resuspended in 500 μL analytical grade DMSO and stored at −20 °C until used in each assay.

2.4. Antimicrobial analysis

2.4.1. Growing conditions

The bacterial strains were activated in HBI broth culture medium for bacteria and Sabouroud culture broth for fungi for 24 h at 37 °C in an "Innova®42 incubator shaker series" incubator. In the same way, the antibiotic Kanamycin (1 mg mL^{-1}) and antimycotic Nystatin (1 mg mL^{-1}) were used as positive controls inhibiting the bacterial and fungi growth respectively.

2.4.2. Inoculation with LAUN's strains extracts

The diffusion disc methodology was used; the experimental design consists of three Petri dishes per microorganism, with HBI agar for bacteria culture and Sabouroud dextrose agar for fungi culture. Two discs were used per each of LAUN's cyanobacteria strain extracts previously desiccated and resuspended in DMSO (1 mg mL^{-1}), and a Petri dish for the negative control with DMSO and the positive control. Each microorganism was seeded by multiple streaks covering the entire surface of the plate. Seeding was performed and exposure to each extract is immediately performed (15 μL per disk) and incubated for 24H at 37 °C [1].

The inhibitory capacity of each extract was quantified as a function of the length of the radius of the inhibition halo around each disc.

3. Results

3.1. Anticancer

3.1.1. LAUN strains cytotoxicity fractions

The tests were carried out on carcinogenic and healthy cell lines (MG063, HCT-116, 3T3L1, and HCMEC). For HCT-116 cell line, it was found that the extract "D" of *Baaleninema* sp. (LAUN 33) showed an inhibition of 66.828 %, and the extract "A" of "*Synechococcales cyanobacterium*" (LAUN 74) showed an inhibition of 65.68 % on the HCT-116 cell line, Identifyin them as most toxic fractions against the colon cancer line (Fig. 1). Despite cytotoxic activity shown by strains *Baaleninema* sp. (LAUN 33) and "*Synechococcales cyanobacterium*" (LAUN 74), the percent of survivor cancer cells is over the DMSO20 % line.

From the 8 fractions derived from each methanol extract of the LAUN strains already mentioned, it was observed that the extract "E" of *Baaleninema* sp. (LAUN 33) has inhibition of 60.72 %, the "F" fraction of *Baaleninema* sp. (LAUN 33) has inhibition of 61.06 %, the "H" extract of the "*Synechococcales cyanobacterium*" (LAUN 55) has inhibition of 61.72 %, and the "F" extract of the "*Synechococcales cyanobacterium*" (LAUN 74) presents inhibition of 61.58 % on the MG063 cell line, being the most toxic fractions against osteosarcoma. (Fig. 2).

On the other hand, tests on cell lines derived from normal tissues showed toxicity in *Baaleninema* sp. (LAUN 47) "B" fraction with a cell inhibition of 56 % for the 3T3L1 line corresponding to fibroblasts (Fig. 3). However, a potential growth-promoting action was observed of the cell line in the presence of *Pleurocapsa* sp. (LAUN 34)"C″ and "*Synechococcales cyanobacterium*" (LAUN 74)"D″ fractions, with cell concentrations of 121.7 % and 112.3 %, respectively, against the control group.

In the same way, *Baaleninema* sp. (LAUN 33) "E" and "*Synechococcales cyanobacterium*" (LAUN 55) "D" fractions show an inhibition of 66 % and 56.4 % (Fig. 4), respectively, against HCMEC cell line, being the most toxic fractions. However, it should be noted that a possible growth-promoting action of the cell line was observed in the presence of *Pleurocapsa* sp. (LAUN 34) "B″ fraction with cell concentrations of 127.1 % compared to the control.

From the above, it was observed that the colon cancer (HCT116) and osteosarcoma (MG063) cell lines tested, exhibited significant sensitivity to cyanobacterial extracts compared to the normal fibroblast and brain endothelial cell lines (3T3L1 and HCMEC respectively).

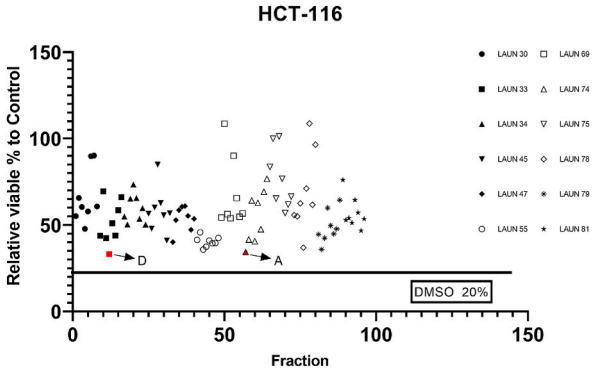

Fig. 1. Toxicity test in HCT-116 cell line. The extracts highlighted in red ((*Baaleninema* sp.) (LAUN 33) "D" with a standard deviation (SD) of 4.9 and "*Synechococcales cyanobacterium*" (LAUN 74) "A" with a SD of 4.0) indicate the fractions with the highest toxicity in the cancer cell line. The tests were conducted in triplicate. (generated using GraphPad Prism 8).

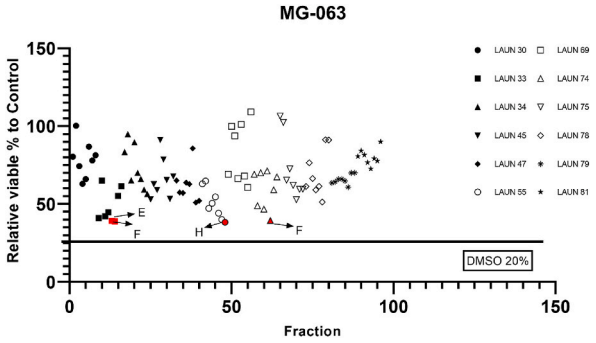

Fig. 2. Toxicity test in MG063 cell line, the extracts highlighted in red ((*Baaleninema* sp.) (LAUN 33) "E", "F" with a SD of 4.1 and 9.9, respectively; "*Synechococcales cyanobacterium*" (LAUN 55) "H" with a SD of 8.5, and "*Synechococcales cyanobacterium*" (LAUN 74) "F" with a SD of 6.1) indicate the fractions with the highest toxicity in the cancer cell line. The tests were conducted in triplicate. (generated using GraphPad Prism 8).

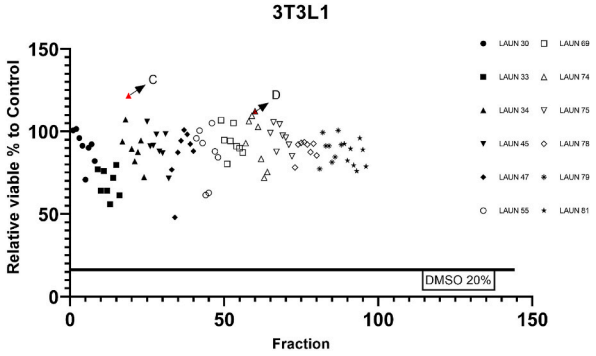

Fig. 3. Toxicity test in the 3T3L1 cell line. The extracts highlighted in red ((*Pleurocapsa* sp. (LAUN 34) "C" with a SD of 10.9 and "*Synechococcales cyanobacterium*" (LAUN 74) "D" with a SD of 10.1) indicate the fractions that demonstrate a possible growth promotion in a cell line. The tests were conducted in triplicate. (generated using GraphPad Prism 8).

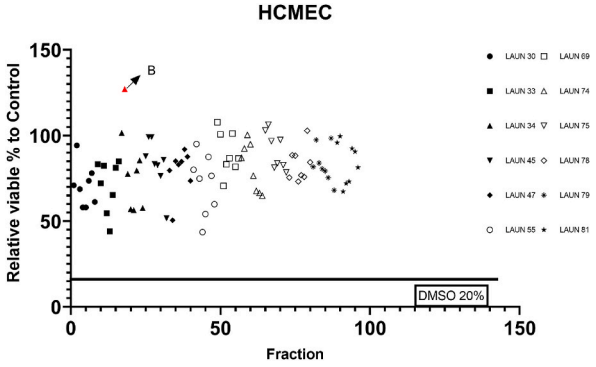

Fig. 4. Toxicity test in the HCMEC cell line. The extract highlighted in red belonging to ((*Pleurocapsa* sp.) (LAUN 34) "B" with a SD of 17.4) indicates the fraction that demonstrates a possible growth promotion in the cell line. The tests were conducted in triplicate. (generated using GraphPad Prism 8).

3.2. Antimicrobial activity

3.2.1. Evaluation of LAUN's strains extracts

The tested LAUN's cyanobacteria strain extracts did not exhibit any antibiotic activity against the selected strains of *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans*.

4. Discussion

The reports of cyanobacteria exhibiting cytotoxic capabilities against cancer cell lines, attributed to the production of secondary metabolites, have been on the rise in the last decade. This positions them as an intriguing source of bioactive compounds. The obtained results in cytotoxic activity in HCT 116 cell lines (colon cancer), shows that the fractions *Baaleninema* sp. (LAUN 33)"D" and "*Synechococcales cyanobacterium*" (LAUN 74) "A" have important bioactivity over 60 % of mortality, similar results were reported by Sousa [5,6]. Cyanobacteria natural toxins by strains bellowing to *Phormidium* and *Nodularia* genera, those exhibit cytotoxicity in cancer cell lines such as HCT 116 targeting mitochondrial metabolism, Parida [17], reported similar results, they tested methanol and acetone extracts from *Lyngbya majuscule* and *Lygbya martenciana* performing it against HepG2 (hepatocellular carcinoma) cells, and found strong anticancer activity in *L. majuscule*. Cegłowska [18], tested Aeruginosamide variants AEG625 and AEG657 over human breast cancer cells T47D and human liver cancer cells Huh7 (Human Hepatome) and found mild toxicity at the higher concentration used in the experiments (60 μg) only in the T47D cell line. By using several chromatographic techniques, Ferreira [19], isolated two new macrolide-type cyanobacteria compounds from *Leptothoe* sp. with high cytotoxic effect on HCT116 human colon cancer cells. Furthermore, M. L. da S. Sousa [20], describes bioactivity against colon cancer cells (HT29 and HCT116) from marine cyanobacteria extracts, they describe several bioactive molecules like nocuolin A from *Nodularia* sp, portoamides A, and B form *Phormidium* sp., hierridins B and C from *Cianobium* sp., bartoloside A from *Nodosilinea* sp., bartoloside B and C from *Synechocystis salina,* and found nocuolin A decreases ATP and oxygen in cells tested propitiating an abnormal mitochondrial respiration function; moreover, portoamides A, and B can induce mild oxidative stress in cancer cells and potent mitochondrial inhibition, at the same time, they found bartoloside C decreases the cancer cells size and finally, hierridines reduces p21 levels and increase p53 and c-myc proteins which trigger the inhibition of growth and division of cancer cells.

In addition, the LAUN's cyanobacteria strain fractions *Baaleninema* sp. (LAUN 33) "E" and "F"; "*Synechococcales cyanobacterium*" (LAUN 55) "H", "*Synechococcales cyanobacterium*" (LAUN 74) "F" have a relatively toxic behavior, with more than 60 % of cell mortality over MG063 (Osteosarcoma) cancer cell line. These results go in the same line as Costa [21], who found several crude extracts and fractions with strong bioactivity from LEGE's cyanobacteria collection strains against multiple cancer cell lines, including MG063 where *Pseudanabaena* and *Leptolyngbya fragilis* strains show mortality yields over 90 %. On the other hand, Hassouani [22], performed anticancer bioassays with *Lyngbya aestuarii* methanolic extracts in the MG063 cell line and found a stimulation effect of the extracts over the cancer cell line, which shows the relevance of our findings to the extent that the strains present toxicity on the cancer line above 60 % mortality.

Regarding the relationship with the positive control (baseline), Ferreira [16], showed 1 extract below the staurosporine control line, proposing this one as a toxic extract. However, Charitos [23], consider the IG50 % as an indicator of the power of the natural cytotoxic agent, therefore, LAUN's bioactive fractions have toxicity over 60 % in approximately 5 mg of all molecules in these fractions over the DMSO20 % baseline. This underscores the rationale for further purifying the bioactive compounds, potentially unveiling a novel potent anticancer compound, given that the concentration of such compounds in the evaluated fraction remains undisclosed.

However, the fractions Pleurocapsa sp. (LAUN 34) "B", Pleurocapsa sp. (LAUN 34) "C", and "Synechococcales cyanobacterium" (LAUN74) "D" exhibit an enhanced metabolic activity effect in healthy cell lines 3T3L1 (fibroblasts) and HCMEC (endothelial brain cells), suggesting the potential for purification and isolation of subfractions to identify bioactive compounds as candidates for new treatments in skin damage and angiogenesis processes. Similarly, Guesmi [24] proposed phytoextracts as re-epithelialization agents for skin burns, while Klinngam [25] demonstrated that polymethoxyflavonoids from black ginger improve epidermal stability and control inflammation in skin explants, also serving as an anti-aging agent. However, there is limited recent information on these aspects concerning cyanobacteria, marking this report as a novel contribution to the field.

Reports in Latin American show that bioinformatic analysis of Nostocal's putative Nocuolin A gene synthetizes an Oxadiazine that triggers apoptosis in human cancer cells [26]. In Colombia, there are a few reports of cytotoxic activity by cyanobacteria and no identification of molecules in the great diversity of cyanobacteria in the country [7,13,27]. In this way, it is necessary to improve the identification of cyanobacteria diversity and their bioactive capacity.

5. Conclusions

In this study, it is possible to elucidate the pharmaceutical potential of Colombian biodiversity in terms of cytotoxicity in cancer lines as well as in tissue growth promoters, which can be proposed as new alternatives for the development of drugs and cosmetic treatments. Likewise, a list of potential strains for mass cultivation is generated for the characterization of the bioactive molecules object of the activities described in this work, and we propose the LAUN strain fractions *Baaleninema* sp. (LAUN 33) "D" and "*Synechococcales cyanobacterium*" (LAUN 74) "A", to re-fractioning looking for pure compounds against colon cancer (HCT-116), *Baaleninema* sp. (LAUN 33) "E", "F"; "*Synechococcales cyanobacterium*" (LAUN 55) "H", "*Synechococcales cyanobacterium*" (LAUN 74) "F" to re-fractioning looking for pure compounds against osteosarcoma (MG063), finally, the fractions *Pleurocapsa* sp. (LAUN 34) "B", *Pleurocapsa* sp. (LAUN 34) "C" and "*Synechococcales cyanobacterium*" (LAUN74) "D", to re-fractioning looking for possible pure growth promotors compounds in fibroblasts (3T3L1) and brain endothelial cells (HCMEC). Knowledge of the natural resources of Colombia, facilitates its competitiveness in industrial and pharmaceutical development processes.

Data availability statement

- Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication.
  o No
- Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication. Has data associated with your study been deposited into a publicly available repository?
  o The authors are unable or have chosen not to specify which data has been used.

CRediT authorship contribution statement

Mohamed T. Darwich-Cedeño: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Luis Carlos Montenegro-Ruiz:** Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mariana Reis:** Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Vitor Vasconcelos:** Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by the Universidad Nacional de Colombia through the Project "Convocatoria de apoyo a iniciativas de intercambio de conocimiento que permitan fortalecer alianzas estratégicas" with HERMES code 52505, Bogotá, Colombia. CIIMAR by the international research collaboration program, and Politécnico do Porto for allowing access to the cell culture laboratory to perform the anticancer bioassays. Export specimen permit code: ANLA-3100.

References

[1] M. Girão, I. Ribeiro, T. Ribeiro, I.C. Azevedo, F. Pereira, R. Urbatzka, P.N. Leão, M.F. Carvalho, Actinobacteria isolated from laminaria ochroleuca: a source of new bioactive compounds, Front. Microbiol. 10 (2019) 1–13, https://doi.org/10.3389/fmicb.2019.00683.
[2] T. Grkovic, R.K. Akee, C.C. Thornburg, S.K. Trinh, J.R. Britt, M.J. Harris, J.R. Evans, U. Kang, S. Ensel, C.J. Henrich, K.R. Gustafson, J.P. Schneider, B.R. O'Keefe, National cancer institute (NCI) program for natural products discovery: rapid isolation and identification of biologically active natural products from the NCI prefractionated library, ACS Chem. Biol. 15 (4) (2020) 1104–1114, https://doi.org/10.1021/acschembio.0c00139.
[3] B. Robles-Bañuelos, L.M. Durán-Riveroll, E. Rangel-López, H.I. Pérez-López, L. González-Maya, Marine cyanobacteria as sources of lead anticancer compounds: a review of families of metabolites with cytotoxic, antiproliferative, and antineoplastic effects, Molecules 27 (15) (2022), https://doi.org/10.3390/molecules27154814.
[4] M.R. Jones, E. Pinto, M.A. Torres, F. Dörr, H. Mazur-marzec, L. Tartaglione, C.D. Aversano, C.O. Miles, D.G. Beach, K. Sivonen, D.P. Fewer, J. Jokela, E. M. Janssen, Comprehensive Database of Secondary Metabolites from Cyanobacteria CyanoMetDB > 2000 Entries Existing Databases & Primary References, C, 2020, pp. 1–16.
[5] M.L.da S. Sousa, Cyanobacterial bioactive metabolites for anticancer drug discovery: characterization of new compounds and molecular mechanisms in physiologically relevant 3D cell culture, Universidade du Porto, 2020, pp. 1–24. https://repositorio-aberto.up.pt/handle/10216/126888.
[6] M.L. Sousa, M. Preto, V. Vasconcelos, S. Linder, R. Urbatzka, Antiproliferative effects of the natural oxadiazine nocuolin A are associated with impairment of mitochondrial oxidative phosphorylation, Front. Oncol. 9 (2019) 1–13, https://doi.org/10.3389/fonc.2019.00224.
[7] J. Cano, Conservación in vitro y cultivo de cyanoprokariotas bentónicas arrecifales de Providencia y Santa Catalina Islas, Colombia. Tesis Maestría en Biología, Universidad Nacional de Colombia, 2018. https://repositorio.unal.edu.co/handle/unal/69410.
[8] M. Nagarajan, V. Maruthanayagam, M. Sundararaman, A review of pharmacological and toxicological potentials of marine cyanobacterial metabolites, J. Appl. Toxicol. 32 (3) (2012) 153–185, https://doi.org/10.1002/jat.1717.
[9] J. Peña, Potential biotecnológico de Cianoprocariotas provenientes de Islas del Rosario, Universidad Nacional de Colombia, Colombia, 2019. https://repositorio.unal.edu.co/handle/unal/76639.
[10] G.R. Pettit, Y. Kamano, P. Brown, D. Gust, M. Inoue, C.L. Herald, Antineoplastic agents. 3. Structure of the cyclic peptide dolastatin 3 from Dolabella auricularia, J. Am. Chem. Soc. 104 (3) (1982) 905–907.
[11] F. Ayala, Búsqueda de compuestos con posible actividad a partir de cianobacterias marinas del Caribe colombiano, Universidad Nacional de Colombia, Colombia, 2017. https://repositorio.unal.edu.co/handle/unal/6.
[12] L. Becerra, Evaluación del perfil metabólico de un consorcio de cianobacterias bentónicas arrecifales del Caribe colombiano bajo condiciones de cultivo, Universidad Nacional de Colombia, Colombia, 2017. https://repositorio.unal.edu.co/handle/unal/62324.
[13] J.A. Prato-Valderrama, Afloramientos de cianobacterias marinas bentónicas en San Andrés, Providencia y las Islas del Rosario (Caribe colombiano): Caracterización y evaluación de su posible papel ecológico, Universidad Nacional de Colombia, 2013. https://repositorio.unal.edu.co/handle/unal/21920.
[14] L. Bayona, Estudio químico y evaluación de la actividad citotóxica de metabolitos secundarios provenientes de cianobacterias bentónicas arrecifales del Caribe colombiano, Universidad Nacional de Colombia, 2014. https://repositorio.unal.edu.co/handle/unal/30357.
[15] R.A. Andersen, Algal culturing techniques, Elsevier 1 (2005).

[16] L. Ferreira, J. Morais, M. Preto, R. Silva, R. Urbatzka, V. Vasconcelos, M. Reis, Uncovering the bioactive potential of a cyanobacterial natural products library aided by untargeted metabolomics, Mar. Drugs 19 (11) (2021), https://doi.org/10.3390/md19110633.
[17] S. Parida, M. Sriram, C. Bhanaja, B. Sahoo, C. Bhanja, In Vitro Screening of Antioxidant, Antimicrobial and Anticancer Activities of Cyanobacteria Found across Odisha Coast, India SATYABRATA DASH Maharaja Sriram Chandra Bhanja Deo University, 2022, pp. 1–19, https://doi.org/10.21203/rs.3.rs-1272821/v1.
[18] M. Cegłowska, P. Kwiecień, K. Szubert, P. Brzuzan, M. Florczyk, C. Edwards, A. Kosakowska, H. Mazur-Marzec, Biological activity and stability of aeruginosamides from cyanobacteria, Mar. Drugs 20 (2) (2022), https://doi.org/10.3390/md20020093.
[19] L. Ferreira, J. Morais, V. Vasconcelos, M. Reis, Discovery of a novel potent cytotoxic compound from Leptothoe sp, Biol. Life Sci. forum 14 (1) (2022) 46, https://doi.org/10.3390/blsf2022014046.
[20] M.L. Sousa, T. Ribeiro, V. Vasconcelos, S. Linder, R. Urbatzka, Portoamides A and B are mitochondrial toxins and induce cytotoxicity on the proliferative cell layer of in vitro microtumours, Toxicon 175 (2020) 49–56, https://doi.org/10.1016/j.toxicon.2019.12.159.
[21] M. Costa, M. Garcia, J. Costa-Rodrigues, M.S. Costa, M.J. Ribeiro, M.H. Fernandez, P. Barros, A. Barreiro, V. Vasconcelos, R. Martins, Exploring bioactive properties of marine cyanobacteria isolated from the Portuguese coast: high potential as a source of anticancer compounds, Mar. Drugs 12 (2014) 98–114, https://doi.org/10.3390/md12010098.
[22] M. Hassouani, B. Sabour, Z. Belattmania, S. El Atouani, A. Reani, T. Ribeiro, V. Ramos, M. Preto, P.M. Costa, R. Urbatzka, P. Leão, V. Vasconcelos, In vitro anticancer, antioxidant and antimicrobial potential of Lyngbya aestuarii (Cyanobacteria) from the Atlantic coast of Morocco, Journal of Materials and Enviromental Sciences 8 (S) (2017) 4923–4933.
[23] G. Charitos, D.T. Trafalis, P. Dalezis, C. Potamitis, V. Sarli, P. Zoumpoulakis, C. Camoutsis, Synthesis and anticancer activity of novel 3,6-disubstituted 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives, Arab. J. Chem. 12 (8) (2019) 4784–4794, https://doi.org/10.1016/j.arabjc.2016.09.015.
[24] F. Guesmi, I. Saidi, R. Abbassi, M. Saidani, N. Hfaiedh, A. Landoulsi, Therapeutic potential of second degree's skin burns by topical dressing of Teucrium ramosissimum that promotes re-epithelialization, Dermatol. Ther. 35 (5) (2022) 1–9, https://doi.org/10.1111/dth.15428.
[25] W. Klinngam, P. Rungkamoltip, S. Thongin, J. Joothamongkhon, P. Khumkhrong, M. Khongkow, K. Namdee, S. Tepaamorndech, P. Chaikul, M. Kanlayavattanakul, N. Lourith, K. Piboonprai, U. Ruktanonchai, U. Asawapirom, T. Iempridee, Polymethoxyflavones from Kaempferia parviflora ameliorate skin aging in primary human dermal fibroblasts and ex vivo human skin, Biomed. Pharmacother. 145 (2022), https://doi.org/10.1016/j.biopha.2021.112461.
[26] A.S. Lorenzi, M.L. Bonatelli, A.M. Varani, M.C. Quecine, M. do C. Bittencourt-Oliveira, Draft genome sequence of the cyanobacterium Sphaerospermopsis aphanizomenoides BCCUSP55 from the Brazilian semiarid region reveals potential for anti-cancer applications, Arch. Microbiol. 204 (1) (2022) 1–7, https://doi.org/10.1007/s00203-021-02602-1.
[27] J.I. Quintana Bulla, Evaluación de la toxicidad y del potential bioactivo de afloramientos de cianobacterias bentónicas arrecifales del Caribe Colombiano, Universidad Nacional de Colombia, 2011. https://repositorio.unal.edu.co/handle/unal/10849.