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ORIGINAL ARTICLE

Biological activities and phytochemicals profiling of different cyanobacterial and microalgal biomass

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Abstract

Potential uses of methanolic extracts derived from cyanobacterial and microalgal biomass were evaluated as promising sustainable sources of bioactive phytochemicals for nutraceutical, cosmetic, and pharmaceutical applications. Among the cyanobacteria and microalgae tested, cyanobacterium *Nostoc* sp. AARL C008 biomass exhibited the highest phytochemicals, correlating with high occurrence of antioxidant activities. The antioxidant potential of *Nostoc* sp. AARL C008 was assessed using 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and potassium ferricyanide reducing antioxidant power (PFRAP) assays, giving ABTS activity of 38.99 mg TE/g extract, DPPH activity of 9.16 mg GAE/g extract and PFRAP activity of 11.48 mg GAE/g extract. *Nostoc* sp. AARL C008 yielded high levels of total phenolic contents (54.10 mg GAE/g extract) and pigments including chlorophyll (6.42 mg/g DW) and carotenoids (1.56 mg/g DW). Interestingly, *Nostoc* sp. AARL C008 showed high potent cytotoxic activity against malignant melanoma skin cancer cells (A375 cells), providing IC50 of 0.42 mg/mL. LC-ESI-QTOF-MS/MS tentatively identified 83 phenolic compounds with favorable bioactivities from the methanolic extract of *Nostoc* sp. AARL C008. Among phytochemical profiles, the most abundant phenolic compound was *p*-coumaric acid (40.70%), indicating valuable biological activities. Results demonstrated that phytochemicals extracted from cyanobacterial biomass can be used as bioactive ingredients, with potential applications in the nutraceutical, cosmetic, and pharmaceutical industries.

Keywords Phytochemicals · Methanolic extract · Bioactivities · Biomass · Cyanobacteria · Microalgae

1 Introduction

The recent dramatic increase in demand for healthy products has stimulated interest in innovative ingredients such as phytochemicals with beneficial bioactivities. Cyanobacterial and microalgal biomass are among the most significant polyphyletic assemblages that contain rich and diverse phytochemicals with high bioactivities. Examples of beneficial phytochemicals include chlorophylls (chlorophyll *a* and *b*), carotenoids (lycopene, β-carotene, and xanthophylls) and phenolic compounds [1]. Abundant evidence confirms that phytochemicals effectively prevent aging and ameliorate various others such as cancer, coronary heart diseases, inflammatory disorders, and neurological degeneration [2, 3]. Hence, phytochemicals have been extensively used as active ingradients in the nutraceutical, cosmetic, and pharmaceutical industries [4]. Among phytochemicals, phenolics, containing one or more aromatic rings bearing hydroxyl groups, have attracted

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considerable attention. Phenolic compounds as the main secondary metabolites in plants are divided into phenolic acids and polyphenols. Phenolics usually combine with mono- and polysaccharides linked to one or more phenolic groups, or can occur as derivatives such as esters or methyl esters [5]. Extracts derived from biomass of cyanobacteria and microalgae contain a plethora of phenolic compounds including gallic acid, protocatechuic acid, caffeic acid and epicatechin, with potential as functional foods [6]. Positive correlation between phenolic compounds and antioxidant capacity has been reported for several cyanobacteria and microalgae [7, 8]. In addition to phenolic acids, polyphenols have also been shown to have excellent antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer properties in in vitro and in vivo investigations [9]. Thus, this study focused on evaluating the phenolic potential of some cyanobacteria and microalgae that were reported as having a variety of bioactivities.

The primary consideration in achieving efficient extraction of phytochemicals is the selection of solvents [10]. Various solvents have been widely used for phytochemicals extraction from cyanobacterial and microalgal biomass, such as *Tetraselmis* sp., *Dunaliella* sp., *Chlorella* sp., *Synechocystis* sp., and *Oscillatoria* sp. Water-soluble solvents including acetone, methanol, and ethanol yielded higher contents of phytochemicals, flavonoids, terpenoids, alkaloids, steroids, and saponins compared to using chloroform [11, 12]. Among water-soluble solvents, methanol proved to be the significant solvent of choice to maximize phytochemical content and provide essential biological properties [13]. The methanol used in this process is readily recovered and utilized through evaporation to reduce total production costs. Accordingly, this research focused on systematically investigating the optimal methanolic extraction that provided a variety of active phytochemicals derived from cyanobacterial and microalgal biomass.

Rapid screening and identification of phytochemicals with high bioactivities against cancer cells were successful using the liquid chromatography-mass spectrometry (LC–MS) approach. High performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS) is a standard method to isolate and characterize phenolic compounds by scrutinizing their molecular weight [14]. The LC–MS instrumentation identified terpenoids, carotenoids, polyphenolics and fatty acid compounds in microalgal *Nannochloropsis oculata* extract that were bioactive molecules against cancer cells [15]. Moreover, over 10 polyphenol compounds with desirable bioactivities were observed in phytochemicals extracted from *Phaedactylium tricornitum*, *Nannochloropsis gaditana*, *Nannochloris* sp., and *Tetraselmis suecica* biomass using LC–MS [16]. Hence, to identify the phytochemical profiles of cyanobacterial and microalgal extracts as fascinating bioactive compounds, particularly phenolics, these compounds were characterized by using LC-ESI-QTOF-MS/MS.

This study evaluated the bioactivities of phytochemicals in methanolic extracts derived from the biomass of cyanobacteria and microalgae. Antioxidant properties including radical scavenging activities of 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the potassium ferricyanide reducing power (PFRAP) activity assays were determined. Total phenolic compounds and cytotoxicity activities against cancer and normal cells were also evaluated. Moreover, the LC-ESI-QTOF-MS/MS analysis was also used to identify phytochemicals in the methanolic extracts.

2 Materials and methods

2.1 Samples

Two species of cyanobacteria and six species of microalgae were obtained from the Applied Algal Research Laboratory (AARL), Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The two species of cyanobacteria were *Nostoc* sp. AARL C008 and *Phormidium* sp. AARL C021, while the six species of green microalgae were *Acutodesmus* sp. AARL G022, *Coelastrum* sp. AARL G133, *Crucigeniella* sp. AARL G135, *Euastrum* sp. AARL G001, *Micractinium* sp. AARL G009, and *Verrucodesmus* sp. AARL G148. The cyanobacteria were cultured in a Blue-Green medium (BG-11), while the green microalgae were cultured in Jaworski's medium (JM). Both cyanobacteria and microalgae were incubated at 25 ± 1 °C under continuous illumination using a fluorescent lamp with an intensity of 31.97 μmol/m^2/s for 30 days.

2.2 Extraction of methanolic extract

Cyanobacterial and microalgal biomass (2.5 g) were extracted using absolute methanol (100 mL) by incubation at 4 °C for 24 h [17]. The mixture was then centrifuged at 6000 rpm and 4 °C for 5 min. The obtained pellets were continually extracted until colorless and the supernatant was collected and subjected to UV–Vis spectrophotometry (250–700 nm). Selected extracts having high phytochemical absorption in UV–Vis wavelengths were then evaporated to dryness in a rotary-evaporator under reduced pressure at 40 °C before bioactivity evaluation.

2.3 Determination of pigment and total phenolic contents of methanolic extracts

The chlorophyll *a* content was determined following the process introduced by Wintermans and De Mots [18] and Saijo [19]. Briefly, the sample suspension was filtered through a

glass microfiber filter. Then, 10 mL of 90% methanol was added to the filtrated samples. This mixture was incubated at 70 °C for 20 min and then centrifuged at 3,000 rpm for 10 min. Values of spectrophotometric absorbance at 630, 645, 665 and 750 nm obtained from the supernatant were recorded. The chlorophyll *a* content (mg/g DW) was calculated using the following equation:

$$Chlorophylla = \{[(11.6(A_{665} - A_{750}) - 1.31(A_{645} - A_{750}) - 0.14(A_{630} - A_{750})) \times v]/[V \times l]\}/DW \quad (1)$$

where v is the volume of methanol (mL), V is the volume of the filtered sample (L), and l is the path length of the spectrophotometer cuvette (cm). A_{630}, A_{645}, A_{665}, and A_{750} correspond to the absorbance at 630, 645, 665, and 750 nm, respectively, and DW is cell dry weight.

The carotenoid content was determined following the method of de Quirós and Costa [20]. Briefly, 0.2 g of finely ground sample was mixed with 10 mL of 90% ethanol. A 1 mL aliquot of 60% potassium hydroxide was added to the mixture before ultrasonication for 15 min. The mixture was then centrifuged at 3,000 rpm for 10 min. The supernatant was poured into a separatory funnel and gently mixed with 15 mL of diethyl ether and 9% sodium chloride. The diethyl ether phase was collected, and the volume was adjusted to 25 mL of diethyl ether. Finally, the supernatant was spectrophotometrically measured at 450 nm. The carotenoid content (mg/g DW) was calculated using the following equation:

$$Carotenoidcontent = [A_{450} \times 25]/[260 \times gDW] \quad (2)$$

where A_{450} is the absorbance at 450 nm and DW is cell dry weight.

The total phenolic content was spectrophotometrically determined according to the Folin-Ciocalteu reagent method [21–23]. Briefly, 20 µL of the sample solution (the tested concentration at 0.2–55.0 mg/mL) was mixed with 20 µL of deionized water and 100 µL of 10% (w/v) Folin-Ciocalteu reagent. The mixture was incubated for 5 min at room temperature. After adding 80 µL of 5% Na_2CO_3, the mixture was incubated for 1 h in dark condition at room temperature. The supernatant was spectrophotometrically measured at 765 nm, with gallic acid used as the reference standard (Supplementary data). Total phenolic content was reported as gallic acid equivalent (GAE) per g extract.

2.4 20-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

The ABTS radical scavenging activity was determined following a modified Bunea et al. [24] and Sampath and Vasanthi [25] method. The ABTS solution was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate (final concentration). The mixture was incubated for 12–16 h in dark condition at room temperature. Then, the absorbance at 734 nm of the ABTS solution was adjusted to 0.700 ± 0.020 using deionized water. A 5 µL aliquot of the sample solution (the tested concentration at 0.2–25.0 mg/mL) was mixed with 195 µL of the ABTS solution in a 96-well plate. The mixture was incubated for 10 min at 37 °C and the reduction of ABTS was measured at 734 nm using a 96-well plate reader. Trolox was used as the reference standard (Supplementary data) and the following equation was used to determine the scavenging activity (%).

$$ABTSradicalscavengingactivity = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of the control reaction (distilled water instead of the sample was used as control) and A_{sample} is the absorbance of the sample. The ABTS activity was reported as Trolox equivalent (TE) per g extract.

2.5 DPPH free radical scavenging assay

The DPPH radical-scavenging activity was determined according to the method described by Bhadoriya et al. [26] and Zhou et al. [27] with minor modifications. A 50 µL aliquot of 1.3 mM DPPH dissolved in methanol was mixed with 100 µL of the sample solution (the tested concentration at 0.2–12.5 mg/mL) in a 96-well plate. The radical stock solution was freshly prepared. The mixture was incubated for 30 min in dark condition at room temperature. The absorbance of DPPH was measured at 517 nm. Gallic acid was used as the reference standard (Supplementary data) and the following equation was used to determine the scavenging activity (%).

$$DPPHradicalscavengingactivity = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (4)$$

where $A_{control}$ is the absorbance of the control reaction (distilled water instead of the sample was used as control) and A_{sample} is the absorbance of the sample. The DPPH activity was reported as gallic acid equivalent (GAE) per g extract.

2.6 Potassium ferricyanide reducing antioxidant power (PFRAP) assay

The PFRAP assay was determined following the methods of Oyaizu [28], Mau et al. [29] and Benslama and Harrar [30] with some modifications. A 130 µL aliquot of the sample solution (the tested concentration at 0.2–50.0 mg/mL) was mixed with 290 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 290 µL of 1% potassium ferricyanide. The mixture was incubated for 20 min at 50 °C. After 290 µL of 10% (w/v) trichloroacetic acid was added, the mixture was

centrifuged at 3,000 rpm for 10 min. The supernatant solution (1 mL) was mixed with 1 mL of distilled water and 200 µL of 0.1% ferric chloride. The absorbance was measured at 700 nm and gallic acid was used as the reference standard (Supplementary data). The PFRAP activity was reported as gallic acid equivalent (GAE) per g extract.

2.7 Cytotoxicity activity

The cytotoxicity of the methanolic extracts on Vero cells and malignant melanoma cells (A375 skin cancer cells) was tested using the MTT assay as described by Umthong et al. [31]. Briefly, 10^5 cell/mL of Vero cells and A375 cells were placed into 96-well plates and incubated for 24 h at 37 °C in a 5% CO_2 incubator. After the cell incubation, each concentration of samples (the tested concentration at 0.4–2.5 mg/mL) was treated with Vero cells or A375 cells and incubated for 48 h at 37 °C in a 5% CO_2 incubator. Then, 2 mg/mL MTT solution (Bio Basic Inc., Markham, ON, Canada) was added and the resulting mixture was incubated for 4 h. The absorbance was measured at 540 and 630 nm. The percentage of cell viability (Supplementary data) and 50% inhibitory concentration (IC_{50}) were calculated.

2.8 LC-ESI-QTOF-MS/MS analysis of phenolic compounds

LC-ESI-QTOF-MS/MS analysis was performed with an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 6545 Accurate-Mass Q-TOF LC–MS (Agilent Technologies, Santa Clara, CA, USA) via an electrospray ionization source (ESI). Separation was achieved by a Poroshell 120 EC-C18, LC Column (2.1 × 100 mm, 2.7 µm) (Agilent Technologies, Santa Clara, CA, USA) at room temperature and the sample temperature was set at 10 °C. LC–MS/MS analyses were performed by modifying the method of Zhong et al. [14]. The mobile phase consisted of water/acetic acid (98:2, v/v; eluent A) and acetonitrile/acetic acid/water (50:0.5:49.5, v/v/v; eluent B). The gradient profile was described as follows: 10–25% B (0–25 min), 25–35% B (25–35 min), 35–40% B (35–45 min), 40–55% B (45–75 min), 55–80% B (75–79 min), 80–90% B (79–82 min), 90–100% B (82–84 min), 100–10% B (84–87 min), and isocratic 10% B (87–90 min). A volume of 6 µL was injected for each standard or sample and the flow rate was set at 0.4 mL/min. Nitrogen gas nebulization was set at 45 psi with a flow rate of 5 L/min at 300 °C and the sheath gas was set at 11 L/min at 250 °C. The capillary and nozzle voltages were set at 3.5 kV and 500 V, respectively. A complete mass scan ranging from *m/z* 50 to 1300 was used, MS/MS analyses were carried out in automatic mode with collision energy (10, 15 and 30 eV) for fragmentation. Peak identification was performed in both positive and negative modes while instrument control, data acquisition and processing were performed using MassHunter Workstation software (Metlin_Metabolites_AM_PCDL.cdb) (Agilent Technologies, Santa Clara, CA, USA).

2.9 Statistical analysis

Triplicate analyses were performed and results were subjected to descriptive statistical analysis using one-way ANOVA (analysis of variance) and Duncan's multiple range tests ($p < 0.05$) using SPSS statistics 17.0. Pearson correlation coefficients between either antioxidant or anticancer capacity of the extract, total phenolic contents, and pigment contents (chlorophylls and carotenoids) were also calculated using SPSS statistics 17.0. The level of probability was set to $p < 0.01$ and $p < 0.05$.

3 Results and discussion

3.1 Screening cyanobacteria and microalgae that produce high contents of phytochemicals

In this study, eight strains of cyanobacteria and microalgae were evaluated for phytochemical production using the UV–Vis spectrophotometric method. The phytochemicals were extracted from their biomass using methanol as a water-soluble solvent. The type of solvents strongly influenced extraction yields and bioactivities due to the solubilization of antioxidant compounds with different chemical structures and polarities [32]. Methanol was reported to be a highly efficient solvent to extract compounds, with high phytochemical yield and antioxidant activity [7]. As shown in Fig. 1, all phytochemical extracts had similar absorption patterns at wavelengths between 250 and 700 nm (UV to visible spectrum). Three major absorption peaks were observed at 270–290 nm, 400–420 nm, and 660–670 nm. These results were supported by previous reports stating that phytochemical extracts contain various phenolic compounds such as pigments that absorb UV–visible light. Cyanobacterial and microalgal pigments like chlorophyll *a*, chlorophyll *b* and beta-carotene absorb light at wavelengths of 400–500 nm and 600–700 nm [33]. Tavares et al. [34] and Kula-Maximenko et al. [35] also reported that chlorophyll *a* produced from microalgae absorbed maximum light at 665 nm, while fucoxanthin absorbed maximum light at 450 nm. In cyanobacteria, phycocyanin (PC) is the principal pigment that absorbs light at 610–620 nm [36]. Interestingly, most cyanobacteria produce UV-protective compounds such as scytonemin-3a-imine with UV–Vis absorption at 237 nm, 366 nm, 437 nm and 564 nm [37]. Mycosporine-like amino acids

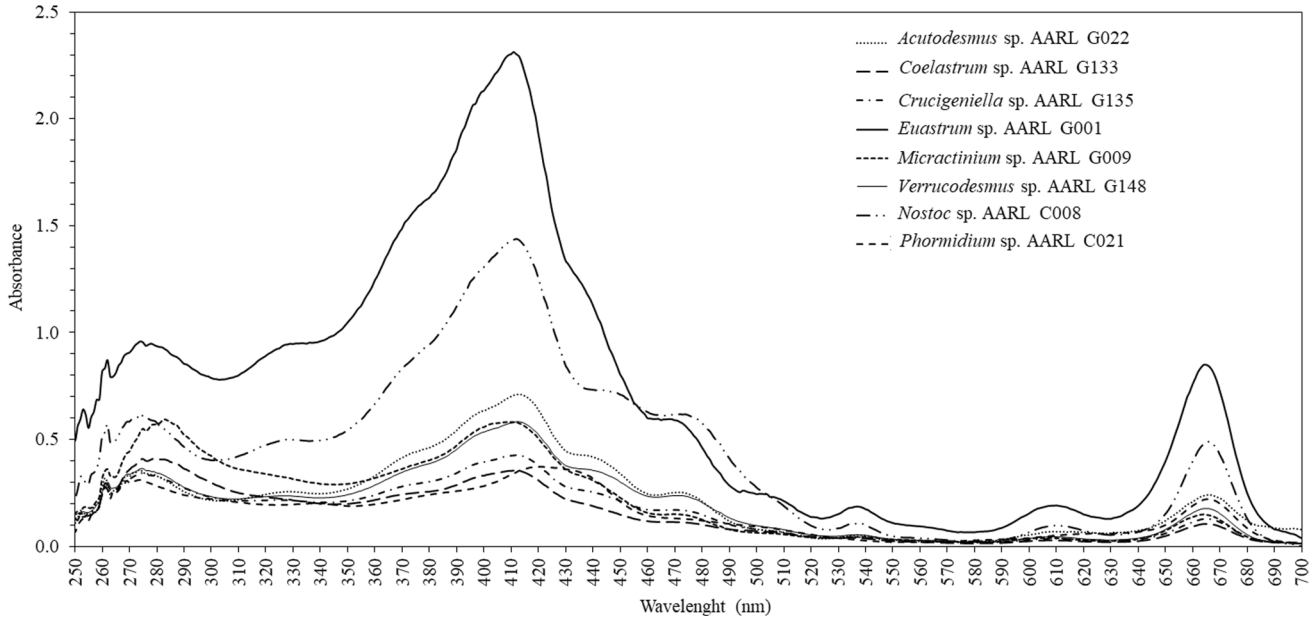

Fig. 1 UV–Vis absorption spectra of methanolic extracts from six species of microalgae, *Acutodesmus* sp. AARL G022, *Coelastrum* sp. AARL G133, *Crucigeniella* sp. AARL G135, *Euastrum* sp. AARL G001, *Micractinium* sp. AARL G009, and *Verrucodesmus* sp. AARL G148, and two species of cyanobacteria, *Nostoc* sp. AARL C008 and *Phormidium* sp. AARL C021

Table 1 Biomass production, biomass productivity, specific growth rate, crude extract yield, total chlorophyll a, carotenoids, and total phenolic content from microalga *Euastrum* sp. AARL G001 and cyanobacteria *Nostoc* sp. AARL C008

Characteristics	*Euastrum* sp. AARL G001	*Nostoc* sp. AARL C008
Biomass production (g/L)	$1.80^{a} \pm 0.011$	$1.09^{b} \pm 0.021$
Biomass productivity (g/L/day)	$0.11^{a} \pm 0.000$	$0.04^{b} \pm 0.001$
Specific growth rate (μ)	$0.71^{a} \pm 0.005$	$0.05^{b} \pm 0.001$
Crude extract yield (%)	$17.02^{a} \pm 2.469$	$3.66^{b} \pm 1.117$
Total chlorophyll *a* (mg/g DW)	$4.23^{a} \pm 0.06$	$6.42^{b} \pm 0.331$
Carotenoids (mg/g DW)	$1.52^{a} \pm 0.106$	$1.56^{a} \pm 0.057$
Total phenolic content (mg GAE/g extract)	$3.87^{a} \pm 0.210$	$54.10^{b} \pm 2.822$

Different small letters at the same row show significant differences ($p \leq 0.05$) between species

(MAAs), a secondary metabolite and UV-protective compound, were also observed at 310–360 nm of the absorption spectrum [38]. Results indicated that methanolic extracts contained these phytochemicals as a promising source of UV protective compounds due to their high UV absorption efficiency. Among the eight strains, the cyanobacterium *Nostoc* sp. AARL C008 exhibited the highest phytochemical absorbance, followed by the microalga *Euastrum* sp. AARL G001. Absorption at 270–290 nm of the extracts was comparable, while absorption of the six strains at other wavelengths was lower than *Nostoc* and *Euastrum* extracts. Higher absorption values were related to extraction yields. Therefore, both *Nostoc* sp. AARL C008 and *Euastrum* sp. AARL G001 were investigated for mass production and evaluated for their bioactivities.

3.2 Mass cultivation and phytochemical content of selected cyanobacteria and microalgae

Table 1 shows the growth and phytochemical content of *Nostoc* sp. AARL C008 and *Euastrum* sp. AARL G001. Results revealed that *Euastrum* sp. AARL G001 had higher biomass production (1.80 g/L), biomass productivity (0.11 g/L/day) and specific growth rate (0.71 day^{-1}) compared to *Nostoc* sp. AARL C008. *Euastrum* sp. AARL G001 also had a higher methanolic extract yield (17.02 g/100 g DW) compared to *Nostoc* sp. AARL C008 (3.66 g/100 g DW). Both selected strains contained chlorophyll *a* and carotenoid ranging from 4.23 to 6.42 mg/g DW and 1.52 to 1.55 mg/g DW, respectively. These results concurred with previous studies that recorded *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Spirulina platensis* with chlorophyll *a* and carotenoid

ranging from 4 to 12 mg/g DW and from 1 to 2 mg/g DW, respectively [39, 40]. Chlorophylls and carotenoids are hydrophobic pigments that are widely found in biomass of cyanobacteria and microalgae. Babadi et al. [41] recorded chlorophyll *a* and *b* as major chlorophylls, pheophorbide-*a*, chlorophyllide-*b*, pheophytin-*a* and pheophytin-*b* in microalgae and cyanobacteria, whereas carotenoid profiles in cyanobacterial and microalgal extracts contained astaxanthin (free and esters), astaxanthin (free and esters), zeaxanthin, lutein, canthaxanthin and β-carotene as the major carotenoids [42]. These compounds are important biological complexes due to their antioxidant activities and UV absorption properties [41, 42]. Interestingly, *Nostoc* sp. AARL C008 had a higher total phenolic content of 54.10 mg GAE/g extract than *Euastrum* sp. AARL G001 with phenolic content of 3.87 mg GAE/g extract. These results agreed with previous studies on *Chlorella vulgaris* (25.92 mg GAE/g extract) [43], *Haematococcus pluvialis* (74.08 mg GAE/g extract) [44], *Spirulina maxima* (45–57 mg GAE/g extract) [45], and *Nostoc linckia* (13–47 mg GAE/g extract) [46].

3.3 Antioxidant activities

Antioxidant activities of the methanolic extracts derived from biomass of cyanobacteria and microalgae were investigated using ABTS, DPPH and PFRAP assays. Results in Fig. 2a–d showed that *Nostoc* sp. AARL C008 had significantly higher antioxidant activities than *Euastrum* sp. AARL G001 based on ABTS and DPPH radical scavenging activities, with IC50 values at 0.20 mg extract/mg ABTS (38.99 mg TE/g extract) and 0.01 mg extract/mg DPPH (9.16 mg GAE/g extract), respectively. These results agreed with the IC50 values for DPPH and ABTS suggested by Rastogi et al. [47] and Ijaz and Hasnain [48]. Methanolic extracts having low IC50 values corresponded to strong antioxidant activities. This result indicated that the methanolic extract of *Nostoc* sp. AARL C008 biomass had a high reducing ability because the hydrogen donors stabilized the DPPH radical. The hydrogen atom of the antioxidant compounds acted as the nitrogen atom of the ABTS radical, resulting in greater stability. The antioxidant activities of the methanolic extract derived from *Euastrum* sp. AARL G001 biomass had less ability to neutralize DPPH and ABTS radicals but the IC50 values were better than previously reported by Assunção et al. [49] and Sivaramakrishnan et al. [50].

The PFRAP potential was assayed to measure the ability of the methanolic extract to reduce TPTZ-Fe^{3+} to TPTZ-Fe^{2+} by the action of electron-donating antioxidants [51]. Results in Fig. 2e showed that the methanolic extract from *Nostoc* sp. AARL C008 biomass had highest PFRAP level of 11.48 mg GAE/g extract, *Euastrum* sp. AARL G001 recorded a PFRAP level of 4.21 mg GAE/g extract. Higher PFRAP activity suggested that the methanolic extract had high reducing power, enabling donation of an electron to the free radical, thereby preventing or retarding the propagation reaction [52]. According to their antioxidant activities, results suggested that methanolic extracts obtained from both cyanobacterial and microalgal biomass showed potential for use as a bioactive ingredient for nutraceutical and pharmaceutical applications.

In the literature, the antioxidant properties of metabolites were related to the prevention of aging and various afflictions such as cancer, coronary heart diseases, inflammatory disorders and neurological degeneration [2, 3]. Phenolics are interesting compounds and classified into four main types based on their different chemical structures [53] as phenolic acids, coumarins, flavonoids and non-flavonoids. Flavonoids can also be divided into flavones, flavonols, isoflavonols, flavanones, flavanols, anthocyanins, and chalcones. Most phenolics are polymerized into larger molecules such as tannins and lignans (polyphenols) [53]. High contents of total phenolic compounds were found in *Nostoc* sp. AARL C008 biomass (54.10 mg GAE/g extract), correlating with high antioxidant activities. Similar results were reported by Yu et al. [54]. Pigments belonging to polyphenol compounds are interesting antioxidant substances, especially chlorophyll and carotenoid, that are found in both *Euastrum* sp. AARL G001 and *Nostoc* sp. AARL C008 biomass. These compounds also exhibited antioxidation, prevention of cancer and cardiovascular disease, and age-related macular degeneration. However, methanolic extracts contain numerous unidentified compounds that may play a role in antioxidant activities. Further studies are required to fully characterize methanolic extracts to expand the current understanding of the complicated occurrences in antioxidant activities.

3.4 Cytotoxicity

Figure 2f shows the cytotoxicity test results of methanolic extracts derived from biomass of *Euastrum* sp. AARL G001 and *Nostoc* sp. AARL C008 on Vero cells and malignant melanoma cells (A375 skin cancer cells). The A375 skin cancer cells, treated with extracts from *Euastrum* sp. AARL G001 and *Nostoc* sp. AARL C008, gave IC50 values of 1.04 and 0.42 mg/mL, respectively. The low IC50 values suggested that the methanolic extract had high anticancer activity that might prevent skin cancer. Our results aligned with Lauritano et al. [4] and Reyna-Martinez et al. [55]. They found that algal extracts rapidly inhibited lymphoma cells and melanoma cancer cells rapidly. Previous studies also found that high phenolic extract promoted cytotoxicity ability due to the presence of some phenolic compounds as attractive cancer inhibitors [54]. Nazir et al. [56] suggested that natural phytochemicals,

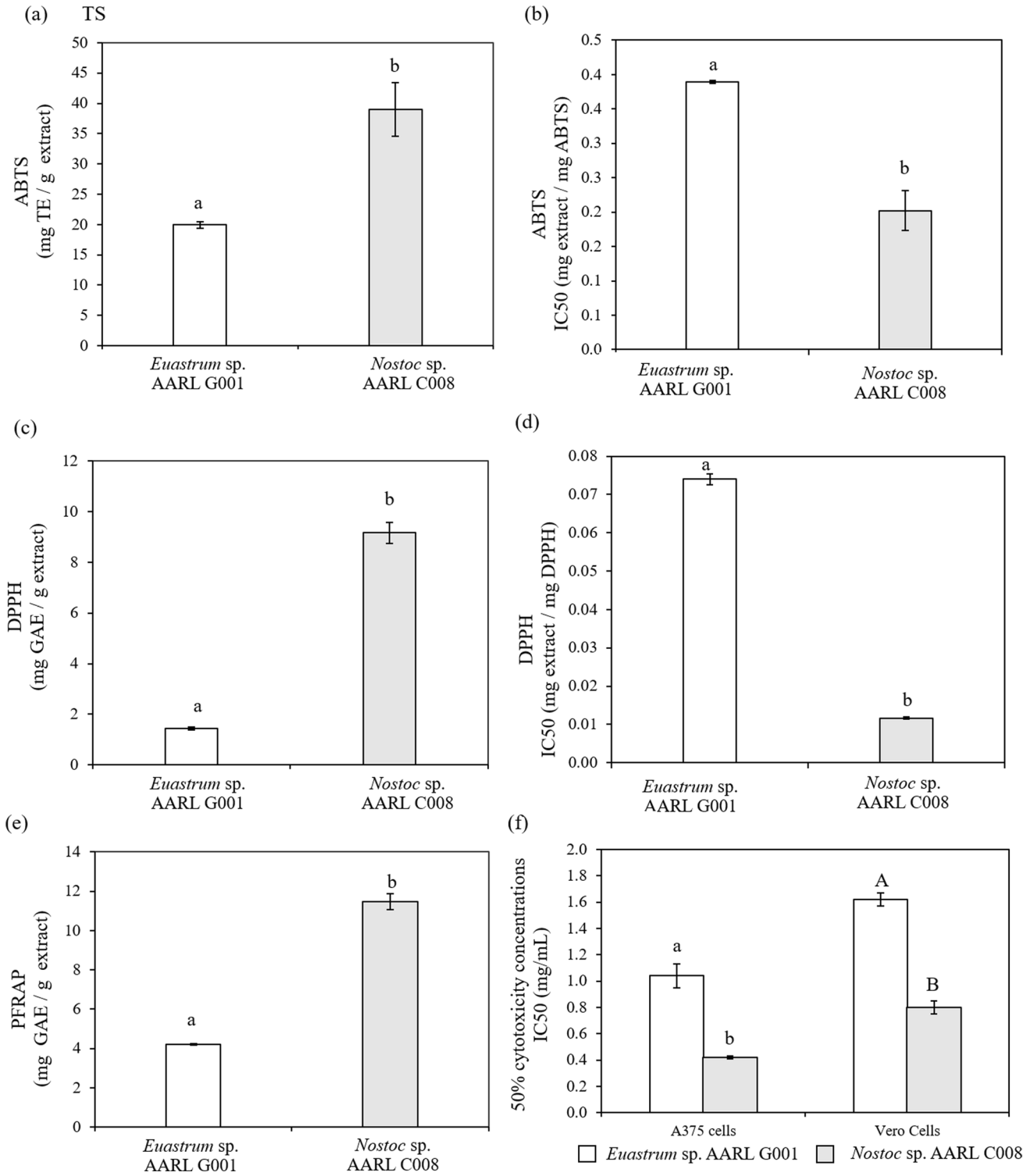

Fig. 2 Equivalent antioxidant capacity of *Euastrum* sp. AARL G001 and *Nostoc* sp. AARL C008 base on: (**a**, **b**) ABTS radical scavenging assay; (**c**, **d**) DPPH radical scavenging assay; (**e**) potassium ferricyanide reducing antioxidant power (PFRAP) assay; and (**f**) fifty percent cytotoxicity concentrations (IC50) of *Euastrum* sp. AARL G001 and *Nostoc* sp. AARL C008 of Vero cells and A375 cells

especially phenolic compounds, exhibited several biological activities including anticancer and antitumor. Pigments also promoted apoptosis in human cancer cell lines, including human prostate cancer cells and human

Table 2 Correlation between phytochemicals and bioactivities of methanolic extract from *Euastrum* sp. AARL G001 and *Nostoc* sp. AARL C008

Bioactivities		Total phenolic content (mg GAE/g extract)	ABTS (mg TE/g extract)	DPPH (mg GAE/g extract)	PFRAP (mg GAE/g extract)	Chlorophyll (mg/g DW)	Carotenoid (mg/g DW)	Anticancer (IC50)
Total phenolic content (mg GAE/g extract)	Pearson correlation	1	.992 (**)	.939 (**)	.995 (**)	.987 (**)	.223	− .981 (**)
	Sig		.000	.005	.000	.000	.672	.001
ABTS (mg TEAC/g extract)	Pearson correlation	.992 (**)	1	.930 (**)	.988 (**)	.969 (**)	.188	− .965 (**)
	Sig	.000		.007	.000	.001	.722	.002
DPPH (mg GAE/g extract)	Pearson correlation	.939 (**)	.930 (**)	1	.969 (**)	.975 (**)	.304	− .916 (*)
	Sig	.005	.007		.001	.001	.558	.010
PFRAP (mg GAE/g extract)	Pearson correlation	.995 (**)	.988 (**)	.969 (**)	1	.995 (**)	.250	− .975 (**)
	Sig	.000	.000	.001		.000	.633	.001
Chlorophyll (mg/g DW)	Pearson correlation	.987 (**)	.969 (**)	.975 (**)	.995 (**)	1	.290	− .976 (**)
	Sig	.000	.001	.001	.000		.577	.001
Carotenoid (mg/g DW)	Pearson correlation	.223	.188	.304	.250	.290	1	− .268
	Sig	.672	.722	.558	.633	.577		.608
Anticancer (IC50)	Pearson correlation	− .981 (**)	− .965 (**)	− .916 (*)	− .975 (**)	− .976 (**)	− .268	1
	Sig	.001	.002	.010	.001	.001	.608	

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

leukemia cell HL-60 [57]. Therefore, results indicated that the methanolic extract from *Nostoc* sp. AARL C008 biomass an interesting substance and effective bioactive component in cosmetic and skincare applications.

The extract had good cytotoxicity activity against A375 skin cancer cells; however, the higher sensitivity of Vero cells compared to cancer cells might damage the growth of normal cells in the human body. Bechelli et al. [58] and Moo-Puc et al. [59] demonstrated that microalgal and cyanobacterial extracts inhibited regular cell lines (namely normal hematopoietic cells and MDCK cells), while Umthong et al. [31] and Trabelsi et al. [60] suggested that adding an extract purification step gave the highest inhibition effect on cancer cell lines but the lowest inhibition on normal cells. Thus, the extract required further purification and examination to determine its potential function before acting as a template for future drug or cosmetic design.

3.5 Correlation between total phenolics, chlorophyll, and carotenoid contents and antioxidant and anticancer activities

Phenolics, chlorophylls and carotenoid compounds are important antioxidant components that contribute significantly to bioactive potential owing to their strong antioxidant activities [61]. Previous reports proved a linear correlation between total phenolic and pigment contents and bioactivities [23]. Results in Table 2 showed high positive correlation between antioxidant activities, total phenolics (ABTS, $r=0.992$; DPPH, $r=0.939$; PFRAP, $r=0.995$), and chlorophylls (ABTS, $r=0.969$; DPPH, $r=0.975$; PFRAP, $r=0.995$) at the 99% confidence level, indicating that phenolic and chlorophyll groups were responsible for the antioxidant activities of the extracts. A statistically significant negative correlation between anticancer activity and total phenolics ($r=-0.995$) and chlorophylls ($r=-0.969$) was also observed at the 95–99% confidence level. Phenolic and chlorophyll groups of *Nostoc* sp. AARL C008 extract highly contributed to anticancer activity. Farasat et al. [62] and Agregán et al. [63] suggested that the positive correlation showed that antioxidant activity enhanced with increasing phytochemicals contents. Survival rates of cancer cells decreased when increasing phytochemical content and the relationship between phytochemicals and anticancer activity was expressed as a negative correlation. The carotenoid group was insignificant contributors of antioxidant and anticancer potential, possibly due to low levels of detected carotenoids and the insignificant difference in carotenoid content

between *Euastrum* sp. AARL G001 and *Nostoc* sp. AARL C008. The Pearson correlation coefficients ($r = -0.976$ to -0.915) showed strong correlation between antioxidant activity and anticancer activity of the extract.

3.6 LC-ESI-QTOF-MS/MS analysis of phenolic compounds

LC–MS has been widely used to characterize the phenolic profiles of different algae, including cyanobacteria [14, 64]. A qualitative analysis of the phenolic compounds from cyanobacterium *Nostoc* sp. AARL C008 extract was achieved by LC-ESI-QTOF-MS/MS analysis in negative and positive ionization modes. Phenolic compounds present in the methanolic extract were tentatively identified from their mass-to-charge ratios (*m/z* values) and MS spectra in both negative and positive ionization modes ($[M+H]^-/[M+H]^+$) using Agilent LC–MS Qualitative Software and Personal Compound Database and Library (PCDL). In this study, LC–MS/MS enabled the tentative identification of 83 phenolic compounds including phenolic acids (12), flavonoids (44), other polyphenols (22), lignans (3), and tannins (2) (Table 3).

3.6.1 Phenolic acids

Phenolic acids have been reported as the most abundant phenolic compounds in algae and cyanobacteria [63] related to antioxidant activities and anticancer properties. Three subclasses of phenolic acids were detected including hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenylacetic acids.

Hydroxybenzoic acid derivatives Four hydroxybenzoic acid derivatives were detected from the methanolic extract of *Nostoc* sp. AARL C008. Compound 1 with $[M+H]^-$ *m/z* at 169.0140 was tentatively characterized as gallic acid. This compound was previously reported as abundant in brown seaweed *Himanthalia elongate* and described in cyanobacterial strains, including *Nostoc* spp. [64, 65]. Gallic acid plays a role in various bioactivities as an antioxidant and an antineoplastic agent, and also induces apoptosis in human skin cancer cells [66–68]. Therefore, the identification of gallic acid in this study supported the antioxidant and anticancer activities of *Nostoc* sp. AARL C00 extract. Gallic acid 4-O-(6-galloylglucoside) (compound 2, *m/z* 483.0902), m-trigallic acid (compound 3, *m/z* 473.0322), and 3,4-O-dimethylgallic acid (compound 4 m/z 197.0474) were tentatively identified in the negative mode.

Hydroxycinnamic acid and other phenolic acid derivatives Five hydroxycinnamic acid derivatives, two hydroxyphenylacetic acid derivatives and one hydroxyphenylacetic acid were tentatively identified. Compound (5) was identified as hydroxycinnamic acid derivative, *p*-coumaric acid with both modes. *p*-Coumaric acid ($[M+H]^+$ *m/z* 165.0548) was detected as the most abundant phenolic profile from *Nostoc* sp. AARL C008 extract. *p*-Coumaric acid is widely found in plants as well as microalgae and cyanobacteria [64]. This compound has shown pharmaceutical activities including antioxidant, anti-inflammatory, antineoplastic and antimicrobial properties [69]. Kong et al. [70] explained that *p*-coumaric acid suppressed tumor growth in vivo by blocking angiogenesis. Results indicated that *p*-coumaric acid contributed high bioactivities from the methanolic extract of *Nostoc* sp. AARL C008. Dihydro-3-coumaric acid (compound 6, *m/z* 167.0674), ferulic acid (compound 7, *m/z* 195.0648) and isopeonidin 3-arabinoside (compound 8, *m/z* 434.1193) were tentatively identified in the positive mode, whereas ferulic acid 4-O glucuronide (compound 9, *m/z* 369.07473) was tentatively identified in the negative mode.

Three other phenolic acids were tentatively identified as dihydroxyphenylacetic acid or 3,4-dihydroxyphenylacetic acid (DOPAC) (compound 10, *m/z* 167.0356), 2-hydroxyphenylacetic acid (compound 11, *m/z* 151.0417) and phenylacetic acid (compound 12, *m/z* 135.0482). 3,4-Dihydroxyphenylacetic acid (DOPAC) was previously reported as having antioxidant and anticancer activity [71, 72].

3.6.2 Flavonoids

Flavonoids are the main class of phenolic compounds. In this study, 44 flavonoids were tentatively identified and further divided into anthocyanins (8), flavanols (14), flavonols (18), and isoflavonoids (4).

Anthocyanins Eight anthocyanins (compounds 13–20) were detected from *Nostoc* sp. AARL C008 extract. Compound 13, delphinidin 3-(6″-malonylglucoside) 5-glucoside ($[M-H]^-$ *m/z* 712.147) was tentatively identified in both modes. Malvidin 3-galactoside (compound 14) was detected with $[M-H]^+$ at *m/z* 494.1479, which described antioxidant and anticancer activities [73–76]. Furthermore, delphinidin derivatives were tentatively classified to compounds 15–20, including delphinidin 3-glucosylglucoside ($[M-H]^-$ *m/z* 626.142), delphinidin 3-(acetylglucoside) ($[M-H]^+$ *m/z* 509.1235), delphinidin 3-O-3″,6″-O-dimalonylglucoside ($[M-H]^-$ *m/z* 636.0864), delphinidin 3-O-β-D-glucoside 5-O-(6-coumaroyl-β-D-glucoside) ($[M-H]^+$ *m/z* 774.198), isopeonidin 3-glucoside ($[M-H]^+$ *m/z* 464.1314), and delphinidin 3-O-(6-O-malonyl-β-D-glucoside) ($[M-H]^+$ *m/z* 552.1136), respectively.

Flavanols In this study, 14 flavanol derivatives (compounds 21–34) were detected from *Nostoc* sp. AARL C008 extract,

Table 3 Characterization of phenolic compounds in methanolic extract of *Nostoc* sp. AARL C008 by using LC-ESI-QTOF-MS/MS

No	Proposed compounds	Molecular formula	Ionization (ESI + / ESI-)	Molecular Weight (g/mol)	Observed (m/z)	Antioxidant capacity	Anticancer capacity	References
Phenolic acid								
Hydroxybenzoic acids								
1	Gallic acid	$C_7H_6O_5$	$[M-H]^-$	170.0215	169.0140	+	+	[66–68]
2	Gallic acid 4-O-(6-galloyl-glucoside)	$C_{20}H_{20}O_{14}$	$[M-H]^-$	484.0853	483.0902	nd	nd	
3	m-Trigallic acid	$C_{21}H_{14}O_{13}$	$[M-H]^-$	474.0434	473.0322	nd	nd	
4	3,4-O-Dimethylgallic acid	$C_9H_{10}O_5$	$[M-H]^-$	198.0528	197.0474	nd	nd	
Hydroxycinnamic acids								
5	*p*-Coumaric acid	$C_9H_8O_4$	*[M + H] +	164.0473	165.0548	+	+	[69, 70]
6	Dihydro-3-coumaric acid	$C_9H_{10}O_3$	[M + H] +	166.0630	167.0674	nd	nd	
7	Ferulic acid	$C_{10}H_{10}O_4$	[M + H] +	194.0579	195.0648	nd	nd	
8	Ferulic acid 4-O glucuronide	$C_{16}H_{18}O_{10}$	$[M-H]^-$	370.0900	369.0747	nd	nd	
9	Isopeonidin 3-arabinoside	$C_{21}H_{21}O_{10}$	[M + H] +	433.1135	434.1193	nd	nd	
Hydroxyphenylacetic acids								
10	3,4-Dihydroxyphenylacetic acid	$C_8H_8O_4$	$[M-H]^-$	168.0423	167.0356	+	+	[71, 72]
11	2-Hydroxyphenylacetic acid	$C_8H_8O_3$	$[M-H]^-$	152.0473	151.0417	nd	nd	
12	Phenylacetic acid	$C_8H_8O_2$	$[M-H]^-$	136.0524	135.0482	nd	nd	
Flavonoids								
Anthocyanins								
13	Delphinidin 3-(6″-malonyl-glucoside) 5-glucoside	$C_{30}H_{33}O_{20}$	$[M-H]^-$	713.1565	712.1470	nd	nd	
14	Malvidin 3-galactoside	$C_{23}H_{25}O_{12}$	[M + H] +	493.1346	494.1479	+	+	[73–76]
15	Delphinidin 3-glucosylglucoside	$C_{27}H_{31}O_{17}$	$[M-H]^-$	627.1561	626.1424	nd	nd	
16	Delphinidin 3-(acetylglucoside)	$C_{23}H_{23}O_{13}$	[M + H] +	507.1139	509.1235	nd	nd	
17	Delphinidin 3-O-3″,6″-O-dimalonylglucoside	$C_{27}H_{25}O_{18}$	*$[M-H]^-$	637.1041	636.0864	nd	nd	
18	Delphinidin 3-O-β-D-glucoside 5-O-(6-coumaroyl-β-D-glucoside)	$C_{36}H_{37}O_{19}$	[M + H] +	773.1929	774.1980	nd	nd	
19	Isopeonidin 3-glucoside	$C_{22}H_{23}O_{11}$	[M + H] +	463.1240	464.1314	nd	nd	
20	Delphinidin 3-O-(6-O-malonyl-β-D-glucoside)	$C_{24}H_{23}O_{15}$	[M + H] +	551.1037	552.1136	nd	nd	
Flavanols								
21	Prodelphinidin A2 3′-gallate	$C_{37}H_{28}O_{18}$	[M + H] +	760.1276	763.1353	nd	nd	
22	Gallocatechin-(4alpha- > 8)-gallocatechin-(4alpha- > 8)-gallocatechin	$C_{45}H_{38}O_{21}$	[M + H] +	914.1906	915.2052	nd	nd	
23	(-)-Epigallocatechin 3-(4-methyl-gallate)	$C_{23}H_{20}O_{11}$	$[M-H]^-$	472.1006	471.0946	nd	nd	
24	Prodelphinidin A1	$C_{30}H_{24}O14$	[M + H] +	608.1160	609.1240	nd	nd	
25	Epigallocatechin-(2b- > 7,4b- > 8)-gallocatechin	$C_{30}H_{24}O_{14}$	$[M-H]^-$	608.1166	607.1071	nd	nd	
26	8,8′-Methylenebiscatechin	$C_{31}H_{28}O_{12}$	$[M-H]^-$	592.1581	591.1517	nd	nd	
27	Epicatechin-(4beta- > 8)-gallocatechin	$C_{30}H_{26}O_{13}$	[M + H] +	594.1373	596.1458	nd	nd	
28	8-C-Ascorbyl epigallocatechin 3-O-gallate	$C_{28}H_{24}O_{17}$	$[M-H]^-$	632.1013	631.0769	+	nd	[77]
29	Gallocatechin-(4alpha- > 8)-epigallocatechin	$C_{30}H_{26}O_{14}$	$[M-H]^-$	610.1323	609.1169	nd	nd	
30	7-Galloylcatechin	$C_{22}H_{18}O_{10}$	[M + H] +	442.0900	443.10	nd	nd	

Table 3 (continued)

No	Proposed compounds	Molecular formula	Ionization (ESI + / ESI-)	Molecular Weight (g/mol)	Observed (m/z)	Antioxidant capacity	Anticancer capacity	References
31	ent-Epicatechin-(4alpha- > 8)-ent-epicatechin 3,3′-digallate	$C_{44}H_{34}O_{20}$	*$[M-H]^-$	882.1643	881.1553	nd	nd	
32	3′-Galloylprodelphinidin B2	$C_{37}H_{30}O_{18}$	$[M-H]^-$	762.1432	761.1293	nd	nd	
33	Epigallocatechin-(4beta- > 8)-epicatechin-3-O-gallate ester	$C_{37}H_{30}O_{17}$	$[M-H]^-$	746.1483	745.1438	nd	nd	
34	8,8′-Methylenebiscatechin	$C_{31}H_{28}O_{12}$	*[M + H] +	592.1581	593.1523	nd	nd	
Flavonols						nd	nd	
35	Tetramethylquercetin 3-Rutinoside	$C_{31}H_{38}O_{16}$	*[M-H]$^-$	666.2160	665.2134	nd	nd	
36	Quercetin 3,7,4′-O-triglucoside	$C_{33}H_{40}O_{22}$	[M + H] +	788.2011	789.2088	nd	nd	
37	Quercetin 3-(4″-acetylrhamnoside) 7-rhamnoside	$C_{29}H_{32}O_{16}$	$[M-H]^-$	636.1690	635.1698	nd	nd	
38	Quercetin 3,7-dimethyl ether	$C_{17}H_{14}O_7$	[M + H] +	330.0740	331.0849	nd	nd	
39	Quercetin 3-(2-caffeoylsophoroside) 7-glucoside	$C_{42}H_{46}O_{25}$	$[M-H]^-$	950.2328	949.2220	nd	nd	
40	Quercetin 3,7,4′-tri-O-sulfate	$C_{15}H_{10}O_{16}S_3$	$[M-H]^-$	541.9131	540.8981	nd	nd	
41	Quercetin 3-(6″-sinapoylsophorotrioside)	$C_{44}H_{50}O_{26}$	$[M-H]^-$	994.2590	993.2253	nd	nd	
42	Quercetin	$C_{15}H_{10}O_7$	$[M-H]^-$	302.0427	301.0341	+	+	[8, 78]
43	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	[M + H] +	464.0955	465.0995	nd	nd	
44	Quercetin 3-O-(6-O-malonyl-β-D-glucoside)	$C_{24}H_{22}O_{15}$	*[M + H] +	550.0959	551.1084	nd	nd	
45	Quercetin 3-O-(6″-malonylglucoside)7-O-glucoside	$C_{30}H_{32}O_{20}$	$[M-H]^-$	712.1487	711.1474	nd	nd	
46	Quercetin 3-(2-glucosylrhamnoside)	$C_{27}H_{30}O_{16}$	$[M-H]^-$	610.1534	609.1454	nd	nd	
47	Quercetin 3-arabinoside	$C_{20}H_{18}O_{11}$	*[M + H] +	434.0849	435.0922	nd	nd	
48	Quercetin 7-glucuronide 3-rhamnoside	$C_{27}H_{28}O_{17}$	$[M-H]^-$	624.1326	623.1283	nd	nd	
49	Quercetin 3-sophoroside	$C_{27}H_{30}O_{17}$	[M + H] +	626.1483	627.1695	nd	nd	
50	Quercetin 3-(2-galloylglucoside)	$C_{28}H_{24}O_{16}$	$[M-H]^-$	616.1064	615.0917	nd	nd	
51	Quercetin 3-O-(6″-acetylglucoside)	$C_{23}H_{22}O_{13}$	[M + H] +	509.1232	639.1153	nd	nd	
52	Quercetin 3-(2-caffeoylglucuronoside)	$C_{30}H_{24}O_{16}$	$[M-H]^-$	640.1064	639.1153	nd	nd	
Isoflavonoids								
53	4′-O-Methyldelphinidin 3-O-beta-D-glucoside	$C_{22}H_{23}O_{12}$	[M + H] +	479.1190	480.1390	nd	nd	
54	4′-O-Methyldelphinidin 3-O-rutinoside	$C_{28}H_{33}O_{16}$	[M + H] +	625.1769	626.1896	nd	nd	
55	Dalbergin	$C_{16}H_{12}O_4$	$[M-H]^-$	268.0736	267.0677	nd	nd	
56	Dihydrobiochanin A	$C_{16}H_{14}O_5$	$[M-H]^-$	286.0841	285.0797	nd	nd	
Other polyphenols								
Lignans								
57	2′-Hydroxyenterolactone	$C_{18}H_{18}O_5$	$[M-H]^-$	314.1154	313.1110	nd	nd	
58	Arctigenin	$C_{21}H_{24}O_7$	*[M + H] +	372.1573	373.1649	nd	nd	
59	8–8′-Dehydrodiferulic acid	$C_{20}H_{18}O_8$	$[M-H]^-$	386.1002	385.1020	nd	nd	
Tannins								
60	3-Methylellagic acid 2-(4-galactosylglucoside)	$C_{28}H_{30}O_{18}$	[M + H] +	654.1432	655.1615	nd	nd	
61	Guibourtinidol-(4alpha- > 6)-catechin	$C_{30}H_{26}O_{10}$	$[M-H]^-$	546.1526	545.1311	nd	nd	

Table 3 (continued)

No	Proposed compounds	Molecular formula	Ionization (ESI + / ESI-)	Molecular Weight (g/mol)	Observed (m/z)	Antioxidant capacity	Anticancer capacity	References
Catechol								
62	5-(3′,4′-Dihydroxyphenyl)-gamma-valerolactone	$C_{11}H_{12}O_4$	*[M + H] +	208.0736	209.0795	+	nd	[80]
Hydroxybenzaldehydes								
63	4-Hydroxybenzaldehyde	$C_7H_6O_2$	$[M-H]^-$	122.0368	121.0303	nd	nd	
64	2,5-Dihydroxybenzaldehyde	$C_7H_6O_3$	$[M-H]^-$	138.0317	137.0269	nd	nd	
Hydroxycoumarins								
65	3-Dimethylallyl-4-hydroxy-benzaldehyde	$C_{12}H_{14}O_2$	[M + H] +	190.0994	191.1066	nd	nd	
66	Isoscopoletin	$C_{10}H_8O_4$	$[M-H]^-$	192.0423	191.0342	nd	nd	
Phenolic glycosides								
67	Dihydrocaffeic acid 3-O-glucuronide	$C_{15}H_{18}O_{10}$	[M + H] +	358.0900	359.0933	nd	nd	
68	2-Hydroxyphenylacetic acid O-b-D-glucoside	$C_{14}H_{18}O_8$	[M + H] +	314.1002	315.1039	nd	nd	
69	Urolithin A 3, 8-O-diglucuronide	$C_{25}H_{24}O_{16}$	$[M-H]^-$	580.1064	579.0959	nd	nd	
70	Urolithin A-3-O-glucuronide	$C_{19}H_{16}O_{10}$	[M + H] +	404.0743	405.0826	nd	nd	
71	2-Hydroxybenzaldehyde O-[xylosyl-(1- > 6)-glucoside]	$C_{18}H_{24}O_{11}$	*$[M-H]^-$	416.1319	415.1304	nd	nd	
72	5-(3′,5′-Dihydroxyphenyl)-gamma-valerolactone 3-O-glucuronide	$C_{17}H_{20}O_{10}$	$[M-H]^-$	384.1056	383.1090	nd	nd	
Phenolic terpenes								
73	Carnosol	$C_{20}H_{26}O_4$	$[M-H]^-$	330.1831	329.1821	+	+	[81, 82]
74	Carnosic acid	$C_{20}H_{28}O_4$	$[M-H]^-$	332.1988	331.1915	+	+	[81, 82]
75	7-Methylrosmanol	$C_{21}H_{28}O_5$	$[M-H]^-$	360.1937	359.1916	nd	nd	
76	11,12-Dimethylrosmanol	$C_{22}H_{30}O_5$	$[M-H]^-$	374.2093	373.1971	nd	nd	
77	6,7-Dimethoxy-7-epirosmanol	$C_{22}H_{30}O_6$	$[M-H]^-$	390.2042	389.1994	nd	nd	
78	Epirosmanol	$C_{20}H_{26}O_5$	$[M-H]^-$	346.1780	345.1768	+	nd	[83, 84]
Tyrosol								
79	Hydroxytyrosol 1-O-glucoside	$C_{14}H_{20}O_9$	*[M + H] +	316.1158	317.1224	+	+	[85, 86]
Other polyphenols								
80	Dihydrocaffeic acid 3-sulfate	$C_9H_{10}O_7S$	$[M-H]^-$	262.0147	261.0027	nd	nd	
81	Phloroglucinol	$C_6H_6O_3$	$[M-H]^-$	126.0317	125.0264	+	+	[14, 87, 88]
82	Dihydrophloroglucinol	$C_6H_8O_3$	$[M-H]^-$	128.0473	127.0397	nd	nd	
83	Leucodelphinidin 3-[galactosyl-(1- > 4)-glucoside]	$C_{27}H_{34}O_{18}$	[M + H] +	646.1745	647.2009	nd	nd	

*Compounds were detected in both negative $[M-H]^-$ and positive $[M+H]^+$ mode of ionization while only single mode data was presented. *RT* stands for "retention time"

nd non detect antioxidant or anticancer capacity, + detect antioxidant or anticancer capacity

belonging to catechins, epicatechins, gallocatechins, and epigallocatechins. Prodelphinidin A2 3′-gallate (compound 21, $[M-H]^+$ *m/z* 763.1353), gallocatechin-(4alpha- > 8)-gallocatechin-(4alpha- > 8)-gallocatechin (compound 22, $[M-H]^+$ *m/z* 915.2052), (-)-epigallocatechin 3-(4-methyl-gallate) (compound 23, $[M-H]^-$ *m/z* 471.0946), prodelphinidin A1 (compound 24, $[M-H]^+$ *m/z* 609.124), epigallocatechin-(2b- > 7,4b- > 8)-gallocatechin (compound 25, $[M-H]^-$ *m/z* 607.1071), 8,8′-methylenebiscatechin (compound 26, $[M-H]^-$ *m/z* 591.1517), epicatechin-(4beta- > 8)-gallocatechin (compound 27, $[M-H]^+$ *m/z* 596.1458), 8-C-ascorbylepigallocatechin 3-gallatem (compound 28, $[M-H]^-$ *m/z* 631.0769), gallocatechin-(4alpha- > 8)-epigallocatechin (compound 29, $[M-H]^-$ *m/z* 609.1169), 7-galloylcatechin (compound 30, $[M-H]^+$ *m/z* 443.1008), ent-epicatechin-(4alpha- > 8)-ent-epicatechin 3,3′-digallate (compound 31, $[M-H]^-$ *m/z* 881.1553), 3′-galloylprodelphinidin B2 (compound 32, $[M-H]^-$ *m/z* 761.1293),

epigallocatechin-(4beta- > 8)-epicatechin-3-O-gallate ester (compound 33, [M-H] $^-$ *m/z* 745.1438), and 8,8′-methylenebiscatechin (compound 34, [M-H]$^+$ *m/z* 593.1523). In addition, 8-C-ascorbyl epigallocatechin 3-O-gallate (compound 28) was described as having antioxidant activity [77].

Flavonols Quercetin and their derivatives were the main flavonols of the *Nostoc* sp. AARL C008 extract. Compound 42 showing precursor ion [M-H] $^-$ at *m/z* 301.0341 in negative mode was tentatively identified as quercetin. High abundance of quercetin in the cyanobacterium *Nostoc ellipsosporum* was also previously described by Singh et al. [8]. Quercetin had been shown to effectively inhibit cancer growth and antioxidant capacity [8, 78]. Additionally, 18 quercetin derivatives were tentatively identified as tetramethylquercetin 3-rutinoside (compound 35, [M-H] $^-$ *m/z* 665.2134), quercetin 3,7,4′-O-triglucoside (compound 36, [M-H]$^+$ *m/z* 789.2088), quercetin 3-(4″-acetylrhamnoside) 7-rhamnoside (compound 37, [M-H] $^-$ *m/z* 635.1698), quercetin 3,7-dimethyl ether (compound 38, [M-H]$^+$ *m/z* 331.0849), quercetin 3-(2-caffeoylsophoroside) 7-glucoside (compound 39, [M-H] $^-$ *m/z* 949.222), quercetin 3,7,4′-tri-O-sulfate (compound 40, [M-H] $^-$ *m/z* 540.8981), quercetin 3-(6″-sinapoylsophorotrioside) (compound 41, [M-H] $^-$ *m/z* 993.2253), quercetin 3-galactoside (compound 43, [M-H]$^+$ *m/z* 465.0995), quercetin 3-O-(6-O-malonyl-β-D-glucoside) (compound 44, [M-H]$^+$ *m/z* 551.1084), quercetin 3-O-(6″-malonylglucoside)7-O-glucoside (compound 45, [M-H] $^-$ *m/z* 711.1474), quercetin 3-(2-glucosylrhamnoside) (compound 46, [M-H] $^-$ *m/z* 609.1454), quercetin 3-arabinoside (compound 47, [M-H]$^+$ *m/z* 435.0922), quercetin 7-glucuronide 3-rhamnoside (compound 48, [M-H] $^-$ *m/z* 623.1283), quercetin 3-sophoroside (compound 49, [M-H]$^+$ *m/z* 627.1695), quercetin 3-(2-galloylglucoside) (compound 50, [M-H] $^-$ *m/z* 615.0917), quercetin 3-O-(6″-acetylglucoside) (compound 51, [M-H]$^+$ *m/z* 639.1153), and quercetin 3-(2-caffeoylglucuronoside) (compound 52, [M-H] $^-$ *m/z* 639.1153). According to the results, quercetin and their derivatives correlated with the bioactivities of *Nostoc* sp. AARL C008 extract.

Isoflavonoid derivatives Four isoflavonoid derivatives were tentatively identified as 4′-O-methyldelphinidin 3-O-beta-D-glucoside (compound 53, [M-H]$^+$ *m/z* 480.139), 4′-O-methyldelphinidin 3-O-rutinoside (compound 54, [M-H]$^+$ *m/z* 626.1896), dalbergin (compound 55, [M-H] $^-$ *m/z* 267.0677), and dihydrobiochanin A (compound 56, [M-H] $^-$ *m/z* 285.0797). Dihydrobiochanin A showed potent antibacterial activity against cariogenic bacteria [79]. The identity was confirmed by comparing with a previous study that characterized dalbergin and dihydrobiochanin A in seaweed (red and green algae) using LC-ESI-QTOF-MS/MS. The spectrum displayed ionic products at *m/z* 267.06666 and 285.0771, respectively [14].

3.6.3 Lignans and tannin derivatives

Three lignan derivatives were tentatively identified as 2′-hydroxyenterolactone (compound 57, [M-H] $^-$ *m/z* 313.111) and arctigenin (compound 58, [M-H]$^+$ *m/z* 373.1649), 8–8′-dehydrodiferulic acid (compound 59, [M-H] $^-$ *m/z* 385.102). According to a previous study, 2′-hydroxyenterolactone and arctigenin were also detected in red seaweed [14]. Tannin derivatives (compounds 60 and 61) were tentatively identified as 3-methylellagic acid 2-(4-galactosylglucoside)) and guibourtinidol-(4alpha- > 6)-catechin according to the precursor ions at [M-H]$^+$ *m/z* 655.1615 and [M-H] $^-$ *m/z* 545.1311, respectively.

3.6.4 Other polyphenols

Twenty-two other polyphenols found were classified as catechol (1), hydroxybenzaldehydes (2), hydroxycoumarins (2), phenolic glycosides (6), phenolic terpenes (6), tyrosol (1), and other polyphenols (4).

Catechol, hydroxybenzaldehydes, and hydroxycoumarin derivatives Compound 62, displaying [M-H]$^+$ at *m/z* 655.1615 was tentatively identified as 5-(3′,4′-dihydroxyphenyl)-gamma-valerolactone with antioxidant activity [80]. This compound played a role in bioactivity of *Nostoc* sp. AARL C008 extract. 4-Hydroxybenzaldehyde (compound 63) and 2,5-dihydroxybenzaldehyde (compound 64) were tentatively identified based on negative mode as [M-H] $^-$ at *m/z* 121.0303 and 137.0269, respectively. Hydroxycoumarin derivatives, 3-dimethylallyl-4-hydroxybenzaldehyde (compound 65, [M-H]$^+$ at *m/z* 191.1066) and isoscopoletin (compound 66, [M-H] $^-$ at *m/z* 191.0342), were tentatively characterized.

Phenolic glycosides Six phenolic glycoside compounds were tentatively identified as dihydrocaffeic acid 3-O-glucuronide (compound 67, [M-H]$^+$ at *m/z* 359.0933), 2-hydroxyphenylacetic acid O-b-D-glucoside (compound 68, [M-H]$^+$ at *m/z* 315.1039), urolithin A 3, 8-O-diglucuronide (compound 69, [M-H] $^-$ at *m/z* 579.0959), urolithin A-3-O-glucuronide (compound 70, [M-H]$^+$ at *m/z* 405.0826), 2-hydroxybenzaldehyde O-[xylosyl-(1- > 6)-glucoside] (compound 71, [M-H] $^-$ at *m/z* 415.1304), and 5-(3′,5′-dihydroxyphenyl)-gamma-valerolactone 3-O-glucuronide (compound 72, [M-H] $^-$ at *m/z* 383.109).

Phenolic terpene derivatives Carnosol (compound 73) and carnosic acid (compound 74) showing as precursor ions at [M-H] $^-$ at *m/z* 329.1821 and 331.1915 were tentatively

identified in negative mode. Loussouarn et al. [81] and Salem et al. [82] also confirmed the presence of carnosol and carnosic acid with antioxidant and anticancer activities. In addition, rosmanol derivatives (compounds 75–77) were tentatively characterized as 7-methylrosmanol, 11,12-dimethylrosmanol, and 6,7-dimethoxy-7-epirosmanol, according to the precursor ions $[M-H]^-$at *m/z* 359.1916, 373.1971, and 389.1994, respectively. Compound (78) with $[M-H]^-$at *m/z* 345.1768 was tentatively identified as epirosmanol with antioxidant capability [83, 84]. Therefore, carnosol, carnosic acid, and epirosmanol were related to the bioactivity of *Nostoc* sp. AARL C008 extract.

Tyrosol and other polyphenols Compound (79), having a precursor ion $[M-H]^-$ m/z at 341.0882, was tentatively characterized as hydroxytyrosol 1-O-glucoside showing antioxidant and anticancer potentials [85, 86]. Other polyphenolics were tentatively characterized as dihydrocaffeic acid 3-sulfate (compound 80, $[M-H]^-$ at *m/z* 261.0027), phloroglucinol (compound 81, $[M-H]^-$ at *m/z* 125.0264), dihydrophloroglucinol (compound 82, $[M-H]^-$ at *m/z* 127.0397), and leucodelphinidin 3-[galactosyl-(1->4)-glucoside (compound 83, $[M-H]^+$ at *m/z* 647.2009). Results supported that phloroglucinol was detected as one of the dominant phenolic compounds from cyanobacterial strains as well as *Nostoc* spp. [48]. Phloroglucinol is the precursor of the well-known phlorotannins present in brown seaweeds that exert diverse biological activities including antioxidant and anticancer [14, 87, 88]. Therefore, the presence of hydroxytyrosol 1-O-glucoside and phloroglucinol in *Nostoc* sp. AARL C008 extract was related to its antioxidant and anticancer activities.

Screening and characterization of polyphenolic compounds showed that some of the polyphenols present in *Nostoc* sp. AARL C008 had antioxidant and anticancer potentials (Table 3). The presence of these antioxidants and anticancer compounds indicated that *Nostoc* sp. AARL C008 could be used as a bioactive phytochemical with potential applications in the nutraceutical, cosmetic, and pharmaceutical industries. These results supported a previous study where phytochemicals of *Nostoc* spp. showed antifibrotic activity and decreased lipid peroxidation with improvement of liver fibrosis and splanchnic hemodynamics without any noxious systemic hemodynamic effects [89]. Shahidi and Ambigaipalan [90] suggested that antioxidative phytochemicals played a role in rancidity development by deferring the formation of toxic oxidation products. Moreover, food supplements with high phytochemicals have potential health benefits due to their bioactivities. Methanolic *Nostoc* sp. AARL C008 extract played an important role in bioactivities. In addition, the complete profile of polar and non-polar phytochemicals may support the overview of bioactive substances in this extract. The list of compounds in the water extract was shown in Supplementary Materials. However, they are other compounds that were still unidentified and may contribute to antioxidant and anticancer properties. Hence, further studies on the full characterization of the extracts are required to expand current understanding of the complicated mechanisms of antioxidant and anticancer activities.

4 Conclusions

Cyanobacterium *Nostoc* sp. AARL C008 extract provided antioxidant capacity based on ABTS, DPPH, and PFRAP assays. Interestingly, *Nostoc* sp. AARL C008 extract also showed cytotoxicity against malignant melanoma cells (A375 skin cancer cells), correlating with a high level of total phenolic contents. Various phytochemical profiles with desirable properties of *Nostoc* sp. AARL C008 were rapidly and successfully identified using the LC-ESI-QTOF-MS/MS approach. Among the phytochemicals, *p*-coumaric acid was determined as the dominant phenolic compound, indicating potential positive pharmacological effects. Results demonstrated that the methanolic extract of *Nostoc* sp. AARL C008 biomass showed potential as a possible future alternative bioactive phytochemical, with potential applications in the nutraceutical, cosmetic, and pharmaceutical industries.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Pereira AG, Otero P, Echave J, Carreira-Casais A, Chamorro F, Collazo N, Jaboui A, Lourenço-Lopes C, Simal-Gandara J, Prieto MA (2021) Xanthophylls from the sea: algae as source of bioactive carotenoids. Mar Drugs 19:188. https://doi.org/10.3390/md19040188
2. Van Vliet S, Provenza FD, Kronberg SL (2021) Health-promoting phytonutrients are higher in grass-fed meat and milk. Front Sustain Food Syst 4:555426. https://doi.org/10.3389/fsufs.2020.555426

3. Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H (2018) Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Front Pharmacol 9:1162. https://doi.org/10.3389/fphar.2018.01162
4. Lauritano C, Andersen JH, Hansen E, Albrigtsen M, Escalera L, Esposito F, Helland K, Hanssen KØ, Romano G, Ianora A (2016) Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. Front Mar Sci 3:68. https://doi.org/10.3389/fmars.2016.00068
5. Minatel IO, Borges CV, Ferreira MI, Gomez HA, Chen CY, Lima GP (2017) Phenolic compounds: functional properties, impact of processing and bioavailability. Phenolic Compd Biol Act 8:1–24. https://doi.org/10.5772/66368
6. Safafar H, Van Wagenen J, Møller P, Jacobsen C (2015) Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. Mar Drugs 13:7339–7356. https://doi.org/10.3390/md13127069
7. Jerez-Martel I, García-Poza S, Rodríguez-Martel G, Rico M, Afonso-Olivares C, Gómez-Pinchetti JL (2017) Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. J Food Qual 2017:2924508. https://doi.org/10.1155/2017/2924508
8. Singh DP, Prabha R, Verma S, Meena KK, Yandigeri M (2017) Antioxidant properties and polyphenolic content in terrestrial cyanobacteria. 3 Biotech 7:1–14. https://doi.org/10.1007/s13205-017-0786-6
9. Besednova NN, Andryukov BG, Zaporozhets TS, Kryzhanovsky SP, Kuznetsova TA, Fedyanina LN, Makarenkova ID, Zvyagintseva TN (2020) Algae polyphenolic compounds and modern antibacterial strategies: current achievements and immediate prospects. Biomedicines 8:342. https://doi.org/10.3390/biomedicines8090342
10. Monteiro M, Santos RA, Iglesias P, Couto A, Serra CR, Gouvinhas I, Barros A, Oliva-Teles A, Enes P, Díaz-Rosales P (2020) Effect of extraction method and solvent system on the phenolic content and antioxidant activity of selected macro-and microalgae extracts. J Appl Phycol 32:349–362. https://doi.org/10.1007/s10811-019-01927-1
11. Chigayo K, Mojapelo PE, Mnyakeni-Moleele S, Misihairabgwi JM (2016) Phytochemical and antioxidant properties of different solvent extracts of Kirkia wilmsii tubers. Asian Pac J Trop Biomed 6:1037–1043. https://doi.org/10.1016/j.apjtb.2016.10.004
12. Chandralega G, Ramadas V (2020) Screening of phytochemicals, fatty acid composition and in-vitro analysis of antioxidant property of green edible seaweed *Caulerpa lentillifera* (family: caulerpaceae). Int J Pharm Sci Res 11:1495–1505. https://doi.org/10.13040/IJPSR.0975-8232
13. Van Weelden G, Bobiński M, Okła K, Van Weelden WJ, Romano A, Pijnenborg J (2019) Fucoidan structure and activity in relation to anti-cancer mechanisms. Mar Drugs 17:32. https://doi.org/10.3390/md17010032
14. Zhong B, Robinson NA, Warner RD, Barrow CJ, Dunshea FR, Suleria HA (2020) LC-ESI-QTOF-MS/MS characterization of seaweed phenolics and their antioxidant potential. Mar Drugs 18:331. https://doi.org/10.3390/md18060331
15. Wali AF, Al Dhaheri Y, Ramakrishna Pillai J, Mushtaq A, Rao PG, Rabbani SA, Firdous A, Elshikh MS, Farraj DAA (2020) LC-MS phytochemical screening, *in vitro* antioxidant, antimicrobial and anticancer activity of microalgae *Nannochloropsis oculata* extract. Separations 7:54. https://doi.org/10.3390/separations7040054
16. Haoujar I, Cacciola F, Abrini J, Mangraviti D, Giuffrida D, Oulad El Majdoub Y, Kounnoun A, Miceli N, Fernanda Taviano M, Mondello L, Rigano F (2019) The contribution of carotenoids, phenolic compounds, and flavonoids to the antioxidative properties of marine microalgae isolated from Mediterranean Morocco. Molecules 24:4037. https://doi.org/10.3390/molecules24224037
17. Rastogi RP, Incharoensakdi A, Madamwar D (2014) Responses of a rice-field cyanobacterium *Anabaena siamensis* TISTR-8012 upon exposure to PAR and UV radiation. J Plant Physiol 171:1545–1553. https://doi.org/10.1016/j.jplph.2014.07.011
18. Wintermans JFGM, De Mots AS (1965) Spectrophotometric characteristics of chlorophylls *a* and *b* and their phenophytins in ethanol. Biochimica *et* Biophysica Acta (BBA)-Biophysics including Photosynthesis 109:448–453. https://doi.org/10.1016/0926-6585(65)90170-6
19. Saijo Y (1975) A method for determination of chlorophyll. Japanese J Limnology 36:103–109
20. de Quirós ARB, Costa HS (2006) Analysis of carotenoids in vegetable and plasma samples: a review. J Food Compos Anal 19:97–111. https://doi.org/10.1016/j.jfca.2005.04.004
21. Chandler SF, Dodds JH (1983) The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of *Solanum laciniatum*. Plant Cell Rep 2:205–208. https://doi.org/10.1007/bf00270105
22. Khan MS, Yusufzai SK, Rafatullah M, Sarjadi MS, Razlan M (2018) Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of *Licuala Spinosa* leaves from sabah, Malaysia. ASM Sci J 11:53–58
23. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N (2019) Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants 8:96. https://doi.org/10.3390/plants8040096
24. Bunea A, Rugina OD, Pintea AM, Sconţa Z, Bunea CI, Socaciu C (2011) Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 39:70–76. https://doi.org/10.15835/nbha3926265
25. Sampath M, Vasanthi M (2013) Isolation, structural elucidation of flavonoids from *Polyalthia longifolia* (Sonn.) Thawaites and evaluation of antibacterial, antioxidant and anticancer potential. Int J Pharm Pharm Sci 5:336–341
26. Bhadoriya U, Sharma P, Solanki SS (2012) *In vitro* free radical scavenging activity of gallic acid isolated from *caesalpinia decapetala* wood. Asian Pac J Trop Dis 2:S833–S836. https://doi.org/10.1016/S2222-1808(12)60274-6
27. Zhou J, Diao X, Wang T, Chen G, Lin Q, Yang X, Xu J (2018) Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species *Rhizophora stylosa* and *R. mucronata* in the South China Sea. PloS one. 13:e0197359. https://doi.org/10.1371/journal.pone.0197359
28. Oyaizu M (1986) Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet 44:307–315. https://doi.org/10.5264/eiyogakuzashi.44.307
29. Mau JL, Lai EY, Wang NP, Chen CC, Chang CH, Chyau CC (2003) Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. Food Chem 82:583–591. https://doi.org/10.1016/j.jfca.2005.04.004
30. Benslama A, Harrar A (2016) Free radicals scavenging activity and reducing power of two Algerian Sahara medicinal plants extracts. Int J Herb Med 4:158–161. https://doi.org/10.22271/flora.2016.v4.i6c.03
31. Umthong S, Phuwapraisirisan P, Puthong S, Chanchao C (2011) *In vitro* antiproliferative activity of partially purified *Trigona laeviceps* propolis from Thailand on human cancer cell lines. BMC Complement Altern Med 11:1–8. https://doi.org/10.1186/1472-6882-11-37

32. Felhi S, Daoud A, Hajlaoui H, Mnafgui K, Gharsallah N, Kadri A (2017) Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. Food Sci Technol 37:483–492. https://doi.org/10.1590/1678-457X.23516
33. Xu Y, Harvey PJ (2019) Carotenoid production by *Dunaliella salina* under red light. Antioxidants 8:123. https://doi.org/10.3390/antiox8050123
34. Tavares RS, Kawakami CM, Pereira KD, do Amaral GT, Benevenuto CG, Maria-Engler SS, Colepicolo P, Debonsi HM, Gaspar LR (2020) Fucoxanthin for topical administration, a phototoxic vs. photoprotective potential in a tiered strategy assessed by In vitro methods. Antioxidants 9:328. https://doi.org/10.3390/antiox9040328
35. Kula-Maximenko M, Zieliński KJ, Ślesak I. The role of selected wavelengths of light in the activity of photosystem II in *Gloeobacter violaceus*. Int. J. Mol. Sci 22:4021. https://doi.org/10.3390/ijms22084021
36. Yacobi YZ, Köhler J, Leunert F, Gitelson A (2015) Phycocyanin-specific absorption coefficient: eliminating the effect of chlorophylls absorption. Limnol Oceanogr-Meth 13:157–168. https://doi.org/10.1002/lom3.10015
37. Simeonov A, Michaelian K (2017) Properties of cyanobacterial UV-absorbing pigments suggest their evolution was driven by optimizing photon dissipation rather than photoprotection. arXiv Prepr. arXiv:1702.03588
38. Vega J, Schneider G, Moreira BR, Herrera C, Bonomi-Barufi J, Figueroa FL (2021) Mycosporine-like amino acids from red macroalgae: UV-photoprotectors with potential cosmeceutical applications. Appl Sci 11:5112. https://doi.org/10.3390/app11115112
39. Abdo SM, Ahmed E, El-Enin SA, El Din RS, El Diwani G, Ali G (2013) Growth rate and fatty acids profile of 19 microalgal strains isolated from river Nile for biodiesel production. J Algal Biomass Util 4:51–59
40. Christaki E, Florou-Paneri P, Bonos E (2011) Microalgae: a novel ingredient in nutrition. Int J Food Sci Nutr 62:794–799. https://doi.org/10.3109/09637486.2011.582460
41. Babadi FE, Boonnoun P, Nootong K, Powtongsook S, Goto M, Shotipruk A (2020) Identification of carotenoids and chlorophylls from green algae Chlorococcum humicola and extraction by liquefied dimethyl ether. Food Bioprod Process 123:296–303. https://doi.org/10.1016/j.fbp.2020.07.008
42. Singh DP, Khattar JS, Rajput A, Chaudhary R, Singh R (2019) High production of carotenoids by the green microalga *Asterarcys quadricellulare* PUMCC 5.1.1 under optimized culture conditions. PloS one 14:e0221930. https://doi.org/10.1371/journal.pone.0221930
43. El-fayoumy EA, Shanab SM, Shalaby EA (2020) Metabolomics and biological activities of *Chlorella vulgaris* grown under modified growth medium (BG11) composition. CMU J Nat Sci 19:91–123. https://doi.org/10.12982/CMUJNS.2020.0007
44. Ruiz-Domínguez MC, Espinosa C, Paredes A, Palma J, Jaime C, Vílchez C, Cerezal P (2019) Determining the potential of *Haematococcus pluvialis* oleoresin as a rich source of antioxidants. Molecules 24:4073. https://doi.org/10.3390/molecules24224073
45. Stramarkou M, Papadaki S, Kyriakopoulou K, Tzovenis I, Chronis M, Krokida M (2021) Comparative analysis of different drying techniques based on the qualitative characteristics of *Spirulina platensis* biomass. J Aquat Food Prod Technol 30:498–516. https://doi.org/10.1080/10498850.2021.1900969
46. El-Fayoumy EA, Shanab SM, Hassan OM, Shalaby EA (2021) Enhancement of active ingredients and biological activities of *Nostoc linckia* biomass cultivated under modified BG-110 medium composition. Biomass Convers Biorefin 1–18. https://doi.org/10.1007/s13399-021-01509-7
47. Rastogi RP, Sonani RR, Madamwar D, Incharoensakdi A (2016) Characterization and antioxidant functions of mycosporine-like amino acids in the cyanobacterium *Nostoc* sp. R76DM. Algal Res 16:110–118. https://doi.org/10.1016/j.algal.2016.03.009
48. Ijaz S, Hasnain S (2016) Antioxidant potential of indigenous cyanobacterial strains in relation with their phenolic and flavonoid contents. Nat Prod Res 30:1297–1300. https://doi.org/10.1080/14786419.2015.1053088
49. Assunção MF, Amaral R, Martins CB, Ferreira JD, Ressurreição S, Santos SD, Varejão JM, Santos LM (2017) Screening microalgae as potential sources of antioxidants. J Appl Phycol 29:865–877. https://doi.org/10.1007/s10811-016-0980-7
50. Sivaramakrishnan T, Swain S, Saravanan KRKS, Sankar K, Roy SD, Biswas L (2017) *In vitro* antioxidant and free radical scavenging activity and chemometric approach to reveal their variability in green macroalgae from south Andaman Coast of India. Turkish J Fish Aquat Sci 17:639–648. https://doi.org/10.4194/1303-2712-v17_3_20
51. Wojtunik-Kulesza KA (2020) Approach to optimization of FRAP methodology for studies based on selected monoterpenes. Molecules 25:5267. https://doi.org/10.3390/molecules25225267
52. Bahari AN, Saari N, Salim N, Ashar SE (2020) Response factorial design analysis on papain-generated hydrolysates from *Actinopyga lecanora* for determination of antioxidant and antityrosinase activities. Molecules 25:2663. https://doi.org/10.3390/molecules25112663
53. Vardhan PV, Shukla LI (2017) Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. Int J Radiat Biol 93:967–979. https://doi.org/10.1080/09553002.2017.1344788
54. Yu M, Gouvinhas I, Rocha J, Barros AI (2021) Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Sci Rep 11:10041. https://doi.org/10.1038/s41598-021-89437-4
55. Reyna-Martinez R, Gomez-Flores R, López-Chuken U, Quintanilla-Licea R, Caballero-Hernandez D, Rodríguez-Padilla C, Beltrán-Rocha JC, Tamez-Guerra P (2018) Antitumor activity of *Chlorella sorokiniana* and *Scenedesmus* sp. microalgae native of Nuevo León State, México. PeerJ 6:e4358. https://doi.org/10.7717/peerj.4358
56. Nazir Y, Saeed A, Rafiq M, Afzal S, Ali A, Latif M, Zuegg J, Hussein WM, Fercher C, Barnard RT, Cooper MA (2020) Hydroxyl substituted benzoic acid/cinnamic acid derivatives: tyrosinase inhibitory kinetics, anti-melanogenic activity and molecular docking studies. Bioorg Med Chem Lett 30:126722. https://doi.org/10.1016/j.bmcl.2019.126722
57. Kotake-Nara E, Kushiro M, Zhang H, Sugawara T, Miyashita K, Nagao A (2001) Carotenoids affect proliferation of human prostate cancer cells. J Nutr 131:3303–3306. https://doi.org/10.1093/jn/131.12.3303
58. Bechelli J, Coppage M, Rosell K, Liesveld J (2011) Cytotoxicity of algae extracts on normal and malignant cells. Leuk Res treatment 2011:373519. https://doi.org/10.4061/2011/373519
59. Moo-Puc R, Robledo D, Freile-Pelegrin Y (2011) Improved antitumoral activity of extracts derived from cultured *Penicillus dumetosus*. Trop J Pharm Res 10:177–185. https://doi.org/10.4314/tjpr.v10i2.66561
60. Trabelsi L, Chaieb O, Mnari A, Abid-Essafi S, Aleya L (2016) Partial characterization and antioxidant and antiproliferative activities of the aqueous extracellular polysaccharides from the thermophilic microalgae *Graesiella* sp. BMC Complement Altern Med 16:1–10. https://doi.org/10.1186/s12906-016-1198-6
61. Iqbal Y, Ponnampalam EN, Suleria HA, Cottrell JJ, Dunshea FR (2021) LC-ESI/QTOF-MS profiling of chicory and lucerne polyphenols and their antioxidant activities. Antioxidants 10:932. https://doi.org/10.3390/antiox10060932
62. Farasat M, Khavari-Nejad RA, Nabavi SMB, Namjooyan F (2014) Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. Iran J Pharm Sci 13:163–170

63. Agregán R, Munekata PE, Franco D, Carballo J, Barba FJ, Lorenzo JM (2018) Antioxidant potential of extracts obtained from macro-(*Ascophyllum nodosum, Fucus vesiculosus* and *Bifurcaria bifurcata*) and micro-algae (*Chlorella vulgaris* and *Spirulina platensis*) assisted by ultrasound. Medicines 5:33. https://doi.org/10.3390/medicines5020033
64. Babić O, Kovač D, Rašeta M, Šibul F, Svirčev Z, Simeunović J (2016) Evaluation of antioxidant activity and phenolic profile of filamentous terrestrial cyanobacterial strains isolated from forest ecosystem. J Appl Phycol 28:2333–2342. https://doi.org/10.1007/s10811-015-0773-4
65. Rajauria G (2018) Optimization and validation of reverse phase HPLC method for qualitative and quantitative assessment of polyphenols in seaweed. J Pharm Biomed Anal 148:230–237. https://doi.org/10.1016/j.jpba.2017.10.002
66. Kim YJ (2007) Antimelanogenic and antioxidant properties of gallic acid. Biol Pharm Bull 30:1052–1055. https://doi.org/10.1248/bpb.30.1052
67. Chia YC, Rajbanshi R, Calhoun C, Chiu RH (2010) Anti-neoplastic effects of gallic acid, a major component of *Toona sinensis* leaf extract, on oral squamous carcinoma cells. Molecules 15:8377–8389. https://doi.org/10.3390/molecules15118377
68. Lo C, Lai TY, Yang JH, Yang JS, Ma YS, Weng SW, Chen YY, Lin JG, Chung JG (2010) Gallic acid induces apoptosis in A375. S2 human melanoma cells through caspase-dependent and-independent pathways. Int J Oncol 37:377–385. https://doi.org/10.3892/ijo_00000686
69. Ferreira PS, Victorelli FD, Fonseca-Santos B, Chorilli M (2019) A review of analytical methods for *p*-coumaric acid in plant-based products, beverages, and biological matrices. Crit Rev Anal Chem 49:21–31. https://doi.org/10.1080/10408347.2018.1459173
70. Kong CS, Jeong CH, Choi JS, Kim KJ, Jeong JW (2013) Antiangiogenic effects of *p*-coumaric acid in human endothelial cells. Phytother Res 27:317–323. https://doi.org/10.1002/ptr.4718
71. Raneva V, Shimasaki H, Ishida Y, Ueta N, Niki E (2001) Antioxidative activity of 3, 4-dihydroxyphenylacetic acid and caffeic acid in rat plasma. Lipids 36:1111–1116. https://doi.org/10.1007/s11745-001-0821-6
72. Marset-Castro A, López-Gallardo Á, López-Muñoz H, Sánchez-Sánchez L, Maya I, López Ó, Fernández-Bolaños JG (2018) Phenolic peptides as antioxidant and anti-proliferative agents. Journal of Molecular and Clinical Medicine 1:237–248. https://doi.org/10.31083/j.jmcm.2018.04.502
73. Huang WY, Zhang HC, Liu WX, Li CY (2012) Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. J Zhejiang Univ Sci B 13:94–102. https://doi.org/10.1631/jzus.B1100137
74. Huang W, Zhu Y, Li C, Sui Z, Min W (2016) Effect of blueberry anthocyanins malvidin and glycosides on the antioxidant properties in endothelial cells. Oxid Med Cell Longev 2016:1591803. https://doi.org/10.1155/2016/1591803
75. Huang W, Yan Z, Li D, Ma Y, Zhou J, Sui Z (2018) Antioxidant and anti-inflammatory effects of blueberry anthocyanins on high glucose-induced human retinal capillary endothelial cells. Oxid Med Cell Longev 2018:1862462. https://doi.org/10.1155/2018/1862462
76. Wang Y, Lin J, Tian J, Si X, Jiao X, Zhang W, Gong E, Li B (2018) Blueberry malvidin-3-galactoside suppresses hepatocellular carcinoma by regulating apoptosis, proliferation, and metastasis pathways *in vivo* and *in vitro*. J Agric Food Chem 67:625–636. https://doi.org/10.1021/acs.jafc.8b06209
77. Hashimoto F, Ono M, Masuoka C, Ito Y, Sakata Y, Shimizu K, Nonaka GI, Nishioka I, Nohara T (2003) Evaluation of the antioxidative effect (*in vitro*) of tea polyphenols. Biosci Biotechnol Biochem 67:396–401. https://doi.org/10.1271/bbb.67.396
78. Khan F, Niaz K, Maqbool F, Ismail Hassan F, Abdollahi M, Nagulapalli Venkata KC, Nabavi SM, Bishayee A (2016) Molecular targets underlying the anticancer effects of quercetin: an update. Nutrients 8:529. https://doi.org/10.3390/nu8090529
79. Osawa K, Yasuda H, Maruyama T, Morita H, Takeya K, Itokawa H (1992) Isoflavanones from the heartwood of *Swartzia polyphylla* and their antibacterial activity against cariogenic bacteria. Chem Pharm Bull 40:2970–2974. https://doi.org/10.1248/cpb.40.2970
80. Unno T, Tamemoto K, Yayabe F, Kakuda T (2003) Urinary excretion of 5-(3 ‘, 4 ‘-dihydroxyphenyl)-γ-valerolactone, a ring-fission metabolite of (−)-epicatechin, in rats and its *in vitro* antioxidant activity. J Agric Food Chem 51:6893–6898. https://doi.org/10.1021/jf034578e
81. Loussouarn M, Krieger-Liszkay A, Svilar L, Bily A, Birtić S, Havaux M (2017) Carnosic acid and carnosol, two major antioxidants of rosemary, act through different mechanisms. Plant Physiol 175:1381–1394. https://doi.org/10.1104/pp.17.01183
82. Salem MA, Radwan RA, Mostafa ES, Alseekh S, Fernie AR, Ezzat SM (2020) Using an UPLC/MS-based untargeted metabolomics approach for assessing the antioxidant capacity and anti-aging potential of selected herbs. RSC Adv 10:31511–31524. https://doi.org/10.1039/d0ra06047j
83. Santos-Gomes PC, Seabra RM, Andrade PB, Fernandes-Ferreira M (2003) Determination of phenolic antioxidant compounds produced by calli and cell suspensions of sage (*Salvia officinalis* L.). J Plant Physiol 160:1025–1032. https://doi.org/10.1078/0176-1617-00831
84. Zeng HH, Tu PF, Zhou K, Wang H, Wang BH, Lu JF (2001) Antioxidant properties of phenolic diterpenes from *Rosmarinus officinalis*. Acta Pharmacol Sin 22:1094–1098
85. Vinha AF, Ferreres F, Silva BM, Valentao P, Gonçalves A, Pereira JA, Oliveira MB, Seabra RM, Andrade PB (2005) Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): influences of cultivar and geographical origin. Food Chem 89:561–568. https://doi.org/10.1016/j.foodchem.2004.03.012
86. Nagao T, Abe F, Okabe H (2001) Antiproliferative constituents in the plants 7. Leaves of *Clerodendron bungei* and leaves and bark of *C. trichotomum*. Biol Pharm Bull 24:1338–1341. https://doi.org/10.1248/bpb.24.1338
87. Quéguineur B, Goya L, Ramos S, Martín MA, Mateos R, Bravo L (2012) Phloroglucinol: antioxidant properties and effects on cellular oxidative markers in human HepG2 cell line. Food Chem Toxicol 50:2886–2893. https://doi.org/10.1016/j.fct.2012.05.026
88. Lopes-Costa E, Abreu M, Gargiulo D, Rocha E, Ramos AA (2017) Anticancer effects of seaweed compounds fucoxanthin and phloroglucinol, alone and in combination with 5-fluorouracil in colon cells. J Toxicol Environ Health Part A 80:776–787. https://doi.org/10.1080/15287394.2017.1357297
89. Moal F, Veal N, Vuillemin E, Barrière E, Wang J, Fizanne L, Oberti F, Douay O, Gallois Y, Bonnefont-Rousselot D, Rousselet MC (2006) Hemodynamic and antifibrotic effects of a selective liver nitric oxide donor V-PYRRO/NO in bile duct ligated rats. World J Gastroenterol 12:6639. https://doi.org/10.3748/wjg.v12.i41.6639
90. Shahidi F, Ambigaipalan P (2015) Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects–a review. J Funct Foods 18:820–897. https://doi.org/10.1016/j.jff.2015.06.018