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Rapid Screening for Mycosporine-like Amino Acids (MAAs) of Irish Marine Cyanobacteria and Their Antioxidant Potential

Norma Browne [1], Paz Otero [2], Patrick Murray [1] and Sushanta Kumar Saha [1,*]

[1] Shannon Applied Biotechnology Centre, Technological University of the Shannon: Midlands Midwest, Moylish Park, V94 E8YF Limerick, Ireland
[2] Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, Universidad de Vigo, E32004 Ourense, Spain
* Correspondence: sushanta.saha@lit.ie; Tel.: +353-61-293536

Abstract: The present study evaluated 53 Irish marine cyanobacteria intending to identify potential producers of mycosporine-like amino acids (MAAs) to meet the increasing demand for replacing chemical sunscreen with bio-sunscreen. The biodiscovery analysis using absorption spectra of methanolic extracts identified eight cyanobacteria as potential MAAs producers with a specific content of 0.114–0.511 A* mg DW^{-1}. *Leptolyngbya tenuis* SABC010201 was found to possess notably higher MAAs content. LC-MS analysis identified a total of eight different types of known MAAs (mycosporine-glutamicol, mycosporine-glutaminol-glucoside, mycosporine-serinol, mycosporine-taurine, palythine, palythine-threonine-sulphate, porphyra-334, and usujirene) in eight cyanobacteria, while four compounds were considered unknown UV-absorbing compounds with specific mass and absorption maximum. For example, two unknown compounds with, respectively, $[M-H]^-$ values of 219.0557 and 289.0730 and lambda max of 314 and 326 nm, were detected in three cyanobacteria *Leptolyngbya tenuis* SABC010201, *Phormidium angustissimum* SABC020801, and *Schizothrix* sp. SABC022401. These two unknown compounds were named M-314 and M-326, respectively. Antioxidant activities of total MAAs of all cyanobacteria showed considerable amounts of DPPH, FRAP, and ORAC activities. Considering the specific MAAs content and antioxidant activities, *Leptolyngbya africana* SABC021601 was considered the best producer of MAAs.

Keywords: antioxidant activities; marine cyanobacteria; LC-MS analysis; mycosporine-like amino acids (MAAs); UV-screening compounds

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1. Introduction

Cyanobacteria are Gram-negative, oxygen-evolving, photosynthetic organisms, which have received much attention in recent years as a source of potentially bioactive molecules [1]. These organisms evolved during the Precambrian era, about 3.5 billion years ago [2], when the absence of the stratospheric ozone layer aided in the penetration of harmful UV radiations and made the aquatic environment stressful.

UV-radiation would have various cytotoxic effects on living organisms including UV-induced DNA damage [3,4], fragmentation of cyanobacterial filaments, decline in cellular growth [5,6], decreased alkaline phosphatase activity [7], and damage to photosynthetic machinery, including degradation of the D1 proteins in photosystem II and nitrogenase enzyme activity required for nitrogen fixation [8–11]. Cyanobacteria have developed appropriate defence mechanisms to prevent themselves from the above harmful effects of UV-radiations. One such mechanism is the biosynthesis of UV-screening compounds, mycosporine-like amino acids (MAAs), which absorb UV radiation and dissipate its energy harmlessly into the environment [12–14].

MAAs are secondary metabolites synthesised inherently by cyanobacteria, microalgae, and other organisms, and their levels are enhanced upon exposure to harmful UV radiation [15,16]. They are colourless, water-soluble, usually low molecular weight compounds

(<400 Da) with absorption maxima between 310 nm and 360 nm. Recently, other types of MAAs have been reported with molecular weights of 478 Da, 508 Da, or 612 Da, depending on the types and numbers of sugars associated [17,18]. Another identified MAA has a molecular mass of 1050 Da with two absorption peaks at 312 and 340 nm [17]. The structure of MAAs constitutes a cyclohexenone or cyclohexenimine chromophore conjugated with a nitrogen substituent of an amino acid or its imino alcohol. The differences between MAAs absorption peaks are due to variations in the attached side groups and their nitrogen substituents [19]. Certain MAAs may also contain sulphate esters or glycosidic linkages through the imine substituents. MAAs possess both antiproliferative and antioxidant activities when tested in vitro from several sources [20–22].

MAAs are considered multifunctional secondary metabolites involved in the overall protection of many marine organisms including cyanobacteria. The inherent ability of cyanobacteria to synthesise MAAs makes them the victors in the "survival of the fittest" under various environmental stresses, particularly under high levels of UV-radiation. Therefore, UV-screening MAAs have been investigated from a biotechnological perspective and used in cosmetic applications [23]. However, only wild marine macroalgae [24], and not cyanobacteria, have been explored for biotechnological benefits. In the present investigation, therefore, we evaluated Irish marine cyanobacteria as potential producers of MAAs and tested their antioxidant activities (DPPH, FRAP, and ORAC) from the point of view of future commercial applications. We have also characterised the extracts for the identification of known MAAs and potentially new UV-absorbing compounds.

2. Materials and Methods

2.1. Cyanobacteria Strains and Cultivation Conditions

A total of 53 cyanobacterial isolates were available at the beginning of this study and were sourced from the Shannon ABC biobank. These isolates belong to the genera of *Anabaena* (1 species), *Calothrix* (2 species), *Chlorogloea* (2 species), *Leptolyngbya* (3 species), *Phormidium* (28 species), *Pseudoanabaena* (1 species), *Plectonema* (2 species) *Hyella* (9 species), *Oscillatoria* (3 species), and *Schizothrix* (1 species). Cultures were maintained in environmental growth chambers (EGC M48, USA) under the photosynthetically active radiation (PAR, 400–700 nm) of 42 µmol photons m^{-2} s^{-1} for 16/8 h light/dark cycle at 20 ± 0.1 °C. In preparation for the preliminary biodiscovery screening of UV-screening compounds analysis, cyanobacteria were actively grown in test tubes (in triplicate) containing 10 mL of Artificial Sea Nutrients medium (ASN-III) for 7–10 days under PAR illumination of 85 µmol photons m^{-2} s^{-1} for 16/8 h light/dark cycle at 20 ± 0.1 °C. Each tube was mildly shaken by hand, every day, for homogenous mixing of cells for uniform reception of available lights.

From the preliminary biodiscovery screen, eight candidates were identified as promising MAAs producers and were selected for large-scale cultivation. These candidates were actively grown in 3 L flasks containing 1 L of the sterile medium, in triplicate, for 15 days at 85 µmol photons m^{-2} s^{-1} for 16/8 h light/dark cycle at 20 ± 0.1 °C with shaking at 120 rpm before harvesting. The biomass from the flask was transferred to a pre-weighed, sterile 50 mL falcon tube and centrifuged at 5000 rpm for 4 min. The supernatant was discarded and the remaining biomass was harvested until all cells were collected. The weight of the tube plus the biomass was recorded and stored at −80 °C. The biomass was freeze-dried (Thermo Electron Heto PowerDry LL3000), weighed, and stored at −20 °C until further use.

2.2. Biodiscovery Screening of Cyanobacteria for MAAs Content

After 10 days of active growth in tubes, cyanobacteria were screened for MAAs content following the method described earlier [25]. Briefly, 50 mg of fresh weight biomass was taken after harvesting by centrifugation at 10,000 rpm for 2 min and re-suspended in 500 µL of 20% (vol/vol) aqueous methanol and left at 4 °C overnight to ensure improved extractability. Thereafter, biomass samples were incubated in a 45 °C water bath for 2.5 h and centrifuged at 5000 rpm for 2 min. We transferred 100 µL of the supernatant to a 96-well

plate and the absorbance spectrum was recorded from 300–700 nm with 2 nm intervals in a plate reader (BioTek Synergy 4). In parallel, the above process of absorption spectrum recording was repeated but started with 90% (vol/vol) aqueous methanol extraction, for confirmation of MAAs content [26]. Cyanobacterial isolates with promising MAAs absorption peaks (between 310–360 nm) found in supernatants of both 20% and 90% methanolic extracts were chosen for further experimentation.

2.3. Optimisation of MAAs Extraction

To determine the optimum conditions for maximum yield of MAAs, the extraction protocol was trialled on various biomass types; freeze-dried biomass, fresh weight biomass, and overnight dried biomass (55 °C oven). Briefly, 100 mg biomass (*Phormidium* sp.) of each of the above conditions were extracted with 10 mL of 20% (vol/vol) aqueous methanol overnight at 4 °C. Later, the samples were incubated in a 45 °C water bath for 2.5 h and the content was at 5000 rpm for 2 min. We transferred100 µL of the supernatant to a 96-well plate and the absorbance spectrum was recorded from 300–700 nm with 2 nm intervals using a plate reader (BioTek Synergy 4). Extracts from freeze-dried biomass with the highest absorbance at 326 nm were chosen as the optimum extraction conditions for MAAs content analysis.

2.4. Large-Scale Extraction of MAAs from Cyanobacteria Candidates

Large-scale extraction of MAAs was carried out using 100 mg of freeze-dried biomass, in triplicates, with 10 mL of 20% (vol/vol) aqueous methanol overnight at 4 °C. The samples were incubated in a 45 °C water bath for 2.5 h and centrifuged at 5000 rpm for 2 min. We transferred 100 µL of the supernatant to a 96-well plate and the absorbance spectrum was recorded from 300–700 nm with 2 nm intervals using a plate reader (BioTek Synergy 4). The same extract was then transferred to a quartz cuvette and the absorbance was read at 260 nm using a spectrophotometer (Thermo Genesys 10 UV-Spectrometer) with 20% methanol as a blank. The remaining supernatant was transferred to a fresh, pre-labelled tube, frozen at −80 °C at a slanted angle, and freeze-dried. The content was reconstituted with 500 µL of ultrapure water and gently mixed to concentrate MAAs content. Then, 5 µL of this homogenous suspension was added to 95 µL of ultrapure water and the absorbance spectrum was recorded as above to evaluate the MAAs yield. The remaining concentrated MAAs were stored at −20 °C for LC-MS identification of MAAs and their antioxidant activity analysis.

2.5. Quantification of Specific MAAs Content

The determination of specific MAAs content was calculated based on corrected absorbance. The absorbance value at 326 nm (minus blank) was used in the expression below [27].

$$\text{Specific MAAs content (A* mg DW}^{-1}) = A_{326\,nm} - 0.2*(A_{260\,nm})$$

Corrections for $A_{260\,nm}$ were made for the equation due to the presence of water-soluble material, not attributed to MAAs, which has previously been calculated by pooled extracts of three species that do not contain MAAs [25].

2.6. LC-MS Identification of Specific MAAs

Freeze-dried extracts after reconstitution with ultrapure water were filtered through a 0.45 µm filter (Millipore Ultrafree-MC centrifugal filter unit) and transferred to amber vials for LC-MS identification of specific MAAs by adopting previous method [28]. We injected 10 µL of the filtrate onto an Agilent C-18 Poroshell 120 column (2.7 µm × 3.0 × 100 mm) to resolve the MAAs profile. The oven temperature of the resolving column was maintained at 30 °C. For negative mode MS analysis, mobile phase A (MP-A) consisted of 2 mM ammonium acetate in water, and 2 mM ammonium acetate in methanol was used as mobile phase B (MP-B). The elution gradients of mobile phases were: (i) 0–20 min, 100% MP-A

and 0% MP-B; (ii) 20–25 min, 0% MP-A and 100% MP-B and (iii) 25–28 min, 100% MP-A and 0% MP-B. The flow rate was adjusted to 0.4 mL min^{-1}. DAD data was recorded at 300, 310, 320, 330, 340, 350, and 360 nm, and absorption spectra (190–700 nm) were recorded every 1.5 s. Then, 100% of the HPLC (Agilent 1260 series) eluent was directed into the electrospray ionisation source of the Q-TOF mass spectrometer (Agilent 6520) that was operated in negative ionisation mode and scanned from 80 to 1200 m/z values. Nitrogen, at a flow rate of 5 L min^{-1} was used as the drying gas at a temperature of 335 °C, and the nebulizer pressure was set to 40 psi. Reference mass ions were constantly flowing to the electrospray source at a rate of approx. 40 µL min^{-1} with accurate masses of 121.050873 and 922.009798.

A database of 22 MAAs compounds was constructed, comprising their relative lambda max, accurate molecular mass, and the $[M-H]^-$ values. LC-MS data were analysed using Agilent Mass Hunter Workstation Qualitative Analysis B.05.00 software. First, the DAD (diode array detector) profile of each sample at 300, 310, 320, 330, 340, 350, and 360 nm was observed to acquire an idea about the MAAs elution pattern. Subsequently, extracted ion chromatogram (EIC) of each known MAAs was obtained from the total ion chromatogram (TIC) by entering the accurate $[M-H]^-$ values. If any EIC peaks were detected, they were then compared with the best DAD profile to match the retention time (RT). Then, the MS spectra profile of interested EIC peaks was obtained to confirm the presence of specific MAAs with accurate mass values.

When no known accurate mass was detected in any MS spectra but showed other predominant mass spectra with characteristic UV peaks (as seen from the DAD profile at specific RT), they were considered unknown UV compounds. We then predicted the chemical formula of the unknown mass spectrum using the same software. Briefly, the MS spectra were copied to "User Spectra". Then, we selected the "interested spectrum peak" and, by right click, selected again "Generate Formulas from Spectrum Peaks", which resulted in a new window with the possible formula for the unknown compound.

2.7. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

The radical scavenging activity of cyanobacterial MAAs extracts against DPPH free radical were assayed based on Blois et al. [29] with minor modifications. A stock solution of 1 mM DPPH reagent was prepared in methanol, stored in the fridge protected from light and air, and used within a week. On the day of analysis, the stock solution was diluted with methanol to obtain 138.88 µM DPPH working solution and used as follows. The reaction mixture contained 30 µL of filtered MAAs extracts or appropriate blank plus 270 µL of 138.88 µM DPPH (final DPPH concentration is 125 µM) that were mixed well and incubated in dark at 30 °C for 30 min. Absorbance was read at 517 nm in a plate reader (BioTek Synergy 4). Trolox at concentrations ranging from 0–125 µM was used to prepare the standard curve (Supplementary Figure S1), and the final values were expressed as µmol TE g^{-1} of dry-weight biomass.

2.8. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay for the determination of the reduction of ferric tripyridyltriazine complex to its ferrous form due to MAAs extracts was carried out according to Benzie and Strain [30]. The FRAP reagent was prepared fresh on the day of the assay by mixing 100 mL of 300 mM sodium acetate buffer (pH 3.6) with 10 mL of 10 mM TPTZ (2,4,6-Tripyridyl-*s*-triazine) and 10 mL of 20 mM $FeCl_3.6H_2O$, which was incubated at 37 °C until required. Thereafter, 280 µL of the FRAP reagent was mixed with 20 µL of the sample, blank (water) or trolox standard in a 96-well microplate, and incubated at 37 °C for 4 min before reading the absorbance at 593 nm. Trolox at concentrations ranging from 0–250 µM was used to prepare the standard curve (Supplementary Figure S2), and the FRAP values were expressed as µmol TE g^{-1} of dry-weight biomass.

2.9. Oxygen Radical Absorbance Capacity (ORAC) Assay

The ORAC assay to determine the protection of fluorescein from free radical oxidation due to MAAs extracts was performed according to Dávalos et al. [31] with minor modifications. The assay was performed on a temperature-controlled, 96-well plate reader. The data collection and analysis were carried out using BioTek Gen5 software. The reaction mixture contained 20 µL of MAAs extracts, standard, or blank (water), and 120 µL of 0.117 µM fluorescein solution (prepared in 75 mM phosphate buffer, pH 7.4) in a black 96-well plate, was pre-incubated at 37 °C for 15 min. Then, the reaction was initiated by adding 60 µL of 40 mM AAPH (2,2′-Azobis(2-amidinopropane) dihydrochloride Sigma) solution, freshly prepared using the above phosphate buffer kept at 37 °C. Then the plate was placed immediately into the reader that was set at 37 °C and the assay was performed at an excitation wavelength of 485 nm and an emission wavelength of 525 nm. Fluorescence was measured every minute for 2 h and the plate was shaken before each measurement. Trolox, a water-soluble analogue of vitamin E, at concentrations ranging from 5–80 µM, was used to prepare the standard curve (Supplementary Figure S3). The antioxidant curve was normalized to standards, and the ORAC-FL values were expressed as µmol TE g^{-1} of dry-weight biomass.

2.10. Statistical Analysis

Data presented for specific MAAs content as well as for antioxidant assays were the averages of triplicates ± standard deviations.

3. Results

3.1. Biodiscovery Screen and Cellular Morphology

A total of 53 different isolates of cyanobacteria from the biobank of Shannon ABC were screened for their MAAs content by analysing the absorption spectra of 20% and 90% methanolic extracts. Out of the 53 samples screened, a distinct peak between 300 to 400 nm indicative of UV-absorbing compounds (MAAs) was detected in 26 isolates. Of these 26 isolates, only eight species, *Anabaena variabilis* SABC011501, *Calothrix contarenii* SABC022701, *Leptolyngbya africana* SABC021601, *Phormidium angustissimum* SABC020801, *Phormidium angustissimum* SABC022612, *Phormidium* sp. SABC022903, *Leptolyngbya tenuis* SABC010201, and *Schizothrix* sp. SABC022401, were found to possess prominent peaks for MAAs with relatively higher absorbance values (Figure 1). These eight isolates (details of geographical location and sampling sites were published earlier [32]) are morphologically distinct from each other in terms of their cell colour, cell size, cell shape, filament morphology, presence or absence of heterocyst, nature of extracellular sheaths, etc. (Supplementary Figure S4), which may be indicative of the biochemical diversity of MAAs.

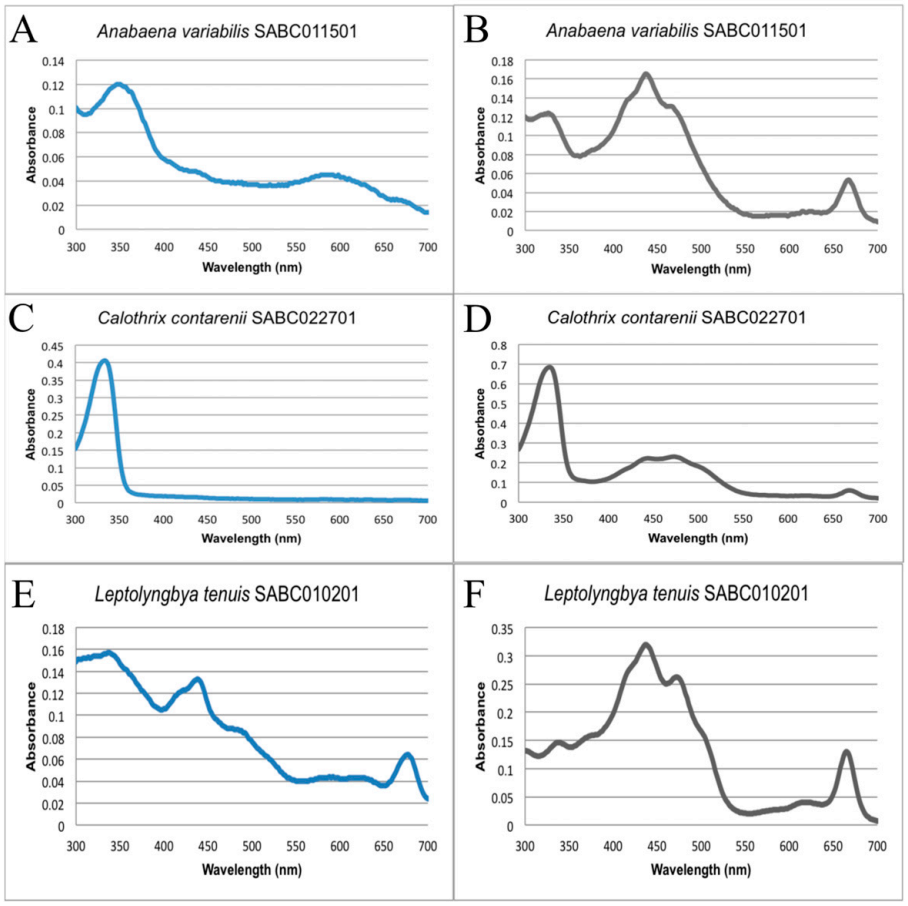

Figure 1. Absorption spectra of 20% (blue) and 90% (black) methanolic extracts of three representative cyanobacterial isolates. (**A**,**B**) *Anabaena variabilis* SABC011501; (**C**,**D**) *Calothrix contarenii* SABC022701; (**E**,**F**) *Leptolyngbya tenuis* SABC010201. Presence of peaks at 310–360 nm indicate the presence of mycosporine-like amino acids (MAAs).

3.2. Determination of Specific MAAs Content

The optimised extraction protocol was used to determine the MAAs content of candidate cyanobacteria and represented as corrected absorbance at 326 nm (Table 1). The aqueous methanol extracts identified various UV absorption peaks, ranging from 318–352 nm. Specific MAAs content varied among the isolates and, based on the A* mg DW^{-1} values, the eight candidate isolates can be categorised into high MAAs content (0.412–0.511, *Calothrix contarenii* SABC022701, *Leptolyngbya africana* SABC021601, and *Leptolyngbya tenuis* SABC010201) and low MAAs content (0.114-0.199, *Anabaena variabilis* SABC011501, *Phormidium angustissimum* SABC020801, *Phormidium angustissimum* SABC022612, *Phormidium* sp. SABC022903, and *Schizothrix* sp. SABC022401) cyanobacteria.

Table 1. Calculated specific MAAs content (A* mg DW^{-1}) at 326 nm of selected cyanobacteria. Data represent the average of triplicates ± SD. GenBank accession numbers of cyanobacteria identified in this study as MAAs producers.

Cyanobacterial Isolates	MAAs Content (A* mg DW^{-1} ± SD)	GenBank Accession Number
Anabaena variabilis SABC011501	0.155 ± 0.009	KX765290
Calothrix contarenii SABC022701	0.445 ± 0.007	KT740998
Leptolyngbya africana SABC021601	0.412 ± 0.006	KT740999

Table 1. *Cont.*

Cyanobacterial Isolates	MAAs Content (A* mg DW^{-1} ± SD)	GenBank Accession Number
Leptolyngbya tenuis SABC010201	0.511 ± 0.014	KX765288
Phormidium angustissimum SABC020801	0.114 ± 0.024	KT740997
Phormidium angustissimum SABC022612	0.156 ± 0.011	KX765287
Phormidium sp. SABC022903	0.123 ± 0.025	KT741000
Schizothrix sp. SABC022401	0.199 ± 0.004	KX765289

3.3. Identification of Specific MAAs by LC-MS

LC-MS analysis identified a total of 12 different types of UV-screening compounds within the tested eight cyanobacteria (Table 2). Of these UV-screening compounds, eight were known MAAs compounds based on their DAD profile, retention times, and accurate $[M-H]^-$ ion values (Figure 2). Mycosporine-taurine was the most common type of MAAs found in eight cyanobacteria followed by Usujirene found in seven cyanobacteria. Mycosporine-glutamicol was found in three *Leptolyngbya* spp. Each mycosporine-glutaminol-glucoside and palythine-threonine-sulphate were found only in two filamentous cyanobacteria. Porphyra-334 was found only in two heterocystous filamentous cyanobacteria (Figure 3). While both mycosporine-serinol and palythine were found only in *Phormidium angustissimum* SABC020801. The remaining four UV-screening compounds were unknown but have a lambda max range of 314–346 nm. An unknown compound with $[M-H]^-$ 289.0730 and a λ max of 326 nm was detected in three cyanobacteria and has been given the trivial name, M-326 (Table 2, Figure 4). Moreover, an unknown compound of $[M-H]^-$ 219.0557 and a λ max of 314 nm was detected in three cyanobacteria (Table 2, Figure 5). Likewise, other unknown compounds have been given the trivial names of M-330 and M-346, depending on their λ max values.

Table 2. Identified MAAs of candidate cyanobacteria with retention time (RT), *m/z* values and λ max. Unknown compounds with RT, detected *m/z* value, λ max, and predicted molecular formulas.

	Phormidium angustissimum SABC020801	*Phormidium angustissimum* SABC022612	*Leptolyngbya africana* SABC021601	*Leptolyngba tenuis* SABC010201	*Phormidium* sp. SABC022903	*Schizothrix* sp. SABC022401	*Anabaena variabilis* SABC011501	*Calothrix contarenii* SABC022701
Mycosporine-serinol	1.198 260.9086 310 nm	-	-	-	-	-	-	-
Mycosporine-taurine	1.186 317.0687 309 nm	1.188 317.0521 309 nm	1.174 317.0504 309 nm	1.170 317.0615 309 nm	1.214 317.0611 309 nm	1.194 317.0607 309 nm	-	1.196 317.0624 309 nm
Usujirene	24.239 283.2665 357 nm	24.229 283.2656 357 nm	24.144 283.2660 357 nm	24.175 283.2675 357 nm	24.172 283.2655 357 nm	24.459 283.2652 357 nm	-	-
Mycosporine-glutamicol	-	-	2.744 302.0658 310 nm	2.645 302.0706 310 nm	-	-	-	-
Mycosporine-glutaminol-glucoside	-	3.159 463.2186 310 nm	-	3.094 463.2215 310 nm	-	-	-	-
Palythine	3.311 243.1741 320 nm	-	-	-	-	-	-	-

Table 2. *Cont.*

	Phormidium angustissimum SABC020801	*Phormidium angustissimum* SABC022612	*Leptolyngbya africana* SABC021601	*Leptolyngba tenuis* SABC010201	*Phormidium* sp. SABC022903	*Schizothrix* sp. SABC022401	*Anabaena variabilis* SABC011501	*Calothrix contarenii* SABC022701
Palythine-threonine-sulphate	-	27.18 367.3812 322 nm	27.13 367.3663 322 nm	-	-	-	-	-
Porphya-334	-	-	-	-	-	-	1.301 345.1368 334 nm	1.196 345.9219 334 nm
UC (M-314)	2.862 219.0557 314 nm $C_6H_{12}N_4O_3S$					2.764 219.0531 314 nm $C_9H_8N_4O_3$		
UC (M-326)				1.347 289.0730 326 nm $C_9H_{15}ClN_6O$		1.43 289.0557 326 nm $C_6H_{15}ClN_4O_7$		
UC (M-330)								19.705 309.2061 330 nm $C_{11}H_{30}N_6O_2S$
UC (M-346)							15.489 321.0005 346 nm $C_{13}H_{10}N_2O_4S$	

Note: Order of information in each box (top to bottom values): retention time in minutes, *m/z* value in dalton, λ max in nanometres, predicted chemical formula. UC, unknown compound.

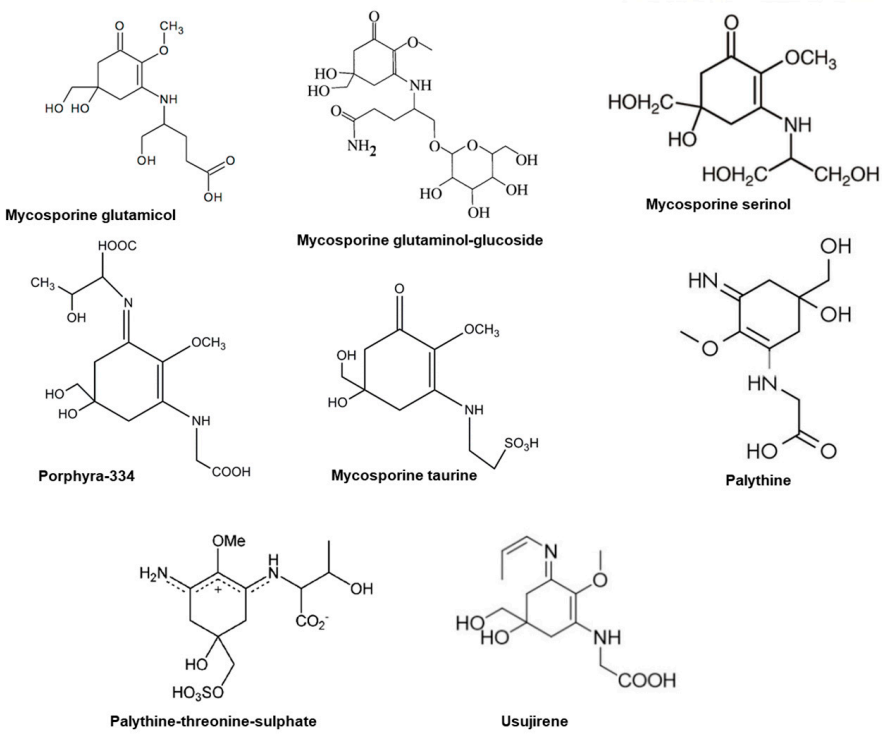

Figure 2. Showing the chemical structures of eight known MAAs identified in eight marine cyanobacteria of Irish habitats.

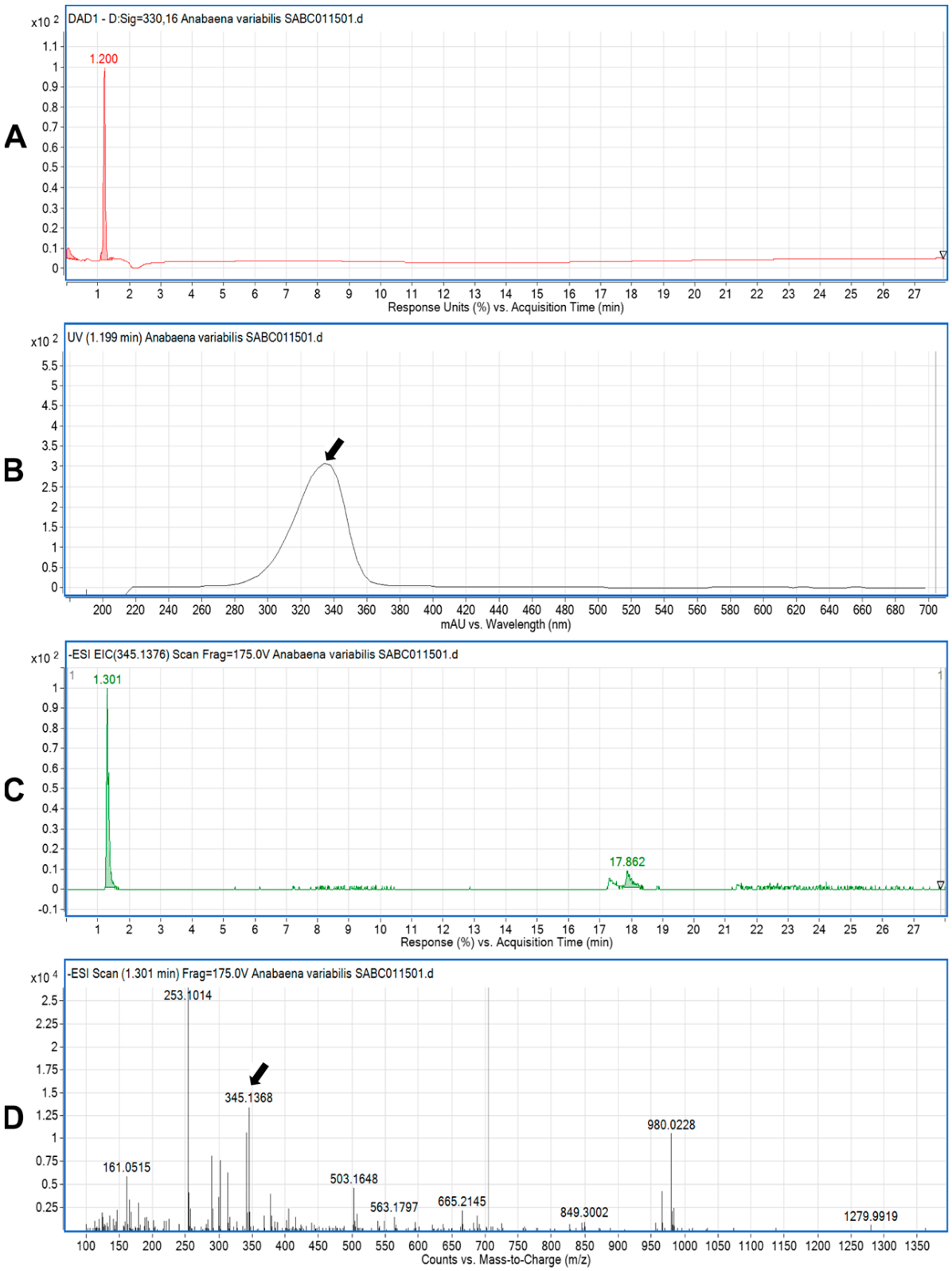

Figure 3. LC-MS analysis showing identification of Porphyra-334 in *Anabaena variabilis* SABC011501. (**A**) DAD profile at 330 nm; (**B**) UV spectra of the DAD profile peak at 1.2 min; (**C**) extracted ions chromatogram of accurate mass for Porphyra-334 in negative mode (345.1376) showing specific peak; and (**D**) ESI scan showing the mass spectra including *m/z* 345.1368 for porphyra-334.

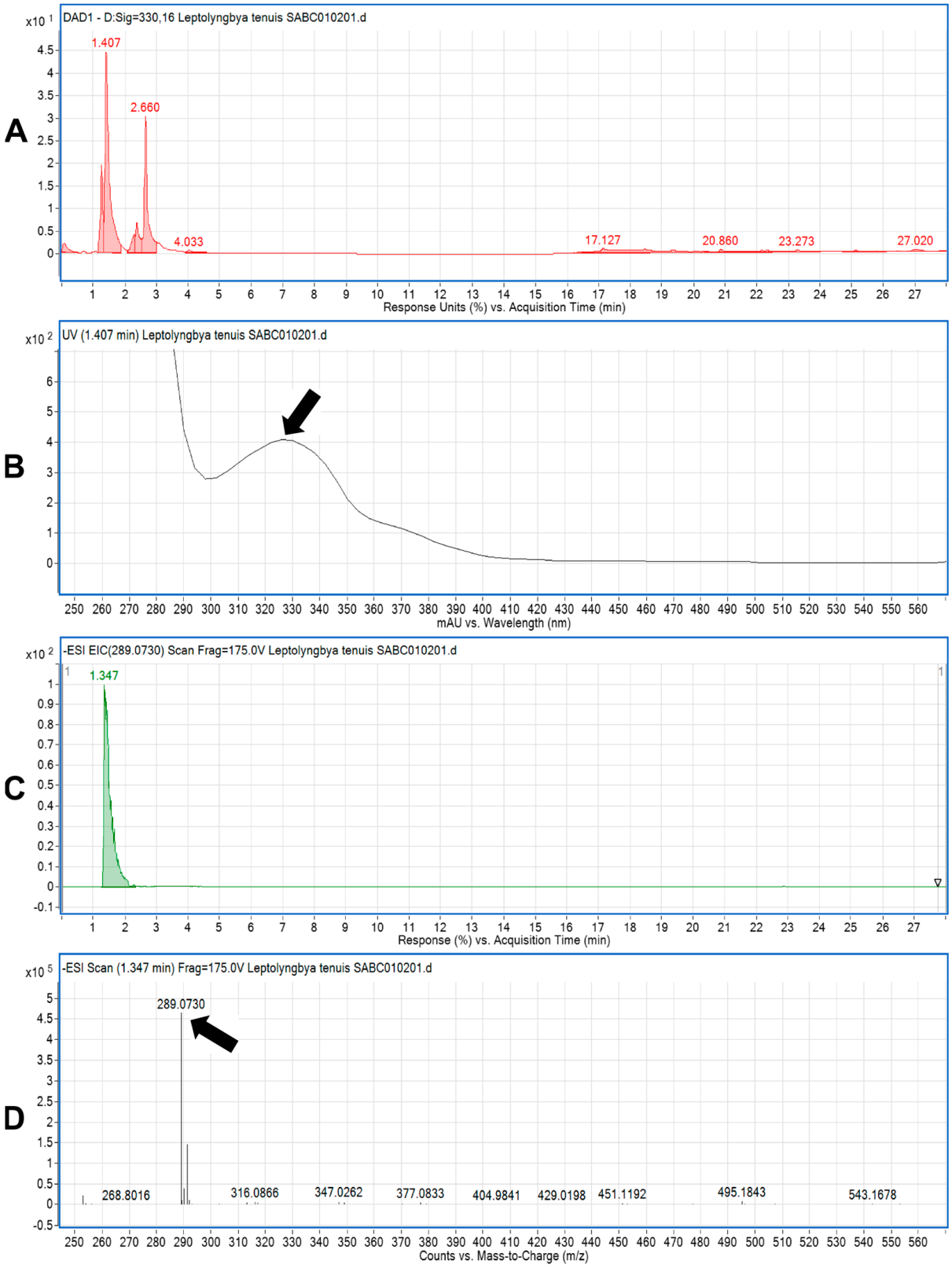

Figure 4. LC-MS analysis showing detection of unknown compound (M-326) in *Leptolyngbya tenuis* SABC010201. (**A**) DAD profile at 330 nm; (**B**) UV spectra of the DAD profile peak at 1.4 min; (**C**) extracted ions chromatogram of mass for unknown compound M-326 in negative mode (289.0730) showing specific peak; and (**D**) ESI scan showing the mass spectra including *m/z* 289.0730 for M-326.

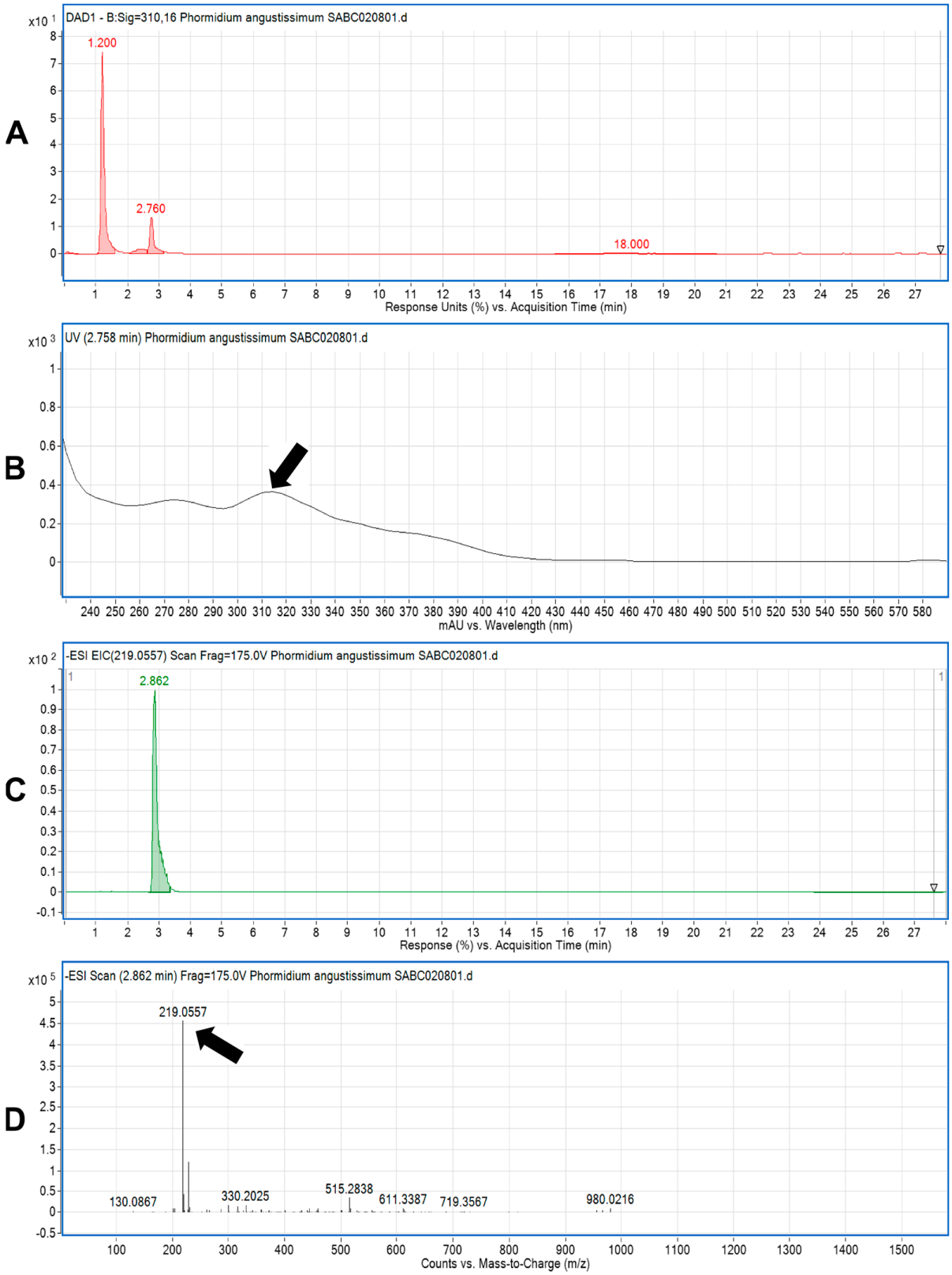

Figure 5. LC-MS analysis showing detection of unknown compound (M-314) in *Phormidium angustissimum* SABC020801. (**A**) DAD profile at 310 nm; (**B**) UV spectra of the DAD profile peak at 2.7 min; (**C**) extracted ions chromatogram of mass for unknown compound M-314 in negative mode (219.0557) showing specific peak; and (**D**) ESI scan showing the mass spectra including *m/z* 219.0557 for M-314.

3.4. Antioxidant Capacity of Total MAAs Content

The antioxidant activities of total MAAs content were assessed for three different mechanisms: (1) DPPH assay for free radical scavenging ability of MAAs by donating a hydrogen atom; (2) FRAP assay for MAAs ability for reduction of an oxidant by donating an electron; and (3) ORAC assay for MAAs ability for protection of fluorescein molecules from free radical oxidation. In each assay, Trolox was used as a reference and the results are shown as µmol Trolox equivalent (TE) g^{-1} dry weight (DW) biomass in Table 3.

Table 3. Calculated antioxidant values of total MAAs content as per DPPH, FRAP, and ORAC assays. Data expressed as averages of triplicates ± SD.

Cyanobacterial Isolates	DPPH (µM TE g DW^{-1})	FRAP (µM TE g DW^{-1})	ORAC (µM TE g DW^{-1})
Anabaena variabilis SABC011501	343.1579 ± 0.0269	325.8333 ± 0.0098	180,405.99 ± 12,615.73
Calothrix contarenii SABC022701	504.5614 ± 0.0190	510.5556 ± 0.0172	111,593.23 ± 381.742
Leptolyngbya africana SABC021601	641.4035 ± 0.0038	603.6111 ± 0.0065	216,537.81 ± 8019.166
Leptolyngbya tenuis SABC010201	388.7719 ± 0.0045	323.0556 ± 0.0136	230,336.89 ± 8985.885
Phormidium angustissimum SABC020801	351.9298 ± 0.0108	410.5556 ± 0.0127	233,694.25 ± 5125.243
Phormidium angustissimum SABC022612	469.4737 ± 0.0026	445.2778 ± 0.0050	107,705.11 ± 554.054
Phormidium sp. SABC022903	764.2105 ± 0.0075	727.2222 ± 0.0015	246,140.97 ± 9370.107
Schizothrix sp. SABC022401	167.7193 ± 0.0030	223.05556 ± 0.0084	241,224.56 ± 8580.119

All MAAs extracts have varied levels of all three tested types of antioxidant activities. DPPH scavenging activity was found in all MAAs samples, with the highest activity recorded in *Phormidium* sp. SABC022903 (764.21 µM TE g^{-1} DW), followed by *Leptolyngbya africana* SABC021601. The lowest DPPH activity was found in *Schizothrix* sp. SABC022401 (167.7 µM TE g^{-1} DW). FRAP assay for the ability to the reduction of ferric-tripyridyltriazine (Ferric III) to ferrous (II) was recorded for all MAAs samples. Like DPPH activity, the highest FRAP activity was recorded in *Phormidium* sp. SABC022903 (727.22 µM TE g^{-1} DW) followed by *Leptolyngbya africana* SABC021601. Moreover, the least FRAP activity was found in *Schizothrix* sp. SABC022401 (223 µM TE g^{-1} DW). ORAC assay for MAA's ability to protect fluorescein from free radical oxidation was found highest in *Phormidium* sp. SABC022903 (246,140.97 µM TE g^{-1} DW) followed by *Schizothrix* sp. SABC022401 (241224.56 µM TE g^{-1} DW), while the least FRAP activity was found in *Phormidium angustissimum* SABC022612 (107705.11 µM TE g^{-1} DW).

4. Discussion

The present study comprises an evaluation of 53 cyanobacterial isolates of Shannon ABC biobank established from collecting samples from the west coast of Ireland. The biodiscovery screen based on absorption spectra with a distinct peak within UV-A/UV-B region (300–400 nm) identified only eight cyanobacteria as potential producers of MAAs with relatively higher absorbance values. These eight candidate cyanobacteria were found morphologically distinct, and were further characterised by molecular marker gene 16S rRNA sequence analysis [32]. In recent years, 16S rRNA gene sequence have been the most highly used genetic marker in determining the identification of cyanobacterial species [33–35]. Both morphological identification and the BLAST analysis of 16S rRNA sequences were comparable, suggesting that the MAAs candidate identified in this study could serve as a base for selecting cyanobacteria based on morphological identification for other MAAs studies.

Of the eight candidates, only *Leptolyngbya tenuis* SABC010201 has the greatest UV-screening potential with the highest specific MAAs content followed by *Calothrix contarenii* SABC022701 (Table 1). Earlier, the specific MAAs content of a *Calothrix* sp. [25] was re-

ported to contain 0.320 A* mg DW^{-1} MAAs, which is much lesser than *Calothrix contarenii* SABC022701 (0.445 A* mg DW^{-1}) of the present study. We used advanced and highly sensitive technologies, such as LC-MS, which utilises known accurate molar masses and retention times for more accurate identification of MAAs in selected cyanobacteria. The analysis revealed *Leptolyngbya tenuis* SABC010201 has one unidentified and four identified MAAs. While *Calothrix contarenii* SABC022701 has only one unidentified and two identified MAAs (Table 2). Known MAAs such as mycosporine-taurine, usujirene, mycosporine-glutaminol-glucoside, and porphyra-334, identified in the above three cyanobacteria, were also found in other cyanobacteria [36–42]. Mycosporine-glutamicol found in all three *Leptolyngbya* spp., mycosporine-serinol found in *Phormidium angustissimum* SABC020801, palythine-threonine-sulphate found in *Phormidium angustissimum* SABC022612 and *Leptolyngbya africana* SABC021601 are new reports for cyanobacterial origin. In a recent study, no MAAs were detected by LC-MS in *Leptolyngbya foveolarum* and *Calothrix* sp., even though the chromatograms had signals with absorption maxima typical for MAAs [43]. They concluded that these could be glycosylated MAAs, as reported earlier in the *Nostoc commune* [17]. Hence, the detection of unknown UV-screening compounds in Irish marine cyanobacteria is of notable interest and illustrates the broad range of MAAs produced by this group of organisms and warrants future investigation.

It is interesting from the biotechnological applications (potential sunscreen with free radical scavenging ability) point of view that all eight cyanobacterial MAAs extracts showed appreciable antioxidant activities as evaluated by three assays (Table 3). This suggested their wide mechanisms of dealing with the oxidation of biomolecules either as free radical scavengers, reductants of an oxidant, or as protectors from free radical oxidation. Surprisingly, although *Leptolyngbya tenuis* SABC010201 possessed notably higher MAAs content, it demonstrated medium radical scavenging activity and reducing antioxidant power in DPPH and FRAP assays. This possibly indicates the types of specific MAAs and other unknown compounds present in the tested extracts. Radical scavenging activity of the MAAs such as shinorine and M-307 (from *Gloeocapsa* sp.), glycosylated MAA (1050-Da from *Nostoc commune*), and total MAAs (palythine, asterina, porphyra, and palythene) from *Nostoc* sp. R76DM has been reported earlier [15,17,44]. FRAP assays for the reducing power of total MAAs (palythine, asterina, porphyra, and palythene) of *Nostoc* sp. R76DM was reported higher compared to the positive control ascorbic acid [44]. While another study with water extracts of four cyanobacteria *Oscillatoria* sp., *Lyngbya* sp., *Microcystis* sp., and *Spirulina* sp. found the highest FRAP and DPPH radical scavenging activities in *Oscillatoria* sp. [45]. ORAC assays have rarely been carried out for cyanobacterial MAAs. However, an investigation with 90% methanolic extracts and defatted water extracts of *Spirulina* powder found higher ORAC values for defatted *Spirulina* extracts [46]. Recently, another study with six MAAs (palythine, asterina-330, shinorine, and palythinol, porphyra-334, and usujirene) present in methanolic extracts of wild-harvested red macroalgae (*Palmaria palmata*, and *Mastocarpus stellatus*) found substantially high ORAC activities [20]. Usujirene was the predominant MAA in both the above macroalgal extracts and was considered responsible for high ORAC activities [20]. Seven out of the eight cyanobacteria tested for antioxidant activities possess usujirene, which might have contributed to their relative ORAC activities.

5. Conclusions

This is the first comprehensive evaluation of UV-screening compounds in Irish marine cyanobacteria, which identified eight cyanobacteria as potential MAAs producers with a specific content of 0.114–0.511 A* mg DW^{-1}. However, *Leptolyngbya africana* SABC021601 can be considered as the best both in terms of specific content and antioxidant activities. To the best of our knowledge, this study also reports for the first time known MAAs, such as mycosporine-glutamicol, mycosporine-serinol, and palythine-threonine-sulphate, from marine cyanobacteria. This study identified a total of eight different types of known MAAs (mycosporine-glutamicol, mycosporine-glutaminol-glucoside, mycosporine-serinol, mycosporine-taurine, palythine, palythine-threonine-sulphate, porphyra-334, and usu-

jirene) and four unknown UV-absorbing compounds, named as M-314, M-326, M-330, and M-346. These unknown MAAs are novel findings and need future studies related to their structural and bio-functional properties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15043792/s1. Figure S1. Standard curve of trolox (μM) plotted against absorbance at 517 nm used for calculating the radical scavenging ability of MAAs extracts; Figure S2. Standard curve of trolox (μM) plotted against absorbance at 593 nm used for calculating the ferric reducing ability of MAAs extracts; Figure S3. Standard curve of trolox (μM) plotted against net area under the curve (Net AUC) for 2 hr used for calculating the ORAC activity of MAAs extracts; Figure S4. Photomicrographs showing morphological variations of candidate eight cyanobacterial isolates for MAAs production.

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