Environ Monit Assess (2020) 192: 244
https://doi.org/10.1007/s10661-020-8207-4

Evaluation of phytotherapeutic activities and phytochemical content of *Phormidium autumnale* Gomont from natural freshwater sources

Dilek Yalcin · Hikmet Türk Katircioğlu · Tülay Özer · Minoo Pourhassan Shamchi · İlkay Acikgoz Erkaya

Received: 10 November 2019 / Accepted: 4 March 2020 / Published online: 20 March 2020

Abstract The information available on microalgae-sourced compounds, especially antibiotics and other bioactive compounds, and their potential commercial applications is still insufficient. In this study, antibacterial activity, metabolites, and molecular characterization of *Phormidium autumnale*, which was isolated from samples collected from different natural freshwater sources in Ankara, Turkey, were investigated. Sequencing results of 16s rDNA confirmed the molecular identification of *P. autumnale* by 99%. It was determined that the peak values of some phenolic compounds and cyclic peptides were consistent with the 1653–1389 cm^{-1} band regions in the FTIR spectra of the species. The antibacterial activities of *P. autumnale* cyanobacteria (CBA) extracts that were obtained by using different solvents were tested on *Escherichia coli*, *Staphylococcus epidermidis*, methicillin-resistant (MR) *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Enterococcus faecalis* by using a disc diffusion method. Also, the minimum inhibition concentration (MIC) and antimicrobial indexes of all extracts were determined. It was found that *P. autumnale* methanol extracts showed antibacterial activity on all test bacteria, whereas acetone extracts showed effects only on *E. coli*. For the inhibition of MR *S. aureus*, the control methanol extract was found to give very similar results to those exhibited by the control antibiotics, and the antimicrobial index results were determined to be 58.7–67.5%. According to the results of the analysis of methanol extract, gentisic acid, vanillic acid, 4-hydroxybenzoic acid, p-coumaric acid, and catechin (especially phenolic compounds) were determined to be the active compounds. It can be concluded that *P. autumnale* is an alternative to current commercial applications as an antibacterial agent in phytotherapy.

Keywords *Phormidium autumnale* · Molecular characterization · Antibacterial activity · Cyanobacteria

Highlights
- There is insufficient knowledge about the bioactive compounds of Cyanobacteria.
- Cyanobacteria have been used in many commercial fields, especially in phytotherapy.
- Many antimicrobial compounds have lost some of their efficacy due to various reasons.
- Cyanobacteria have become important subjects of alternative biodegradable and broad spectrum for researchers.
- *Phormidium autumnale* is an alternative to current commercial applications as an antibacterial agent in phytotherapy.

D. Yalcin (✉) · H. Türk Katircioğlu · M. Pourhassan Shamchi
Faculty of Education, Secondary School Science and Mathematics Education, Biology Education, Gazi University, Ankara, Turkey
e-mail: dilekduygu06@hotmail.com

T. Özer
Kaman High School of Applied Science, Kırşehir Ahi Evran University, Kırşehir, Turkey

İ. Acikgoz Erkaya
Faculty of Architecture and Engineering, Department of Environmental Engineering, Kırşehir Ahi Evran University, Kırşehir, Turkey

Introduction

Cyanobacteria are one of the oldest organisms in the world and have spread to very large habitats including freshwater, sea, and land (Malathi et al. 2014; Mundt et al. 2001; Thajuddin and Subramanian 2005). In recent years, the metabolites and the biomass produced by Cyanobacteria are gaining attention as new commercial sources. Cyanobacteria are used in commercial areas such as animal and human nutrition, cosmetics, agriculture, and especially phytotherapeutic applications (Heidari et al. 2012; Pandey 2015). Cyanobacteria are natural sources of antiviral, antibacterial, antifungal, anti-inflammatory, and anticancer compounds due to their biologically active secondary metabolites (e.g., phenolics, terpenes, pigments, phytochemicals) (Namikoshi and Rinehart 1996; Jaki et al. 2000; Sundararaman and Sekar 2001; Ghasemi et al. 2003). Also, they provide new and useful pharmaceuticals which are hard to produce synthetically due to their structural complexity (Schlegel et al. 1999).

Secondary metabolites produced by cyanobacteria, such as *Lyngbya majuscula* (Burja et al. 2001), *Phormidium tenue* (Rajeev and Xu 2004), *Nostoc commune* (Bhadury and Wright 2004), and *Anabaena circinalis* (Rajeev and Xu 2004), and antimicrobial activities of these metabolites were successfully tested on various microorganism groups. Similarly, Cyanobacterial strains of the genera *Spirulina*, *Tolypothrix*, *Anabaena*, *Cylindrospermopsis*, *Tychonema*, *Microcystis*, *Aphanizomenon*, *Oscillatoria*, and *Trichodesmium* have been reported mostly for antibacterial activities (Deshmukh and Puranik 2010, Sundaramanickam et al. 2015). Cyanobacteria and their associated bacteria are also utilized successfully in wastewater treatment (Martins et al. 2011). Some cyanobacteria species, such as *Spirulina, Anabaena*, and *Nostoc*, have been consumed in several countries as potential nutritional supplements since antiquity (Panjiar et al. 2018).

Many antimicrobial compounds currently used have lost some of their efficacy due to various reasons including an increase in the number of pathogenic microorganisms, toxicity, lack of efficacy, cost increases, and the development of resistant strains caused by frequent use. Therefore, a need for the development of alternative biodegradable and broad-spectrum alternative agents has emerged. Microalgae and cyanobacteria have become important subjects for researchers in the search for new raw materials (Abedin and Taha 2008; Demiriz et al. 2011).

Studies of the antibacterial activity of *Phormidium* taxa, which play an important role in Cyanobacteria, have demonstrated that these taxa show high biological activity and can be more effective on many microorganisms at various rates compared with other Cyanobacteria species (Pabuçcu and Demiriz Yücer 2018). As can be seen above, the antimicrobial properties of cyanobacteria have been studied on different species, but no study has been found in the literature on the antimicrobial activity of *Phormidium autumnale*. In this study, the antibacterial activity of *P. autumnale* isolated from natural freshwater sources of Turkey was investigated, and molecular characterization was performed. *P. autumnale* was evaluated in terms of phytochemical content and phytotherapeutic activities against several pathogenic bacteria.

Materials and methods

Sample isolation and preparation

Samples were collected from different natural freshwater sources in Ankara, Turkey. These cultures were allowed to incubate at room temperature after inoculation in pre-enrichment nutrition media. This media contained $MgSO_4 \cdot 7H_2O$ (2.50 g), KNO_3 (5.0 g), KH_2PO_4 (1.25 g), $FeSO_4 \cdot 7H_2O$ (0.009 g), and distilled water (1000 ml). *Phormidium autumnale* (C. Agardh) Trevisan ex Gomont was isolated from these samples. *P. autumnale* was identified at the species level with microscopic examination after incubation (Huber-Pestalozzi 1938, 1955, 1982; Hur et al. 2015). From the mixed species in the pre-enrichment medium, *P. autumnale* was isolated through a micromanipulation technique (CSIRO 2017). The subcultures were prepared by putting 30 ml BG-11 nutrient media into 50-ml Erlenmeyer flasks and adding approximately 5–10% culture depending upon the intensity of cells (Hur et al. 2015). The BG-11 medium contained (in g/l) $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; EDTA, 0.001; and Na_2CO_3, 0.02. This medium was amended with 1 ml trace solution of composition (in g/l) H_3BO_3, 2.86; $MnCl_2$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $Na_2MoO_4 \cdot 2\ H_2O$, 0.39; $CuSO_4 \cdot 5\ H_2O$, 0.079; and $Co(NO_3)_2 \cdot 6H_2O$, 0.0494. All the chemicals were obtained from Merck, Germany. Those containing 30 ml cultures were incubated at 25 °C and under fluorescent

lamps at photon flux density of 50 μmol photons m^{-2} s^{-1} with a photocycle of a light for 16 h and darkness for 8 h (Guillard 2005). The pH was adjusted to be 6.8 (Andersen and Kawachi 2005). This strain is conserved in the Ahi Evran University of Culture Collections of Algae (AEU-CCA) and is encoded as *CCA01Ph02*.

Pigment extraction analysis

Accordingly, 0.5 g of fresh sample was weighed, and 25 ml of methanol (96%) was added. The mixture was homogenized at 1000 rpm in a centrifuge for 1 min, and homogenate was filtered. Absorbances of chlorophyll-a (630, 645, 665, and 750 nm) and total carotenoid (480 nm) were measured with UV-vis spectrophotometry (Thermo Scientific GENESYS 10S UV-Vis). The chlorophyll-a concentrations were calculated using the equations below (Eqs. 1 and 2). In order to determine the total carotenoid, the absorbance at 480 nm was measured, and Eq. 3 was used to determine the total carotenoid content (Parsons and Strickland 1963).

$$\text{Chlorophyll}-a = 11.6 \times D665 - 1.31 \times D645 - 0.14 \times D630 \quad (1)$$

$$\mu\frac{g}{L} = C \times \frac{v}{1} \times V \quad (2)$$

$$\text{Total Carotenoid } \mu\frac{g}{L} = D480 \times 10 \quad (3)$$

where C is the chlorophyll-a content from the first equation, V is the filtered water (l), v is the volume of acetone (ml), and 1 is the path length of the cuvette (cm).

Molecular characterization

FTIR spectroscopy

For spectral analysis (and all measurements), samples were prepared during their exponential phases. From these samples, 0.5 ml aliquots were resuspended in deionized water, and droplets of the culture suspension were spread out all over "Low-e" reflectance slides (Kevley Technologies) for monolayer preparations. Samples were subsequently air-dried under a laminar flow system at room temperature and stored in a desiccator until analysis. Infrared analysis was carried out at the Nanotechnology Laboratory of Bilkent University (UNAM), Ankara (Turkey), using a Vertex 70 with Hyperion microscope fitted with a Bruker Tensor 37 FTIR spectrometer. Spectral resolution of the mercury cadmium telluride (MCT) detector was 4 cm^{-1}, with 128 co-added scans. Each view on the microscope was chosen from the transmission region between 400 and 5000 cm^{-1} wave number range, 4 cm^{-1} resolution, and aperture of 20 × 20 μm^2 (Sigee et al. 2002; Duygu et al. 2012). Firstly, samples were examined and analyzed by bright-field microscopy in the dry state, without any mounting medium or coverslip. Afterwards, infrared absorption spectra were collected from a clear field (background) and from algae colonies, and a ratio was obtained of the sample-to-background spectrum. Spectra were obtained from 33 individual colonies of *P. autumnale* (one spectrum per colony). The manipulation of spectra was carried out using the Bruker OPUS 6.5 software. The spectra were baseline corrected, using the automatic baseline correction algorithm, and were normalized to amide I. Spectral absorption bands were identified in relation to the published information (Sigee et al. 2002).

DNA extraction and PCR analysis

DNA extraction and PCR analysis were carried out based on an unpublished study on other algae species. At the first stage of analysis, DNA was extracted from the algal cells using a DNA extraction kit from Mo Bio Laboratories. The DNA was then cleaned using a Qiagen QIAquick PCR purification kit and run on a 1% gel to visualize the cleaned-up DNA. PCR was carried out using a Roche Expand High Fidelity PCR kit. 16S rRNA gene amplicons were amplified by the universal primers 27F and 1492R (~ 1.5 kb amplicon size). The 16s PCR product was then cleaned using a Qiagen QIAquick PCR purification kit to remove salt/enzymes from the PCR reaction. The DNA was measured on a nanodrop (http://www.nanodrop.com/Productnd2000overview.aspx), and the data are presented in the following table.

Microalgae	260/280	260/230	ng/μl DNA
P. autumnale	1.90	2.37	26.7

As the algae sample gave a concentration close to the required range, it was sent to GATC-Biotech for single-read sequencing (http://www.gatc-biotech.com/en/index.

html). Subsequent sequence analysis was performed by BLAST.

Identification of *P. autumnale* compounds by HPLC-TOF

Extracts were analyzed in an Agilent 1260 Infinity HPLC system coupled with an Agilent 6210 TOF-MS detector and a VYDAC C18 column (25 mm × 300 mm 10 μm) (Eser et al. 2017). The methanol extract was obtained from the aerial parts of *P. autumnale* and analyzed using HPLC-TOF. The identification of individual compounds was based on the data from the standards. Eight compounds were identified as phenolic acids from the extract.

Antimicrobial activity

Preparation of the sample extracts

For antibacterial activity observations, *P. autumnale* biomass was harvested after 15 days of incubation by centrifugation at 20 min, dried, and powdered using a blender (Prakash et al. 2012). The dried biomass was extracted with organic solvents (methanol (ME) and acetone (ACE)). Prepared suspensions were dried in a rotary evaporator (Heidolph, Germany). The dried extracts were resuspended in 3 ml of each solvent and preserved at 4 °C until further use in antibacterial assays (Deshmukh and Puranik 2010).

Test microorganisms

In vitro antibacterial studies were carried out against the pathogens *Escherichia coli* 4240620, *Staphylococcus epidermidis* 4243606, methicillin-resistant (MR) *Staphylococcus aureus* 4239589, *Streptococcus agalactiae* 4239460, and *Enterococcus faecalis* 4241508. Pathogens were obtained from the Culture Collection of Gazi University.

Disc diffusion assay

Antibacterial activity of *P. autumnale* extracts against the test organisms was examined by a disc diffusion assay (Deshmukh and Puranik 2010). Whatman No. 1 filter paper discs of 6-mm diameter were sterilized by autoclaving for 15 min at 121 °C. The sterile discs were impregnated with different extracts (100 μl). Agar plates were surface-inoculated uniformly from the broth culture of the tested microorganisms. Sufficient inocula were added until the turbidity equaled 0.5 McFarland standards (Sasidharan et al. 2010).

The impregnated discs were placed on the Mueller-Hinton medium, suitably spaced apart, and the plates were incubated at 37 °C for 24 h. All the organic solvents that were used in extract preparation were used as negative controls, while commercial antibiotic discs (cefoxitin 30 mcg, vancomycin 30 mcg, and cefotaxime 30 mcg) were applied as positive controls. Antibacterial activity was assessed by measuring the diameter of the growth inhibition zones around the discs. All the experiments were carried out in triplicate.

Determination of minimum inhibitory concentrations (MICs)

Minimum inhibitory concentrations (MICs) were measured by determining the lowest concentration of extract or standard antibiotic needed to inhibit the visible growth of a test microorganism. The test was performed in 96-well microtiter plates, so that several replicates of each sample could be run. All isolates were grown in nutrient broth at 37 °C overnight, and then the bacterial cultures were added into 96-well plates containing diluted samples of algal extract (10–100 μg/ml).

Each sample was tested in triplicate and the results were recorded after 24-h incubation periods at 37 °C.

Antimicrobial index

The antimicrobial indexes of individual *P. autumnale* extracts were calculated by the formula below (Ghasemi et al. 2003).

$$\text{Antimicrobial index} = (\text{Extract inhibition zone}/\text{Antibiotic inhibition zone}) \times 100$$

Statistical analysis

Wet weight, pigment content, agar well diffusion, and MIC were performed in triplicate, and the results were expressed as means (±) ($n = 3$) plus the standard deviation of the means. Statistical analysis was performed using Microsoft Excel.

Results and discussion

Taxonomic identification

The genus *Phormidium* belongs to the family Oscillatoriaceae and is a filamentous genus of Cyanobacteria. *Phormidium* is a blue-green colored alga that is morphologically unbranched filamentous, usually colonial, thin, smooth, and microscopic (with a macroscopic appearance in the case of a colony). They are facultative organisms that can live under extraordinary conditions and have free trichomes. Trichomes consist of isodiametric or long cylindrical-shaped cells. Although there are some folds in the trichomes, heterocyst and akinete-like structures are not observed (Pabuçcu and Demiriz Yücer 2018). Approximately 170 species have been classified, but there have been challenges with describing the genera of *Phormidium*, and molecular analyses have shown different results from morphological approaches indicating that they are not monophyletic (Meijer 2017). In Fig. 1, the taxonomic classification (left) of *P. autumnale* (Guiry and Guiry 2018), its appearance under a microscope (middle), and a representative FTIR image (right) are given.

Detection of *P. autumnale* growth and pigment content

In this study, pure cultures of isolated *P. autumnale* were first prepared. The BG11 medium was used for culturing the strains, and pigment analysis was performed to determine if there was sufficient biomass density following the incubation. The total biomass volume, fresh weight, chlorophyll-a, and carotenoid values obtained as a result of the 15-day incubation of *P. autumnale* cultures are given in Table 1. According to the results of the pigment analysis performed at the end of the 15th day, the chlorophyll-a content was 3.18 μg/ml, while the carotenoid content was 69.8 μg/ml. Asadi et al. (2011) have reported that the chlorophyll-a content of *Phormidium* sp. was 1.282 μg/mg, while Fresnedo et al. (1991) have reported the chlorophyll-a content of *Phormidium* sp. as 10.9 mg/mg and carotenoid content as 3.95 mg/mg. Santhose et al. (2011) have reported that the chlorophyll-a and carotenoid values of *Phormidium* sp. were 12.40 μg/ml and 76.2 μg/ml, respectively. These differences were associated with species structure and incubation conditions.

FTIR spectroscopy

The molecular characterization of *P. autumnale* was performed using FTIR spectroscopy (Fig. 2), and the band assignments of the FTIR spectra are shown in Table 2. In the 400–5000 cm^{-1} wavelength range, 11 clear bands of *P. autumnale* were determined, and each peak of these bands was assigned to a functional group. These bands were temporarily identified on the basis of reference standards published for phytoplankton and bacteria (Sigee et al. 2002; Nauman 2002; Dean et al. 2007). Two strong bands represented in 1653 cm^{-1} (amide I) and 1545 cm^{-1} (amide II) were defined as protein spectra. These bands are

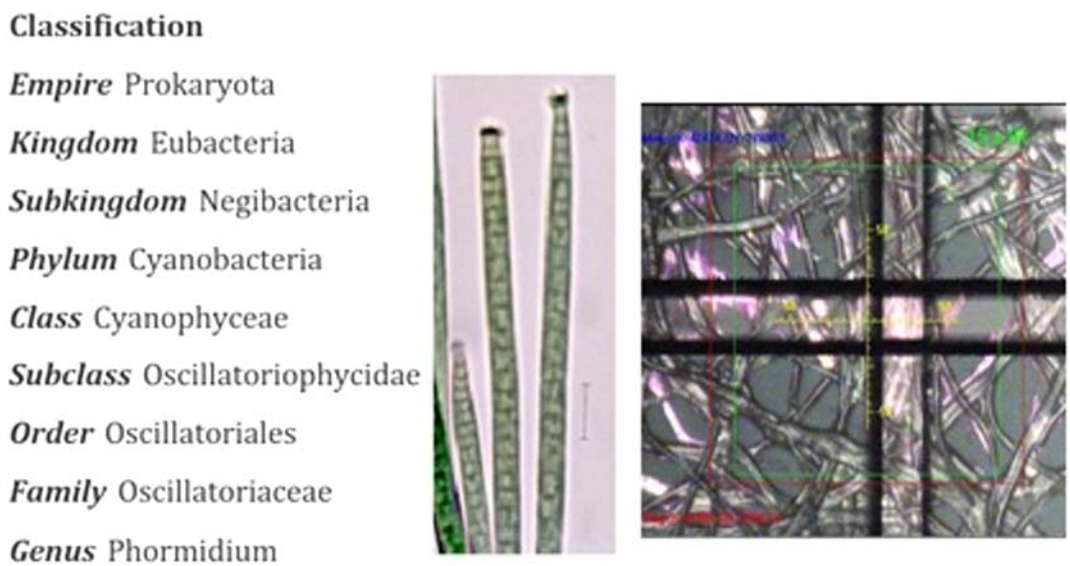

Fig. 1 Classification, microscopic, and FTIR images of *P. autumnale*

Table 1 Total biovolume, wet weight, chlorophyll-a, and carotenoid content of *P. autumnale* at the end of 15 days

Total biovolume (%)	Wet weight (gr)	Chlorophyll-a (μg/ml)	Carotenoid (μg/ml)
0.25	0.012 ± 0.0005	3.18 ± 1.3	69.8 ± 2.3

Data are given as mean ± standard deviation of triplicates. Mean values, $n = 3$

caused by a combination of C=O stretching vibration and N-H and C-N stretching vibrations in the amide complexes (Ponnuswamy et al. 2013). Lipid spectra were characterized by two sets of strong vibrations: C-H at 2923 cm^{-1} and the C=O mode of the side chain from the ester carbonyl group at 1740 cm^{-1}. The wavelengths of 1152 cm^{-1}, 1077 cm^{-1}, and 1050 cm^{-1} detected in the FTIR spectrum were carbohydrate absorption bands attached to the C-O-C of polysaccharides. Functional groups such as nucleic acids that are the stretching of phosphodiesters in the same region of the spectrum (1077 cm^{-1}) were identified (Table 2). FTIR spectra of some phenolic compounds showed the wavenumbers of C-H stretching and C=O asymmetric stretching vibrations. In this study, the absorbance values of the peaks in band regions 4–7 (especially amide I and amide II) were high. Initially, although the FTIR expressions were not planned according to specific molecules such as phenolic compounds or cyclic peptides, these band regions of *P. autumnale* resemble most of the wavelengths of FTIR spectra of some phenolic compounds and cyclic peptides (Signh et al. 2007; Swisłocka et al. 2012; Trivedi et al. 2015; Poon et al. 2012). Cyclic peptides and phenolics have attracted a great deal of attention for the development and design of new antimicrobial agents due to their antimicrobial activities and high stability (Flanga et al. 2017).

Genetic characterization of *P. autumnale*

The 16s sequencing results were returned in FASTA format as shown below. The results confirmed the molecular identification of *P. autumnale* by 99%.

>Phormidium autumnale

TTGGAAACGACTGCTAAGCCCCGATGTACCGA
AAGGGAAAATATTTATAGCCTGAAGATGAGCTCGCG
TCCGATTAGCTAGTNGGCGGAGTAAAAGCCCACCAA
GGCGACGATCGGTANCTGGTCTGAGAGGACGATCAG
CCACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGAA
AGCCTGACGGAGCAAGACCGCGTGGGGGAA
GAAGGCTCTTGGGTTGTAAACCCCTTTTCTCTGGGA
AGAAAGTTGTGAAAGCAACCTGACGGTACC
AGAGGAATCAGCATCGGCTAACTCCGTGCCAGCAGC
CGCGGTAAGACGGAGGATGCAAGCGTTATCCGGAAT
GATTGGGCGTAAAGCGTCCGCAGGTGGCAGTTCAAG
TCTGCTGTCAAAGACCGGGGCTTAACTTCGGAAAGG
CAGTGGAAACTGAACAGCTAGAGTATGGTAGGGGCA
GAGGGAATTCCTGGTGTAGCGGTGAAATGCGTAGAG
ATCAGGAAGAACATCGGTGGCGAAGGCGCTCTGCTG
GACCATAACTGACACTCAGGGACGAAAGCTAGGGGA
GCGAATGGGATTAGATACCCCAGTAGTCCTAGCCGT
AAACGATGGATACTAGGTGTTGTCTGTATCGACCCG
GACAGTGCCGTAGCTAACGCGTTAAGTATCCCGCCT
GGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTT
TAATTCGATGCAACGCGAAGAACCTTACCAGGACTT
GACATGTCGCGAATCTTTTGGAAACAGAAGAGTGCC
TTCGGGAGCGCGAACACAGGTGGTGCATGGCTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTCGTGTTTAGTTGCCATCATTA
AGTTGGGCACTCTAAACAGACTGCCGGTGACA

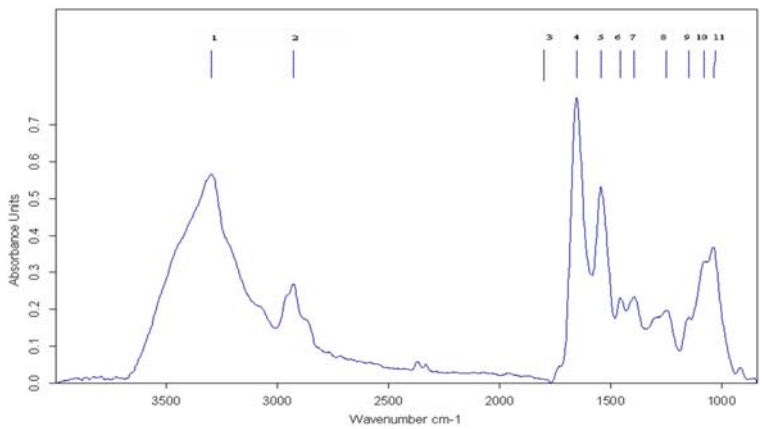

Fig. 2 FTIR absorption spectrum of *P. autumnale*

Table 2 Tentative assignment of bands found in FTIR spectra of air-dried preparations of *P. autumnale*[1]

Band number	Band wavenumber (cm^{-1})	Wavenumber range (cm^{-1})	Tentative assignment of bands to major macromolecules
1	3426	3029–3639	Water ν(O-H) stretching protein ν(N-H) stretching (amide A)
2	2923	2809–3012	Lipid–carbohydrate Mainly $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ stretching
3	1740	1763–1712	Cellulose–fatty acids ν(C=O) stretching of esters
4	1653	1583–1709	Protein amide I band Mainly ν(C=O) stretching
5	1545	1481–1585	Protein amide II band Mainly δ(N-H) bending and ν(C-N) stretching
6	1452	1425–1477	Protein $\delta_{as}(CH_2)$ and $\delta_{as}(CH_3)$ bending of methyl Lipid $\delta_{as}(CH_2)$ bending of methyl
7	1389	1357–1423	Protein $\delta_s(CH_2)$ and $\delta_s(CH_3)$ bending of methyl Carboxylic acid ν_s(C-O) of COO^- groups of carboxylates
8	1238	1191–1356	Nucleic acid (and other phosphate-containing compounds) ν_{as}(>P=O) stretching of phosphodiesters
9	1152	1134–1174	Carbohydrate ν(C-O-C) of polysaccharides
10	1077	1072–1099	Carbohydrate ν(C-O-C) of polysaccharides Nucleic acid (and other phosphate-containing compounds) ν_s(>P=O) stretching of phosphodiesters
11	1050	980–1072	Carbohydrate ν(C-O-C) of polysaccharides

[1] Band assignment based on Sigee et al. (2002), Nauman (2002), and Dean et al. (2007)

Antimicrobial activity

In recent years, the resistance of the pathogenic bacteria to antibacterial agents and the side effects of drugs have led researchers to try to find new sources and other antimicrobial options (Shamchi 2016). Although in phytotherapeutic applications, higher plants are important as biological materials, rich metabolite contents produced by algae and cyanobacteria, and the development of algae biotechnologies have made research on this subject popular. In particular, cyanobacteria have become potential sources of new active substances for drugs and pharmaceuticals and have become available as phytotherapeutic agents (Asadi et al. 2011; Aliyazıcıoğlu et al. 2018). It was determined that *P. autumnale* contains various phenolic compounds. According to the antimicrobial test results of *P. autumnale* CBA extracts, screening the methanol extract by the HPLC-TOF method, phenolic compounds, gentisic acid, 4-hydroxybenzoic acid, caffeic acid, β-quartzic acid, 4-hydroxybenzaldehyde, salicylic acid, catechin, and vanillic acid was identified among a total of 23 parameters. Some other phenolics and flavonoids such as gallic acid and cinnamic acid were not determined. Some of the phenolic acids detected in *P. autumnale* (especially gentisic acid, vanillic acid, 4-hydroxybenzoic acid, and p-coumaric acid) were found to be similar to those found in some other *Phormidium* or terrestrial cyanobacterial species (Babić et al. 2015; Jerez-Martel et al. 2017).

For the phytotherapeutic evaluation of *P. autumnale*, Gr (+) and Gr (−) test strains (*Staphylococcus epidermidis*, MR *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus faecalis*, and *Escherichia coli* clinical isolates) were used to determine the essential antibacterial activity. The data on inhibition abilities of extracts, obtained from *P. autumnale*, and test antibiotics (zone diameter: given in mm) are listed in Table 3. The highest inhibition ability of methanol extracts was found with *E. faecalis* 4241508 (15 ± 0.4 mm), while the inhibitory effect on MR *S. aureus* 4239589 (13.5 ± 0.5 mm) suggests that the extract has a broad spectrum. Furthermore, the fact that only distilled methanol (13.5 ± 0.72 mm) extract was effective on *S. epidermidis* 4243606 shows that volatile compounds of *P. autumnale* obtained after evaporation can possess antibacterial effects. The extracts obtained with acetone were found to be effective only on *E. coli* 4240620 (9.8 ± 0.43 mm). According to the results of the analysis of methanol extract, the active compounds

Table 3 Antimicrobial activities of different extracts of *P. autumnale* compared with commercial antibiotics

Bacterial strains	Inhibition zone (mm)				Antibiotic positive control		
	ME[1]	DM[2]	ACE[3]	DAC[4]	FOX[a]	VA[b]	CTX[c]
E. coli 4240620	4.1 ± 0.68	2 ± 0.37	9.8 ± 0.43	2.5 ± 0.45	22	-	20
S. epidermidis 4243606	-	13.5 ± 0.72	-	-	16	19	-
MR *S. aureus* 4239589	13.5 ± 0.5	11.5 ± 0.58	-	-	23	20	-
S. agalactiae 4239460	5.3 ± 0.35	3 ± 0.47	-	-	21	19	-
E. faecalis 4241508	15 ± 0.4	-	-	-	25	18	-

[1] Methanol extract

[2] Distilled methanol extract

[3] Acetone extract

[4] Distilled acetone extract

[a] Cefoxitin 30 mcg

[b] Vancomycin 30 mcg

[c] Cefotaxime 30 mcg

Data are given as mean ± standard deviation of triplicates. Mean values, $n = 3$

were determined to be the phenolic compounds gentisic acid, vanillic acid, 4-hydroxybenzoic acid, p-coumaric acid, and catechin.

Antibacterial activity tests for the evaluation of *P. autumnale* from a phytotherapeutic point of view showed that this strain could be evaluated as an alternative. Many studies on the inhibition data from extracts obtained from higher plants and algae have reported that the differences are generally associated with the active compound ratios and are caused by solvent differences. In the literature, it has been reported that the methanol extracts of different algae showed higher antibacterial activity than other extracts (Shamchi 2016). Acetone and distilled acetone extracts of *P. autumnale* were found to be effective only on *E. coli*. The data on the minimum inhibition concentrations of the extracts are shown in Table 4. The MIC values for bacterial strains, which are sensitive to the extracts, were in the range of 40–80 μg/ml.

To investigate the antimicrobial activity of Cyanobacteria, various species have been investigated. Malathi et al. (2014) investigated *Tolypothrix tenuis*, *Anabaena variabilis*, and *Cylindrospermum* species against *Bacillus subtilis* and *Pseudomonas aeruginosa*; Tiwari and Sharma (2013) investigated *Anabaena variabilis* and *Synechococcus elongatus* species against *E. coli*, *Enterococcus*, and *Klebsiella*; Rania and Taha (2008) investigated *Spirulina platensis* for the inhibition of Gram-positive and Gram-negative bacteria and reported positive results. Rodriguez-Meizoso et al. (2008) have reported that *Phormidium* sp. had an antibacterial effect on *Staphylococcus aureus* and *Escherichia coli*. Demiriz et al. (2011) determined that *Phormidium tenue* extract had good antibacterial effects on *Escherichia coli* O157:H7. In the literature, there has been no study yet to our knowledge on the biological activity of *P. autumnale*.

The comparison of the antibacterial activity of *P. autumnale* extracts with commercial antibiotics was evaluated as the antimicrobial index. These data are shown in Table 5. According to the index data, the efficacy of the methanol extract obtained from *P. autumnale*, especially on MR *S. aureus*, was 58.7–67.5% similar to the efficacy of currently used antibiotics. Antimicrobial indexes were found to be noteworthy compared with those of commercial antibiotics.

Conclusion

Screening of extracts or isolated compounds from different natural sources is a common way to explore bioactive metabolites. In this study, it is noteworthy that Cyanobacteria are a rich source of bioactive compounds. These CBA-origin compounds have been a subject of great interest in pharmaceutical therapy and commercial applications. It has been established in this work that *P. autumnale* organic solvents have antibacterial effects against the tested pathogen bacteria. Our data revealed that considerable inhibition zones were

Table 4 Minimum inhibitory concentrations (MICs) of *P. autumnale*'s different extractions

Microalgae	Test microorganisms	MIC(μg/ml)			
		ME[1]	DM[2]	ACE[3]	DAC[4]
P. autumnale	*Escherichia coli* 4240620	70	80	50	80
	Staphylococcus epidermidis 4243606	-	40	-	-
	MR *Staphylococcus aureus* 4239589	60	-	-	-
	Streptococcus agalactiae 4239460	40	-	-	-
	Enterococcus faecalis 4241508	50	-	-	-

[1] Methanol extract

[2] Distilled methanol

[3] Acetone

[4] Distilled acetone

Data are given as mean values, $n = 3$

formed for the methanol and distilled methanol extracts, and these zones are comparable with those formed by commercial antibiotics. However, further investigations regarding toxicity, stability, and metabolism of *P. autumnale* and its components must be undertaken.

Table 5 *P. autumnale* extractions antimicrobial index

Bacterial strains	Antibiotic	Antimicrobial index in percentage			
		ME[1]	DM[2]	ACE[3]	DAC[4]
Escherichia coli 4240620	FOX[a]	18.2	9	43.2	11.4
	VA[b]	-	-	-	-
	CTX[c]	20	10	47.5	12.5
Staphylococcus epidermidis 4243606	FOX[a]	-	84	-	-
	VA[b]	-	71	-	-
	CTX[c]	-	-	-	-
MR *Staphylococcus aureus* 4239589	FOX[a]	58.7	50	-	-
	VA[b]	67.5	57.5	-	-
	CTX[c]	-	-	-	-
Streptococcus agalactiae 4239460	FOX[a]	26.2	14.3	-	-
	VA[b]	28.8	15.8	-	-
	CTX[c]	-	-	-	-
Enterococcus faecalis 4241508	FOX[a]	60	-	-	-
	VA[b]	83.3	-	-	-
	CTX[c]	-	-	-	-

[1] Methanol extract

[2] Distilled methanol extract

[3] Acetone extract

[4] Distilled acetone extract

[a] Cefoxitin 30 mcg

[b] Vancomycin 30 mcg

[c] Cefotaxime 30 mcg

Data are given as mean values, $n = 3$

Acknowledgments We would like to thank Prof. Dr. İbrahim Demirtaş from Çankırı Karatekin University for the HPLC analysis and Andrew P. Dean from the University of Manchester for the PCR analysis.

Funding information This study was supported by the Gazi University Scientific Projects Research Management Funds (No. 04-2007-28).

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals.

References

Abedin, R. M. A., & Taha, H. M. (2008). Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of media components by Plackett- Burman design for antimicrobial activity of *Spirulina platensis*. *Global Journal of Biotechnology & Biochemistry, 3*(1), 22–31.

Aliyazıcıoğlu, R., Korkmaz, N., Akkaya, Ş., Şener, Ş. Ö., Özgen, U., & Karaoğlu, Ş. (2018). Investigation of antioxidant, antimicrobial and tyrosinaz inhibitor activities on the aerial parts of *Dactilorhiza osmanica*. *Firat Medical Journal, 23*(2), 50–57.

Andersen, R. A., & Kawachi, M. (2005). Traditional microalgae isolation techniques. In R. A. Andersen (Ed.), *Algal culturing techniques* (pp. 83–100). London: Elsevier Press.

Asadi, A., Khavari-Nejad, R., Soltani, N., Najafi, F., & Molaie-Rad, A. (2011). Physiological and antimicrobial characterizations of some cyanobacteria isolated from the rice fields in Iran. *Journal of Agricultural Technology, 7*(3), 649–663.

Babić, O., Kovač, D., Rašeta, M., & Šibul, F. (2015). Evaluation of antioxidant activity and phenolic profile of filamentous terrestrial cyanobacterial strains isolated from forest ecosystem. *Journal of Applied Phycology, 28*, 2333–2342. https://doi.org/10.1007/s10811-015-0773-4.

Bhadury, P., & Wright, P. C. (2004). Exploitation of marine algae: biogenic compounds for potential antifouling applications. *Planta, 219*, 561–578.

Burja, A. M., Banaigs, B., Abou-Mansour, E., Burgess, J. G., & Wright, P. C. (2001). Marine cyanobacteria–a prolific source of natural products. *Tetrahedron, 57*, 9347–9377.

Dean, A. P., Martin, M. C., & Sigee, D. C. (2007). Resolution of codominant phytoplankton species in a eutrophic lake using synchrotron-based Fourier transform infrared spectroscopy. *Phycologia*. https://doi.org/10.2216/06-27.1.

Demiriz, T., Cokmus, C., & Pabuccu, K. (2011). Antimicrobial activity of some algal species belonging to cyanobacteria and chlorophyta. *Asian Journal of Chemistry, 23*(3), 1384–1386.

Deshmukh, D. V., & Puranik, P. R. (2010). Application of Plackett-Burman design to evaluate media components affecting antibacterial activity of alkaliphilic cyanobacteria isolated from Lonar Lake. *Turkish Journal of Biochemistry, 35*(2), 114–120.

Duygu, D., Udoh, A. U., Özer Baykal, T., Akbulut, A., Erkaya Açıkgöz, I., Yıldız, K., & Deniz, G. (2012). Fourier transform infrared (FTIR) spectroscopy for identification of *Chlorella vulgaris* Beijerinck 1890 and *Scenedesmus obliquus* (Turpin) Kützing 1833. *African Journal of Biotechnology, 11*(16), 3817–3824.

Eser, F., Yaglioglu, S. A., Aktas, E., Onal, A., Demirtas, I. (2017). Phytochemical content of centaurea polypodiifolia boiss. var. polypodiifolia. *International Journal of Secondary Metabolite*. https://doi.org/10.21448/ijsm.376888.

Flanga, A., Nigro, E., De Biasi, M. G., et al. (2017). Cyclic peptides as novel therapeutic microbicides: engineering of human defensin mimetics. *Molecules*. https://doi.org/10.3390/molecules22071217.

Fresnedo, O., Gomez, R., & Serra, J. L. (1991). Carotenoid composition in the cyanobacterium *Phormidium laminosum* effect of nitrogen stravation. *FEBS, 282*(2), 300–304.

Ghasemi, Y., Tabatabaei Yazdi, M., Shokravi, S., Soltani, N., & Zarrini, G. (2003). Antifungal and antibacterial activity of paddy-fields cyanobacteria from the north of Iran. *Journal of Sciences Islamic Republic of Iran, 14*(3), 203–209.

Guillard, R. R. L. (2005). Purification methods for microalgae. In R. A. Andersen (Ed.), *Algal culturing techniques* (pp. 117–132). London: Elsevier Press.

Guiry, M. D., Guiry, G. M. (2018). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org. Accessed 04 November 2018.

Heidari, F., Riahi, H., Yousefzadi, M., & Asadi, M. (2012). Antimicrobial activity of cyanobacteria isolated from hot spring of Geno. *Middle-East Journal of Scientific Research, 12*(3), 336–339.

Huber-Pestalozzi, G. (1938). Das Phytoplankton Des Süβwassers, 1. Teil E. Schweizerbartsche Verlagsbuchhandlung, Germany.

Huber-Pestalozzi, G. (1955). Das Phytoplankton Des Süβwassers, 4. Teil Euglenophyceen, E. Schweizerbart'sche Verlagsbuchhandlung, Germany.

Huber-Pestalozzi, G. (1982). Das Phytoplankton Des Süβwassers, 8. Teil Conjugatophyceae, Zynematales and Desmidiales, E. Schweizerbart'sche Verlagsbuchhandlung, Germany.

Hur, S. B., Bae, J. H., Youn, J. Y., Jo, M. J. (2015). KMMCC-Korea marine microalgae culture center: list of strains, 2nd edition. *Algae*, S1-S188.

Jaki, B., Heilmann, J., Linden, A., Volger, B., & Sticher, O. (2000). Novel extra cellular diterpenoids with biological activity from the cyanobacterium *Nostoc commune*. *Journal of Natural Products, 63*, 339–343.

Jerez-Martel, I., Garcia-Poza, S., Rodriguez-Martel, G., Rico, M., Afonso-Olivares, C., & Gómez-Pinchetti, J. L. (2017). Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. *Hindawi Journal of Food Quality*. https://doi.org/10.1155/2017/2924508.

Malathi, T., Ramesh Babu, M., Mounika, T., Snehalatha, D., & Digamber Rao, B. (2014). Screening of cyanobacterial strains for antibacterial activity. *Phykos, 44*(2), 6–11.

Martins, J., Peixe, L., & Vasconcelos, V. M. (2011). Unraveling cyanobacteria ecology in wastewater treatment plants (WWTP). *Microbial Ecology, 62*, 241–256.

Meijer, N. (2017). The relationship between enhanced Phormidium growth and fine sediment depositon in New Zealand Rivers. Desertion, University College of Southeast Norway Faculty of Arts and Science.

Mundt, S., Kreitlow, S., Nowotny, A., & Effmert, U. (2001). Biological and pharmacological investigation of selected cyanobacteria. *International Journal of Hygiene and Environmental Health, 203*, 327–334.

Namikoshi, M., & Rinehart, K. L. (1996). Bioactive compounds produced by Cyanobacteria. *Journal of Industrial Microbiology, 17*, 373–384.

Nauman, D. (2002). Infrared spectroscopy in microbiology. In R. A. Meyers (Ed.), *Encyclopedia of analytical chemistry* (pp. 102–131). Chichester: John Wiley & Sons, Ltd..

Pabuçcu, K., & Demiriz Yücer, T. (2018). Investigation of the medical features of *Phormidium* species. *Gaziosmanpasa Journal of Scientific Research, 7*(1), 101–115.

Pandey, V. D. (2015). Cyanobacterial natural products as antimicrobial agents. *International Journal of Current Microbiology and Applied Sciences, 4*(1), 310–317.

Panjiar, N., Mishra, S., Yadav, A. N., & Verma, P. (2018). Functional foods from cyanobacteria: an emerging source for functional food products of pharmaceutical importance. In V. K. Gupta, H. Treichel, V. Shapaval, L. A. Oliveira, & M. G. Tuohy (Eds.), *Microbial functional foods and nutraceuticals* (First ed.). USA: John Wiley & Sons. https://doi.org/10.1002/9781119048961.ch2.

Parsons, T. R., & Strickland, J. D. H. (1963). Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. *Journal of Marine Research, 21*(3), 115–163.

Ponnuswamy, I., Madhavan, S., & Shabudeen, S. (2013). Isolation and characterization of green microalgae for carbon sequestration, waste water treatment and bio-fuel production. *International Journal of Bio-Science and Bio-Technology, 5*(2), 17–26.

Poon, C. K., Chapman, R., Jolliffe, K. A., & Perrie, S. (2012). Pushing the limits of copper mediated azide–alkyne cycloaddition (CuAAC) to conjugate polymeric chains to cyclic peptides. *Polymer Chemistry, 3*, 1820–1826.

Prakash, N. K., Bhuvaneswari, S., et al. (2012). A study on antibacterial activitiy of common weeds in northern districts of Tamil Nadu, India. *Research Journal of Medical Plant.* https://doi.org/10.3923/rjmp.2012.341.345.

Rajeev, K. J., & Xu, Z. (2004). Biomedical compounds from marine organisms. *Marine Drugs, 2*, 123–146.

Rania, M. A., & Taha, A. H. M. (2008). Antibacterial and antifungal activity of Cyanobacteria and green microalgae, evaluation of medium components by Placket-Burman design for antimicrobial activity of *Spirulina platensis*. *Global Journal of Biotechnology & Biochemistry, 3*(1), 22–31.

Rodriguez-Meizoso, I., Jaime, L., Santoyo, S., Cifuentes, A., Garcia-Blairsy Reina, G., Senorans, F. J., & Ibanez, E. (2008). Pressurized fluid extraction of bioactive compounds from *Phormidium* species. *Journal of Agricultural and Food Chemistry, 56*, 3517–3523.

Santhose, I., Gnanadoss, J., Ramganesh, S., & Elumalai, S. (2011). Enhanced carotenoid synthesis of *Phormidium* sp. in stressed conditions. *Journal of Experimental Science, 2*(3), 38–44.

Sasidharan, S., Darah, I., & Noordin, M. K. (2010). In vitro antimicrobial activity against Pseudomonas aeruginosa and acute oral toxicity of marine algae *Gracilaria changii*. *New Biotechnology.* https://doi.org/10.1016/j.nbt.2010.02.002.

Schlegel, I., Doan, N. T., de Chazal, N., & Smith, G. D. (1999). Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *Journal of Applied Phycology, 10*(5), 471–479.

Shamchi, M. P. (2016). Investigation of cyanobacteria and some algal species bioactive compounds. Desertion, Hacettepe University.

Sigee, D. C., Dean, A., Levado, E., & Tobin, M. J. (2002). Fourier-transform infrared microscopy of *Pediastrum dublex*: characterization of a micro-population isolated from a eutrophic lake. *European Journal of Phycology, 37*, 19–26.

Signh, Y. P., Das, R., & Singh, R. A. (2007). Numerical simulation of the internal vibrations of COOH group in amino-salicylic acids. *African Journal of Biochemistry Research, 1*(2), 19–23.

Sundaramanickam, A., Palanivel, S., Shekhar, S., Kumaresan, S., & Balasubramanian, T. (2015). In vitro evaluation of antimicrobial activity of some selected cyanobacterial extracts against human pathogens. *IJAPBC, 4*(1), 36–43.

Sundararaman, M., & Sekar, S. (2001). *Biotechnological potentials of cyanobacteria*. Jaipur: Algal Biotechnology, Pointer Publishers.

Swisłocka, R., Kowczyk-Sadowy, M., & Lewandowski, W. (2012). Spectroscopic (FT-IR, FT-Raman, 1H and 13C NMR) and theoretical studies of *p*-coumaric acid and alkali metal *p*-coumarates. *Spectroscopy.* https://doi.org/10.3233/SPE-2012-0568.

Thajuddin, N., & Subramanian, G. (2005). Cyanobacterial biodiversity and potential applications in biotechnology. *Current Science, 89*(1), 47–57.

Tiwari, A., & Sharma, D. (2013). Antibacterial activity of bloom farming Cyanobacteria against clinically isolated human pathogenic microbes. *Journal of Algal Biomass Utilization., 4*(1), 83–89.

Trivedi, M. K., Branton, A., Trivedi, D., Shettigar, H., Bairwa, K., & Jana, S. (2015). Fourier transform infrared and ultraviolet-visible spectroscopic characterization of biofield treated salicylic acid and sparfloxacin. *Natural Products Chemistry & Research.* https://doi.org/10.4172/2329-6836.1000186.