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Antimicrobial Activity of the Heterocytous Cyanobacterium *Westiellopsis prolifica* and Assessment of Its Microcystin–LR Biosynthesis

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THE HETEROCYTOUS cyanobacterium *Westiellopsis prolifica* has been recently documented in Egypt; however, our understanding of its diverse biotechnological and biomedical applications remains a limited and challenging topic. In this study, a *W. prolifica* strain isolated from hyper-arid desert habitats was investigated to test its antimicrobial potential against six foodborne pathogenic bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) and eight mycotoxigenic fungi (*Aspergillus carbonarius*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *A. westerdijikia*, *Fusarium proliferatum*, and *Penicillium verrucosum*), as well as to evaluate its probable toxicity and biosynthesis of the most common microcystin variant (MC–LR) using high-performance liquid chromatography (HPLC). The antimicrobial activity of various *W. prolifica* extracts was assessed using the disk–diffusion method, and the minimum inhibitory concentrations (MICs) were also determined. Our findings revealed that the extracts of dryland-dwelling *W. prolifica* had significant antimicrobial activities against all tested bacterial and fungal strains, with average MIC values of 0.4–6.7 and 0.3–9.2mg.mL^{-1}, respectively. This antimicrobial potential can be ascribed to the combined synergistic effects of its biologically active and structurally diverse metabolites. Moreover, this species was a non-producer of MC–LR and was non-toxic based on the brine shrimp bioassay. Thus, the cyanoprokaryote *W. prolifica* can be considered a sustainable multifunctional candidate in the development of the pharmaceutical industry in Egypt.

Keywords: Antibacterial and antifungal potential, Cyanoprokaryotes, Microcystin–LR, *Westiellopsis prolifica*.

Introduction

The rapid growth of resistance in pathogenic bacteria and fungi has recently led to the necessity for a new strategy in drug development by depending on antimicrobial products of natural origin (e.g., El-Sheekh et al., 2008; Righini & Roberti, 2019; Besednova et al., 2020). Members of the phylum Cyanobacteria, in particular the heterocytous genera and taxa, have been characterized as keystone producers of biologically active and structurally diverse compounds with high-value pharmaceutical importance (El-Sheekh et al., 2008; Saurav et al., 2019; Khalifa et al., 2021). However, our understanding of the antimicrobial potential of the dryland-inhabiting taxa, such as the poorly studied *Westiellopsis prolifica* (Hapalosiphonaceae, Nostocales), remains scarce in this area of research.

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The ability of cyanobacterial taxa to produce cyanotoxins has been documented in different genera, mostly allocated within the orders Chroococcales, Oscillatoriales, and Nostocales (Cirés et al., 2017; Kust et al., 2018; Shishido et al., 2019; Nowruzi & Porzani, 2021). Microcystins (MCs), which are cyclic heptapeptides and the most prevalent class of cyanotoxins, are primarily characterized by the presence of the unique amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6 dienoic acid (Adda) (Sivonen, 1990). MCs are biosynthesized nonribosomally via an MC synthetase gene cluster (*mcy*) consisting of a set of polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), and tailoring enzymes (Tillett et al., 2000). This *mcy* gene cluster is often modified through point mutations, insertions and deletions, or a series of genetic recombination events that affect the activity of MC peptide synthetases, consequently leading to the chemical structures found in nature (Puddick et al., 2016). To date, 279 MC variants have been identified, with the most common types being MC–LR, MC–LA, and MC–RR (Bouaïcha et al., 2019). The first variant has been reported as the most potent hepatotoxin (Nowruzi & Porzani, 2021). The presence of MCs in the true-branching heterocytous cyanobacterium *W. prolifica* remains an unclear and challenging topic, especially regarding the strains isolated from desert soils (Abed et al., 2013).

The main objectives of this research were to assess the antimicrobial potential of the poorly investigated desert-soil cyanobacterium *W. prolifica* against several foodborne bacterial and mycotoxigenic fungal strains, and to evaluate its probable toxicity and the presence/absence of the most common microcystin variant, MC–LR.

Materials and Methods

Origin, growth conditions, and biomass harvesting of the W. prolifica strain

The true-branching heterocytous cyanobacterium *W. prolifica* (Hapalosiphonaceae, Nostocales) was obtained from the algal culture collection of the Phycology Unit No. 341 at the Botany Department, Faculty of- Science, Ain Shams University, Cairo, Egypt, which was curated by Abdullah A. Saber (Saber, 2016). The original specimens were isolated from the hyper-arid desert soils of the Western Desert Oases in Egypt, and were characterized using an integrative polyphasic approach (Saber et al., 2017). An axenic strain of *W. prolifica* (Fig. 1A–D), which was obtained by streaking and repeated sub-culturing on agarized medium, was cultured in 5-L Erlenmeyer flasks containing 3000mL of BG-11_0 (*N-free*) medium each (Rippka et al., 1979), and inoculated at an initial optical density (OD_{680}) of 0.01±0.002. The inoculated flask was kept at 24±1°C and a 16:8 h light:dark photoperiod using 20W cool white fluorescent lamps at an irradiance of 55µmol photons m^{-2} s^{-1}, and bubbled with sterile air using a 0.2-µm-pore bacterial filter. The growth of the *W. prolifica* biomass growth was monitored every 2–3 days by measuring changes in optical density at 680nm, which is the highest absorbance peak of chlorophyll *a*, using a Unicum UV-300 UV/Vis spectrophotometer (Spectronic Unicam, Rochester, USA). The biomass was harvested at the late exponential growth phase, after approximately 3 weeks in culture (Fig. 2), by filtering using sterile 0.45µm Whattman® filter papers. The biomass harvested was washed three times with distilled water, re-suspended in sterilized distilled water (to remove any traces of the growth medium), and finally dried at the room temperature in the dark. Bright-field and autofluorescent photomicrographs were acquired using a Zeiss Axioskop 2 microscope (Zeiss, Jena, Germany) equipped with an Axiocam digital camera and a BEL® photonics biological microscope (BEL® Engineering, Monza, Italy) fitted with a Canon Powershot G12 (Japan) digital camera.

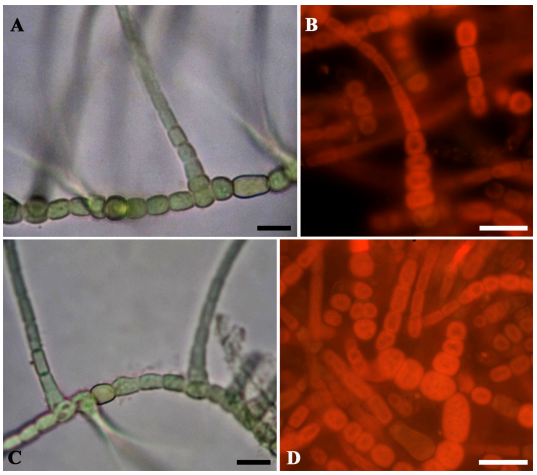

Fig. 1(A–D). Detailed morphotaxonomic diagnostic features of the *Westiellopsis prolifica* specimens included in this study [Scale bars= 10µm]

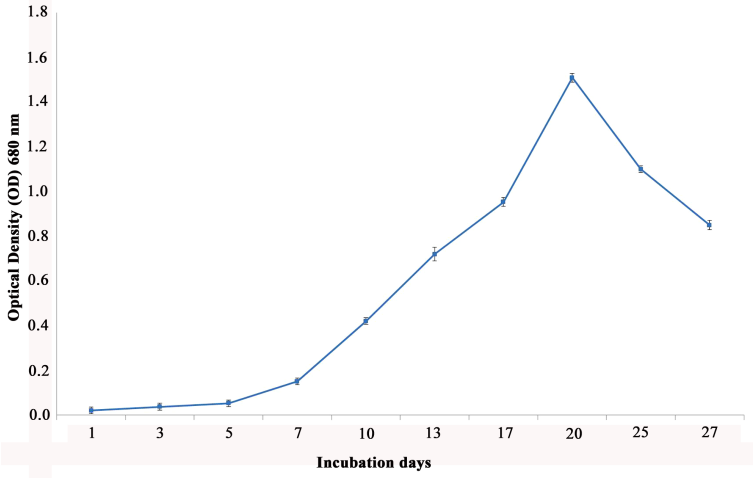

Fig. 2. Growth curve of the heterocytous cyanobacterium *Westiellopsis prolifica* in BG-11$_0$ (N-free) medium during the 27-day incubation period

Antimicrobial activity of W. prolifica extracts

Tested microorganisms

Six strains of foodborne pathogenic bacteria were tested, including *Bacillus cereus* EMCC 1080 and *Staphylococcus aureus* ATCC 13565 as Gram–positive bacteria, and *Salmonella typhi* ATCC 25566, *Escherichia coli* 0157 H7 ATCC 51659, *Pseudomonas aeruginosa* NRRL B–272, and *Klebsiella pneumoniae* LMD 7726 as representatives of Gram–negative bacteria. These bacterial strains were obtained from the Holding Company for Biological Products and Vaccines (VACSERA), Egypt. The stock cultures were grown on nutrient agar slants at 37°C for 24h and then kept in a refrigerator until use. Eight fungal strains were used for the antifungal assay: *Aspergillus flavus* NRR 3357, *A. parasiticus* SSWT 2999, *A. ochraceus* ITAL 14, *A. niger* IM I288550, *A. westerdijikia* CCT 6795, *A. carbonarius* ITAL 204, *Fusarium proliferatum* MPVP 328, and *Penicillium verrucosum* BFE 500. These fungal strains were obtained from the Applied Mycology Department, Cranfield University, UK. The stock cultures were grown on potato dextrose agar slants at 25°C for 5 days and then kept in the refrigerator until use.

Disk–diffusion technique

The antibacterial activities of the different *W. prolifica* extracts, i.e., water, ethanol, diethyl ether, chloroform, and hexane (1:4, *w/v*), was assessed against the different bacterial cultures using the Kirby–Bauer technique (Bauer et al., 1966). Dimethyl sulfoxide (DMSO) was used as the negative control, whereas the standard antibacterial drug "ceftriaxone" (1mg.mL^{-1}, *w/v*) was used as the positive control. The inoculated plates were incubated at 37°C for 24h, and the inhibition zones were measured (Marrez et al., 2019). Regarding the mycotoxigenic fungal strains, a spore suspension of each fungus was prepared in 0.01% Tween 80 solution. The fungal suspension was compared with the 0.5 McFarland standard, and the turbidity of each inoculum suspension represented about 2× 10^{8}cfu.mL^{-1}. The negative control was prepared using DMSO, and the standard commercial fungicide "miconazole" (1mg.mL^{-1}) was used as the positive control. The inoculated plates were incubated at 25°C for 24-48h. The antifungal activity of the *W. prolifica* extracts was assessed by measuring the inhibition zones (Medeiros et al., 2011). All treatments were carried out in triplicate. The results are expressed as the mean values ± standard error.

Determination of the minimum inhibitory concentrations (MICs)

The MIC of each extract of *W. prolifica* was determined using the broth microdilution assay (Andrews, 2001). Two-fold serial dilutions of each cyanobacterial extract, ranging from 5 to 0.02mg.mL^{-1}, were used. Equal volumes of each bacterial strain (10^{5}cfu.mL^{-1}) were added to each well. The MIC values were obtained from the lowest concentration that inhibited the bacterial growth after culture for 24h at 37°C. In turn, the MIC values of the mycotoxigenic fungi were assessed using the method of Sokmen et al. (2004) and Marrez & Sultan (2016). The *W. prolifica* extracts, at different concentrations,

were separately dissolved in 0.5mL of 0.1% Tween 80 mixed with 9.5mL of melting potato dextrose agar medium at 45°C, and then poured into Petri dishes (6cm). The plates were centrally inoculated with 3µL of each fungal suspension (10^8cfu.mL^{-1}; 0.5 McFarland standard). The inoculated plates were incubated at 25°C for 24–48h. At the end of the incubation period, mycelial growth was monitored and MIC values were determined.

Assessment of the toxicity of W. prolifica

Brine shrimp bioassay

Brine shrimp eggs (*Artemia salina* Leach) were supplied by Avocet Artemin Inc., Utah, USA. Larvae were used within 24 h of hatching. One gram of the dried biomass of the cyanobacterium *W. prolifica* was extracted with 10mL of dH_2O using an ultrasonic cell disrupter equipped with a 400W microtip probe (Ultrasonic Get 750, USA), then centrifuged at 4000× g (Sigma Laborzentrifugen Gmbh, Germany) for 5min; eventually, the supernatant was eliminated using a rotary evaporator (Meyer et al., 1982). For toxic activity detection, the dried cyanobacterial extract was dissolved in seawater (36g of sea salts per liter), and the following concentrations were prepared: 250, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, and 5000µg.mL^{-1}. The number of dead shrimps that was placed in five vials (10 shrimps per vial) was counted and the percentage of mortality was calculated.

MC–LR determination using high-performance liquid chromatography (HPLC)

The standard cyanobacterial toxin MC–LR, which was used throughout the present study, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Assessment of the microcystin variant MC–LR was conducted according to the protocol reported by Amé et al. (2003). Briefly, 20mg of *W. prolifica* dried biomass was placed in an Eppendorf tube, extracted with 1.5mL of 5% acetic acid, and sonicated for 5min using a 400W ultrasonic microtip probe. Subsequently, the suspension was centrifuged at 4000× g for 7min. The supernatant was collected and the residue was re-extracted to ensure thorough extraction. The mixed supernatants were centrifuged at 4000× g for 20min. The final extract of *W. prolifica* was applied to a C-18 solid-phase extraction cartridge (strata C18, 500mg. 3mL^{-1}, Phenomenex Inc., California, USA), which was previously conditioned with 10mL of methanol and 10mL of 5% acetic acid. The cartridge was washed three times with 10mL of 10%, 20%, and 30% methanol (*v/v*) and the MC–LR toxin was eluted with 10 ml of methanol of HPLC analytical grade. The elute was evaporated to dryness at 40°C and then re-suspended in 200µL of methanol, prior to HPLC analysis. The HPLC system used for MC–LR determination was a Perkin-Elmer series 200 system (USA) equipped with a quaternary pump, a UV diode array detector set at 238nm, and a C18 column chromatography ODS Phenomenex (250× 4.6mm, 5µm). The HPLC analysis was performed using acetonitrile with 0.05% trifluoroacetic acid (TFA) (*v/v*) as the mobile phase A and water with 0.05% TFA (*v/v*) as the mobile phase B, in the following gradient mode: 0–5min: 30% A + 70% B; 5–15min: 35% A + 65% B; 15–17min: 70% A + 30% B; 17–19min: 100% A + 0.0% B; and 19–22 min: 30% A + 70% B. The flow rate was set at 1mL.min^{-1}.

Statistical analysis

All treatments were carried out in triplicate and the results are expressed as the mean ± standard error (SE). Statistical analysis was primarily conducted using one-way analysis of variance. All significance statements were based on $P< 0.05$.

Results

Antimicrobial activity of the W. prolifica extracts

The antibacterial activities of the crude *W. prolifica* extracts against the various strains of foodborne pathogenic bacteria are illustrated in Table 1. The cyanobacterial ethanol, diethyl ether, chloroform, and hexane extracts showed significant antibacterial effects toward all tested bacterial strains, whereas the *W. prolifica* aqueous extract had antibacterial activity against *S. aureus* exclusively (also exhibiting the lowest inhibition zone among all treatments: 7.3±0.058mm). The highest antibacterial activities were obtained from the chloroform, diethyl ether, and hexane extracts against *P. aeruginosa*, with average inhibition zone values of 16.3±2.51, 15.8±2.05, and 15.2±1.04mm, respectively, followed by the hexane extract toward *S. typhi* and *B. cereus*, with average inhibition zone values of 15±0.86 and 14.5±2.65mm, respectively.

The minimum inhibitory concentrations of the different crude *W. prolifica* extracts against the foodborne pathogenic bacteria are shown

in Table 2. More interestingly, the most potent antibacterial activities were distinctly recorded against *St. aureus*, *S. typhi*, and *P. aeruginosa* from the hexane extract (average MIC values, 0.4±0.06, 0.4±0.08, and 0.4±0.04mg.mL^{-1}, respectively), followed by the diethyl ether and chloroform extracts against *P. aeruginosa*, with average MIC values of 0.4±0.06 and 0.5±0.04mg.mL^{-1}, respectively. Moreover, the aqueous extract in general exhibited the lowest antibacterial activity toward all tested strains, particularly *B. cereus*, *E. coli*, and *K. pneumoniae*, for which the average MIC values were 6.7±0.48, 5.8±52, and 5.8±0.52mg.mL^{-1}, respectively.

The antifungal activity of the crude *W. prolifica* extracts against the different mycotoxigenic fungi investigated in the present study is presented in Table 3. The cyanobacterial aqueous, diethyl ether, chloroform, and hexane extracts showed a broad range of significant antifungal activities against all tested fungal strains. However, the ethanol extract had antifungal potential toward all fungi but *A. parasiticus* and *A. ochraceus*. More specifically, the highest antifungal activities were recorded against *A. carbonarius* and *F. proliferatum* using the chloroform and hexane extracts, with average inhibition zone values of 15.2±1.25 and 14.5±0.5mm, respectively. The lowest antifungal effects were observed against *P. verrucosum* and *A. parasiticus* from the ethanol and chloroform extracts, with average inhibition zone values of 7.3±0.58 and 7.3±0.28mm, respectively.

TABLE 1. Antibacterial activity of the crude *W. prolifica* extracts against the foodborne pathogenic bacteria investigated in the present study

Bacterial strains	Inhibition zone (mm)*					
	Ceftriaxone (+ve control)	Aqueous	Ethanol	Diethyl ether	Chloroform	Hexane
B. cereus	17.2±1.04^{a}	0.0	12.7±2.25^{c}	11.3±1.04^{d}	13.5±1.50^{bc}	14.5±2.65^{b}
St. aureus	15.8±1.44^{a}	7.3 ± 0.58^{e}	11.8±1.44^{c}	10.8±2.02^{d}	12.8±1.04^{b}	12.5±1.50^{b}
E. coli	13.0±2.18^{a}	0.0	12.7±1.25^{ab}	11.0±0.50^{c}	12.2±1.25^{b}	13.2±0.58^{a}
S. typhi	19.2±1.25^{a}	0.0	12.0±0.50^{c}	9.5±0.50^{d}	10.0±0.50^{d}	15.0±0.86^{b}
P. aeruginosa	28.2±1.25^{a}	0.0	13.2±1.04^{d}	15.8±2.05^{bc}	16.3±2.51^{b}	15.2±1.04^{c}
K. pneumoniae	17.7±1.04^{a}	0.0	12.3±1.25^{c}	9.7±0.76^{d}	10.5±0.50^{d}	13.7±1.25^{b}

*Data are expressed as the mean±SE (n= 3).

Mean values with different superscript letters within the same row were significantly different at P< 0.05.

TABLE 2. Minimum inhibitory concentration (MIC) values of the crude *W. prolifica* extracts against the foodborne pathogenic bacteria tested in this study

Bacterial strains	MIC values (mg.mL^{-1})*				
	Aqueous	Ethanol	Diethyl ether	Chloroform	Hexane
B. cereus	6.7±0.48^{a}	0.8±0.14^{bc}	0.9±0.08^{b}	0.6±0.14^{d}	0.7±0.09^{c}
St. aureus	2.0±0.21^{a}	0.8±0.14^{bc}	0.9±0.08^{b}	0.7±0.12^{c}	0.4±0.06^{d}
E. coli	5.8±0.52^{a}	0.8±0.14^{bc}	0.9±0.08^{b}	0.6±0.14^{c}	0.7±0.08^{c}
S. typhi	4.2±0.36^{a}	0.8±0.06^{b}	0.9±0.14^{b}	0.8±0.11^{b}	0.4±0.08^{c}
P. aeruginosa	3.3±0.48^{a}	0.8±0.11^{b}	0.4±0.06^{c}	0.5±0.04^{c}	0.4±0.04^{c}
K. pneumoniae	5.8±0.52^{a}	0.9±0.14^{c}	1.5±0.28^{b}	0.8±0.14^{cd}	0.7±0.08^{d}

*Data are expressed as the mean±SE (n= 3).

Mean values with different superscript letters within the same row were significantly different at P < 0.05.

TABLE 3. Antifungal activity of the crude *W. prolifica* extracts against the mycotoxigenic fungi investigated in the present study

Fungal strains	Inhibition zone (mm)*					
	Miconazole (+ve control)	Aqueous	Ethanol	Diethyl ether	Chloroform	Hexane
A. flavus	21.5±1.0^{a}	8.5±0.86^{d}	9.2±0.28^{c}	9.8±0.58^{b}	8.0±0.50^{e}	8.3±0.58^{de}
A. parasiticus	20.2±2.25^{a}	9.7±0.76^{b}	0.0	8.5±0.50^{c}	7.3±0.28^{d}	8.5±0.86^{c}
A. niger	20.2±1.52^{a}	11.2±0.76^{d}	8.8±0.28^{e}	13.3±1.04^{b}	13.8±0.76^{b}	12.3±0.76^{c}
A. carbonarius	15.5±1.0^{a}	12.8±1.04^{c}	7.7±0.58^{d}	13.5±0.50^{bc}	15.2±1.25^{a}	14.0±0.50b
A. ochraceus	14.3±1.04^{a}	12.0±0.50^{b}	0.0	9.2±0.58^{d}	9.8±0.76^{c}	8.0±0.50^{e}
A. westerdijikia	13.7±1.04^{a}	9.7±0.76^{b}	7.8±0.28^{d}	8.5±0.50^{c}	7.5±0.50^{d}	9.3±1.04^{b}
F. proliferatum	10.8±0.58^{bc}	10.5±0.5^{c}	10.7±1.04^{bc}	11.0±0.5^{b}	8.5±0.5^{d}	14.5±0.5^{a}
P. verrucosum	19.8±2.56^{a}	11.7±1.04^{b}	7.3±0.58^{e}	10.0±0.5^{c}	10.3±0.76^{c}	8.2±0.76^{d}

*Data are expressed as the mean±SE (n= 3).

Mean values with different superscript letters within the same row were significantly different at P< 0.05.

As shown in Table 4, the highest antifungal activities of the crude *W. prolifica* extracts were detected against *A. carbonarius* and *F. proliferatum* from the chloroform and hexane extracts, with an average MIC value of 0.3±0.04mg.mL^{-1}, followed by the hexane extract toward *A. niger*, with an average MIC value of 0.4±0.06mg.mL^{-1}. In contrast, the cyanobacterial ethanol and diethyl ether extracts had the highest MICs toward *A. parasiticus* and *A. flavus* with average values of 9.2±0.48 and 5.8±0.48mg.mL^{-1}, respectively.

Brine shrimp bioassay

The assessment of the toxicity of the heterocytous cyanobacterium *W. prolifica* using the brine shrimp bioassay showed that the highest percentage in shrimp mortality (83.3%) was detected at a concentration of 5000µg.mL^{-1}. Furthermore, all *W. prolifica* concentrations lower than 2500µg.mL^{-1} caused a mortality below 50%. The lethal dose (LC_{50}) of the *W. prolifica* extracts was 2800µg.mL^{-1}.

MC–LR assessment

Based on the HPLC chromatogram analysis (Fig. 3A), the standard MC–LR was detected at a retention time of 7.58 min, whereas this peak was not detected for the *W. prolifica* extract at the same time (Fig. 3B).

TABLE 4. Minimum inhibitory concentration (MIC) values of the crude *W. prolifica* extracts against the mycotoxigenic fungi tested in this study.

Fungal strains	MIC values (mg.mL^{-1})*				
	Aqueous	Ethanol	Diethyl ether	Chloroform	Hexane
A. flavus	2.0±0.28^{c}	1.5±0.21^{d}	5.8±0.48^{a}	0.9±0.14^{e}	3.3±0.48^{b}
A. parasiticus	1.4±0.32^{d}	9.2±0.48^{a}	2.0±0.28^{c}	4.2±0.52^{b}	1.5±0.32^{d}
A. niger	0.75±0.12^{b}	3.3±0.48^{a}	0.6±0.08b^{c}	0.7±0.14^{b}	0.4±0.06^{c}
A. carbonarius	0.6±0.08^{c}	2.0±0.28^{a}	0.8±0.14^{a}	0.3±0.04^{d}	0.7±0.09b^{c}
A. ochraceus	0.9±0.14^{e}	4.2±0.86^{a}	1.5±0.36^{d}	2.0±0.30^{c}	3.3±0.48^{b}
A. westerdijikia	1.5±0.32^{d}	3.3±0.52^{b}	4.2±0.48^{a}	2.0±0.28^{c}	1.5±0.21^{d}
F. proliferatum	0.9±0.14^{b}	0.75±0.08^{c}	2.0±0.32^{a}	0.9±0.14^{b}	0.3±0.04^{d}
P. verrucosum	0.75±0.08^{e}	4.2±0.48^{a}	1.5±0.28^{c}	0.9±0.14^{d}	3.3±0.40^{b}

*Data are expressed as the mean ± SE (n= 3).

Mean values with different superscript letters within the same row were significantly different at P< 0.05.

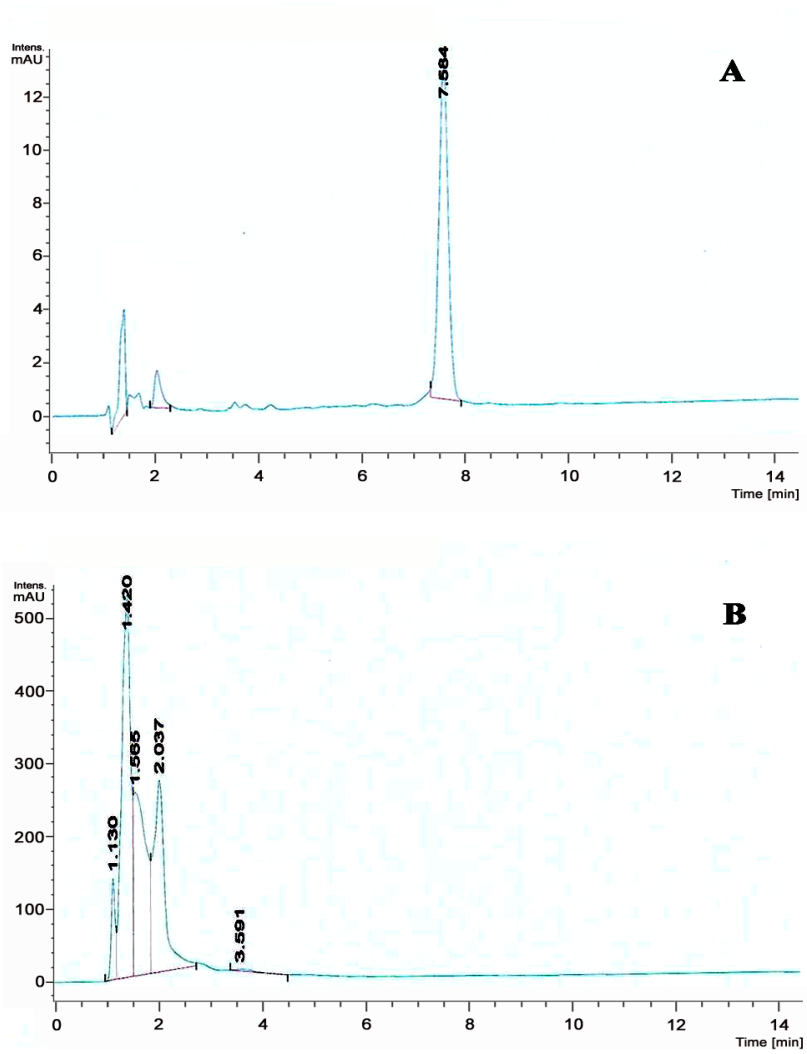

Fig. 3. HPLC chromatograms of the standard MC–LR (A) and the *W. prolifica* extract (B)

Discussion

Cyanobacteria, in general, possess structurally diverse metabolites with immense bioactive potential for use in drug synthesis (Sharma et al., 2011; Galica et al., 2017; Khalifa et al., 2021). In the present study, the different crude extracts of *W. prolifica* significantly exhibited potent antibacterial and antifungal activities toward all tested strains. This antimicrobial potential can be attributed to the wide array of its biologically active metabolites. A polyphasic study recently published by Saber et al. (2017) on this cyanobacterial species unveiled an array of low unsaturated and short fatty acyl chains, typically C16 and C18, which possibly played a role in the antimicrobial effect observed here. The antimicrobial activities of the fatty acids extracted from cyanobacteria, and also seaweeds (e.g., Mohamed & Saber, 2019 and the references therein), have already been discussed in several previous studies (e.g., Soltani et al., 2005; El-Sheekh et al., 2008 and the references therein). The mode of action of these antimicrobial substances is basically different from the metabolite *per se*. Nowruzi & Porzani (2021) suggested that the cyanobacteria-derived bioactive metabolites could interfere with signal transduction (either via the activation or blockage of ionic channels or by targeting signaling proteins), resulting in apoptosis via the disruption of cytoskeletal proteins and the inhibition of membrane transporters, receptors, and enzymes.

In the present study, *W. prolifica* extracts had an LC_{50} value above 1000µg.mL^{-1}, as assessed using the brine shrimp bioassay (USEPA, 1984);

thus, it can be considered as a "non-toxic" species. According to Meyer's toxicity index, extracts with an LC_{50} dose< 1000µg.mL^{-1} are considered as toxic, whereas those with an LC_{50}>1000µg.mL^{-1} are considered as non-toxic (Meyer et al., 1982). Accordingly, Clarkson et al. (2004) pointed out that plant extracts with an LC_{50} of 500–1000µg. mL^{-1} are weakly toxic, those with an LC_{50} of 100–500µg.mL^{-1} are moderately toxic, and those with an LC_{50} of 0–100µg.mL^{-1} are highly toxic.

The *W. prolifica* strain isolated from the hyper-arid desert habitats of Egypt did not produce MC–LR, as assessed based on HPLC analysis. However, Abed et al. (2013) suggested the biosynthesis of microcystins in three freshwater *W. prolifica* strains isolated from the Tigris River based on the detection and amplification of the *mycE* gene. In this sense, we can consider that cyanobacteria from the desert drylands are a reasonable target for future research work on the detection of cyanotoxins. In agreement with our observations, Kust et al. (2018) assessed the presence and/or absence of cyanotoxins (including MCs) in 311 non-planktic nostocalean taxa and found the rare occurrence of the common cyanotoxins, which is in contrast with the cyanotoxin producers of the phylogenetically closely related planktic species. In a similar pattern, Řeháková et al. (2014) reported that *Nodularia* species with a soil origin were rarely recognized as nodularin producers. Powell et al. (2015) and Cirés et al. (2017) also confirmed the lack of desert-dwelling cyanobacterial isolates, and molecular studies showed strong evidence of the presence of cyanotoxin producers in the biological soil crusts of drylands. Furthermore, it has been established that the biosynthesis of MCs is primarily strain dependent and that their structural diversity is substantially linked to variability in the coding of the MC synthetase genes among the different cyanobacterial strains (Dittmann et al., 2015), as well as to environmental triggers (e.g., Monchamp et al., 2014; Puddick et al., 2016).

Conclusions

The heterocytous cyanobacterium *W. prolifica* produces a wide range of potent antifungal and antibacterial metabolites. However, further in-depth studies are necessary to identify the mechanism and mode of action of these biologically active metabolites, to broaden our understanding of this phenomenon and to strengthen our findings at the molecular level. Moreover, this interesting species did not produce the microcystin variant MC–LR and was "non-toxic," as assessed using the brine shrimp bioassay. Therefore, it can be recommended as a sustainable multifunctional candidate in the pharmaceutical industry in Egypt.

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النشاط المضاد للميكروبات للبكتيريا الخضراء المزرقة ذات الحويصلات المغايرة *Westiellopsis prolifica* وتقييم قدرتها على التخليق الحيوي للمادة السامة microcystin–LR

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مؤخرًا تم تسجيل البكتيريا الزرقاء المخضرة ذات الحويصلات المغايرة *Westiellopsis prolifica* في مصر، لكن معرفتنا للتطبيقات التكنولوجية والطبية الحيوية المتنوعة لهذا النوع من البكتيريا الزرقاء المخضرة لا يزال موضوعًا قيد الدراسة. في هذا البحث، تم اختبار القدرة المضادة للميكروبات لسلالة *W. prolifica* معزولة من بيئة صحراوية شديدة الجفاف ضد ستة أنواع مختلفة من البكتيريا المسببة للأمراض والمنقولة بالغذاء (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*)، وثمانية أنواع مختلفة من الفطريات السامة (*Aspergillus carbonarius*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *A. westerdijikia*, *Fusarium proliferatum*, and *Penicillium verrucosum*)، بالإضافة إلى تقييم السمية المحتملة لهذا النوع والتخليق الحيوي للمادة السامة الأكثر شيوعًا microcystin–LR بإستخدام تقنية الكروماتوجرافيا السائلة ذات الأداء العالي. تم تقييم النشاط المضاد للميكروبات للمستخلصات المختلفة للبكتيريا الزرقاء المخضرة *W. prolifica* بإستخدام طريقة إنتِشار القُرُص، كما تم تحديد الحد الأدنى من فَعَّالِيَّة التراكيزات المثبطة (MICs). كشفت النتائج التي تم التوصل إليها إلى أن مستخلصات البكتيريا الزرقاء المخضرة *W. Prolifica* لها قدرة فعالة مضادة للميكروبات ضد جميع السلالات البكتيرية والفطرية بمتوسط قيم تتراوح من 0.4–6.7 و0.3–9.2 ملليجرام/ ملي على التوالي. هذه القدرة المضادة للميكروبات يمكن أن تُعزى إلى التأثيرات التآزرية المشتركة للمركبات المختلفة والنشطة بيولوجيًا لهذا النوع. علاوة على ذلك، وجد أن سلالة *W. prolifica* غير منتجة للمادة السامة microcystin–LR وأيضًا غير سامة بناءً على الاختبار الحيوي للجمبرى. وبناء عليه يمكن اعتبار البكتيريا الزرقاء المخضرة ذات الحويصلات المغايرة *Westiellopsis prolifica* مرشحًا مستداما متعدد الوظائف في تطوير صناعة الأدوية في مصر.