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Original article

Anti-enterococcal and anti-oxidative potential of a thermophilic cyanobacterium, *Leptolyngbya* sp. HNBGU 003

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A R T I C L E   I N F O

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A B S T R A C T

Enterococci, the opportunistic pathogens, pose several serious and life-threatening infections such as urinary tract infections, sepsis, and endocarditis. The situation is worsening due to the development of drug resistance in these pathogens against several antibiotics. The addition of anti-enterococcal compounds with antioxidant activity in fermented and packaged food may help prevent the transmission of food-borne enterococcal infections. Scientists are in continuous search of such compounds from various sources. Hence, the present study has tested the diethyl ether extracts of thermophilic cyanobacteria, selected based on a previous study, against the multidrug-resistant and -sensitive strains of *Enterococcus faecium*. Out of the eleven tested extracts, 72% have shown anti-enterococcal activity against both strains. Among the extracts with anti-enterococcal activity, the diethyl ether extract of *Leptolyngbya* sp. (DEEL-3) inhibited the growth of VRE in a dose-dependent manner with a minimum inhibitory concentration of 2.0 mg mL^{-1}. The DEEL-3 has also shown its antioxidant potential in terms of DPPH scavenging with an IC_{50} of 3.16 mg mL^{-1}. The organism was named *Leptolyngbya* sp. HNBGU 003 based on 16SrRNA sequence homology analysis and morphological features. Further, the GC–MS analysis of the DEEL-3 has revealed the predominance of two phenolic compounds, phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) and tris(2,4-di-*tert*-butylphenyl) phosphate, in it. Thus, the anti-enterococcal and antioxidant activity of DEEL-3 may be attributed to these phenolics, which may be isolated and developed as food additives.

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1. Introduction

Enterococci have emerged as the third most common source of hospital-acquired bloodstream infections (Oprea and Zervos, 2007). Besides, these organisms can also cause urinary tract infections, sepsis, and endocarditis (Sood et al., 2008). More than twelve species are known to cause enterococcal infections in humans worldwide. However, *Enterococcus faecium* and *Enterococcus faecalis* are the most common species responsible for about 90–95% of enterococcal infections (García-Solache and Rice, 2019). Further, the development of multidrug resistance including vancomycin resistance has left physicians with very limited choices of antibiotics for the treatment of infections caused by *Enterococci* (Karaiskos et al., 2019). The vancomycin-resistant enterococci impose severe economic loss to society as they pose serious threats to the patients admitted in intensive care units (ICUs) as well as those with compromised immunity (MacDougall et al., 2020; Ben Braïek and Smaoui, 2019; Puchter et al., 2018). These organisms have been categorized in the high priority list of antibiotic-resistant bacteria by World Health Organization (Melese et al., 2020).

Enterococci are food-borne pathogens and can be transmitted in humans through the consumption of contaminated food materials. These organisms can colonize in both raw and fermented food products such as meat, vegetables, cheese, and sausages (Giraffa, 2002). The use of natural food additives having both anti-enterococcal and antioxidant properties in food products can easily reduce the transmission of enterococci through the food chain (Lucera et al., 2012). Microorganisms have always been the major source of novel natural products with such bioactive properties

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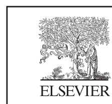

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(Cragg and Newman, 2013). Among the microorganisms, cyanobacteria, the oxygenic photosynthetic organisms, have emerged as the newer and least explored source of secondary metabolites with both anti-enterococcal and antioxidant compounds (Singh et al., 2015, 2011; Tyagi et al., 2018). The metabolite spectrum of cyanobacteria vary due to the adaptation of strains in their respective habitats and offers significant diversity to be explored by the researchers for various purposes (Demay et al., 2019).

Therefore, the present study has been carried out to determine the anti-enterococcal potential of diethyl ether extracts obtained from eleven different thermophilic cyanobacteria. The selected extract with potent anti-enterococcal activity was tested for its anti-oxidant potential as well as chemical profiling by GC–MS analysis.

2. Materials and methods

2.1. Cyanobacterial strains and extract

The diethyl ether extracts of eleven cyanobacterial strains isolated from Taptkund hot spring, Badrinath (30°74′48″ N and 79°49′18″ E; elevation 3250 m) situated in Garhwal Himalaya, Uttarakhand, India, were used in the present study. The cyanobacterial strains were characterized based on their morphological and morphometric features. These extracts were selected based on their activity against a drug-sensitive strain of *S. aureus* in a primary screening (Tyagi and Singh, 2020). All the cyanobacterial strains used in this study are being maintained in the Cyano-Biotech Lab, Department of Microbiology, HNB Garhwal University, Srinagar (Garhwal), India.

2.2. Cultivation of cyanobacteria and preparation of extract

All the eleven cyanobacterial strains were cultivated in Castenholz- D media as per the conditions described by Tyagi and Singh (Tyagi and Singh, 2020). All the chemicals used for the preparation of culture media were procured from Hi-Media Laboratories Pvt. Ltd, Mumbai, India. The cyanobacterial biomass was harvested in the late stationary phase, dried at 50 ± 2 °C, powdered, and extracted by the freeze–thaw method using the diethyl ether as the solvent. The extracts were collected, evaporated to dryness, re-dissolved in 10% DMSO (Di-methyl-sulfoxide) maintaining the concentration of 100 mg mL^{-1}, and stored at 4 °C until further use.

2.3. Identification of the selected cyanobacterium

The selected cyanobacterium was identified using colonial appearance, morphology, morphometric and molecular features as described earlier (Singh et al., 2015; Tyagi and Singh, 2020). For molecular identification, the partial 16S rDNA region of the organism was amplified using Cya106F and Cya781R primers (Eurofin, India) and sequenced using the sanger sequencing method. The obtained sequence was matched with the 16S rDNA sequences available in the NCBI database using BLASTn analysis. The closest sequences were aligned and used for the preparation of the phylogenetic tree with MEGA X software (version 10.1.6).

2.4. Enterococcal strains

The drug-sensitive and multidrug-resistant strains of *Enterococcus faecium* (EF-DS and EF-DR) used in the present study were obtained from VCSGG Institute of Medical Sciences, Srinagar (Garhwal), Uttarakhand, India, and characterized in the laboratory based on standard biochemical tests as well as their antibiogram (CLSI, 2010).

2.5. Anti-Enterococcal activity

2.5.1. Qualitative assay

The anti-enterococcal activity of cyanobacterial extracts was tested by the agar well diffusion method. Briefly, the 200 μl enterococcal suspension with the turbidity as per 0.5 McFarland standard was uniformly spread on the surface of Mueller Hinton Agar (Hi-Media, India) to prepare enterococcal lawn and dried. Subsequently, the wells of 08 mm diameter were created in agar employing agar borer. Each test-well received 200 μl of diluted cyanobacterial extract, whereas the positive, and negative control wells received the same volume of amikacin/chloramphenicol solution, and dimethyl sulfoxide, respectively. The plates containing enterococcal lawn and cyanobacterial extracts were kept at 4 °C for the diffusion of extracts, and subsequently at 37 ± 2 °C for 20–24 h to detect the enterococcal growth inhibition zones.

2.5.2. Quantitative assay

The quantitative anti-enterococcal assay was performed to determine the minimum inhibitory concentration (MIC) of the selected cyanobacterial extract using the macro broth dilution method as described in CLSI guidelines (CLSI, 2010). The minimum concentration of the extract responsible for the disappearance of visible enterococcal growth was considered MIC. The enterococcal culture treated with MIC of the cyanobacterial extract was subcultured on fresh nutrient agar (Hi-Media, India) plates to detect the bactericidal/ bacteriostatic nature of the extract.

2.6. Antioxidant activity using DPPH free radical

Antioxidant activity of the selected cyanobacterial extract was measured with DPPH (2.2-diphenyl-1-picrylhydrazyl) scavenging assay using the method of Assuncao (Assunção et al., 2017). Concisely, 1.8 ml of 0.06 mM DPPH (CDH, India) dissolved in methanol was added to the 0.2 ml of the selected cyanobacterial extract with six different concentrations (1, 5, 10, 15, 20 and 30 mg/mL). A blank solution was prepared by adding 0.2 ml of DMSO to 1.8 ml DPPH solution. The absorbance of the reaction mixture, as well as the blank, was measured after 15 min at 515 nm using the UV–VIS double beam spectrophotometer (Systronics AU 2701, India). While the same amount of DPPH mixed with same volume of synthetic antioxidant 3, 5-di-*tert*-butyl-4-hydroxytoluene (BHT) solution was used as the positive control. The DPPH radical scavenging ability of the extract and the positive control was expressed in terms of inhibition percentage and calculated with the equation given below.

$$Inhibition(\%) = \frac{(A_{DPPH} - A_{sample})}{A_{DPPH}} \times 100$$

where A_{DPPH} is the absorbance of blank and A_{sample} is the absorbance of the reaction mixture containing cyanobacteria extract after 15 min.

A curve was plotted between the concentration of extract and corresponding inhibition percentage. The concentration (%, v/v) of the extract that reduces the initial absorbance of DPPH by 50% was calculated by logarithmic regression with R^2 value of 0.98 and considered as IC_{50}.

2.7. GC–MS analysis

The selected cyanobacterial extract was subjected to chemical profiling by the coupled GC–MS (Agilent GC 7890B; MS 5977B MSD, USA) instrument following the method of Abdel-Aal with

slight modifications (Abdel-Aal et al., 2015). The GC–MS instrument was operated with the following conditions; DB-5 MS (5% phenyl methyl polysiloxane) column with 30 m × 250 μm × 0.25 μm dimensions, 3 min solvent delay, 1 μl injection volume-, and helium as carrier gas - with the pulsed split-less mode at 3 ml.min^{-1}. Total run time was 42.5 min where the initial temperature was 60 °C with rising rate of 8 °C min^{-1} and maximum up to 280 °C with the hold time of 10 min. The ion source temperature was set at 230° C (maximum 250) and the quadruple temperature was set at 150 °C (maximum 200).

An inbuilt mass detector was used for the mass detection of the peaks. The detector was operated with the ionizing energy of 70 eV with the scanning range of 30–800 *m*/*z* values. The electron multiplier voltage (EM voltage) was maintained at 1065.7 with a gain factor of 1.00. Mass-Hunter/NIST17 software with database library was used for the identification of the observed peaks.

2.8. Statistical analysis

One-way ANOVA (Analysis of variance) followed by LSD (Least significant difference) was used for comparing the sizes of enterococcal growth inhibition zone and DPPH scavenging activities.

3. Results

3.1. Antibiogram of VRE

Fig. 1 shows the antibiogram of multidrug sensitive (EF-DS) and resistant (EF-DR) strains of *E. faecium* used in the study. The results indicate that the strain EF-DS is sensitive to all the standard antibiotics tested as the size of growth inhibition was > 18 mm in all cases. The growth inhibition zones of < 18 mm, i.e., antibiotic resistance, were observed in EF-DR strain against all the tested antibiotics except amikacin and chloramphenicol (CLSI, 2010).

3.2. Anti-enterococcal activity

Out of the 11 diethyl ether extracts prepared from different cyanobacterial strains, 09 extracts exhibited anti-enterococcal activity at the dose of 2.0 mg against both multidrug sensitive (EF-DS) and resistant (EF-DR) strains of *E. faecium* (Table 1). Among these only 02 extracts showed the growth inhibition zone of ≥ 18 mm against EF-DS while only one extract, i.e., diethyl ether extracts of *Leptolyngbya* sp. (DEEL-3), resulted in the same size (≥18 mm) of growth inhibition zone against EF-DR.

A dose-dependent increase was observed in the anti-enterococcal potential of DEEL-3 in the range of 2.5–3.5 mg against EF-DR strain ($p < 0.05$; Fig. 2a). The size of enterococcal growth inhibition caused by DEEL-3 at doses 2.0 and 2.5 mg was comparable to that caused by the standard antibiotics chloramphenicol and amikacin. The quantitative bioassay with the same extract revealed the minimum inhibitory concentration (MIC) of 2.0 mg mL^{-1} against the VRE (Fig. 2b). Further, the subculture of the pathogen treated with DEEL-3 at MIC indicated the bactericidal nature of the extract (Fig. 2c).

3.3. Antioxidant activity

The antioxidant activity of any biological product may be determined by its capability to inhibit the activity of free radicals like DPPH, ABTS (3-ethylbenzothiazoline- 6-sulfonic acid), and hydroxyl radicals. Among these free radicals, DPPH is more commonly used as it is relatively more stable in comparison to others. The free radical scavenging activity of DEEL-3 using DPPH resulted in >80% inhibition at the concentration of 10 mg/mL (Fig. 3). The logarithmic regression of concentration-dependent inhibition of DPPH activity determined an IC_{50} of 3.16 mg/mL for DEEL-3 while 26 μg/mL for the positive control, BHT.

3.4. Molecular characterization of the selected cyanobacterium

The identity of the selected cyanobacterium, *Leptolyngbya* sp. was confirmed by its 645 bp long partial 16 s rDNA sequence homology analysis and morphological features. Fig. 4a shows the photomicrograph of the organism. The phylogenetic study of this cyanobacterium based on the partial 16S rDNA sequence showed that it was closest to *Leptolyngbya* sp. PKUAC-SCTA141 (Fig. 4b). The partial 16S rDNA gene sequence of the of *Leptolyngbya* sp. HNBGU 003 was submitted to the NCBI GenBank under the accession number, MT683109. This cyanobacterial strain is publically available through the National Agriculturally Important Microbial Culture Collection (NAIMCC) with accession number NAIMCC-C-00337.

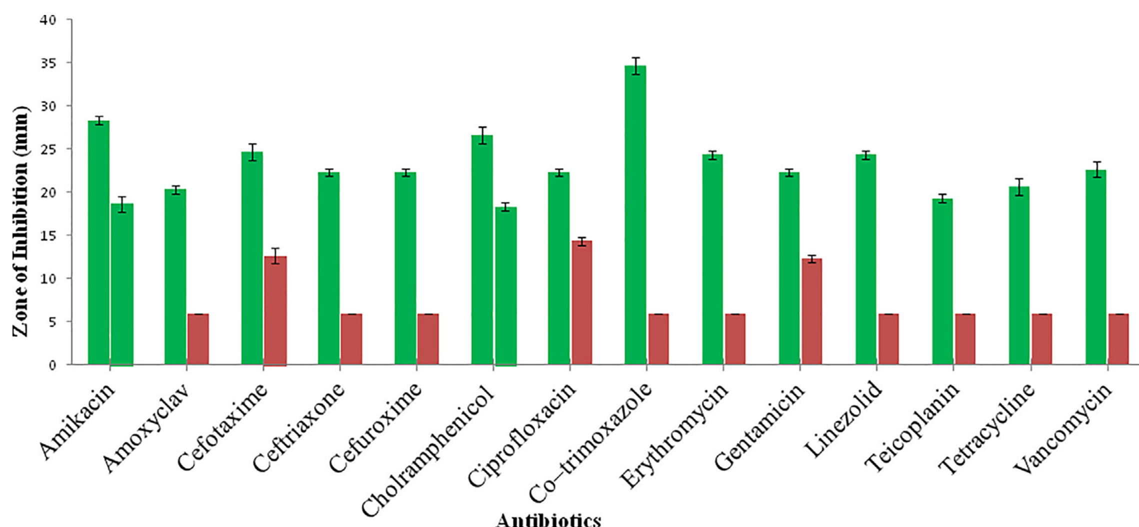

Fig. 1. Antibiogram of drug-sensitive and vancomycin-resistant *Enterococcus faecium* strains. The red colored bar represents the resistance whereas the green colored bar indicate the sensitivity to the particular antibiotics as per guidelines of CLSI.

Table 1
Antienterococcal activity of standard antibiotics and diethyl-ether extracts prepared from different cyanobacteria against drug sensitive and vancomycin resistant *Enterococcus faecium* (VRE) strains.

S.N	Antibiotics/Cyanobacteria	Dose	Size of growth Inhibition zone (mm) against	
			Drug sensitive E. faecium	Multidrug resistant *E. faecium*
1.	Chloramphenicol	30 µg	26.66 ± 1.52	18.33 ± 0.57
2.	Amikacin	10 µg	28.33 ± 1.15	18.66 ± 0.57
3.	*Leptolyngbya* sp. HNBGU 003	2.0 mg	19.66 ± 0.57	18.66 ± 0.57
4.	*Lyngbya lutea*	2.0 mg	17.66 ± 0.57	17.33 ± 0.57
5.	*Lyngbya mesotricha*	2.0 mg	ND	ND
6.	*Oscillatoria jasorvensis*	2.0 mg	ND	ND
7.	*Oscillatoria unigranulata*	2.0 mg	18.33 ± 0.57	18.33 ± 0.57
8.	*Phormidium* sp. 01	2.0 mg	18.66 ± 0.57	17.66 ± 1.15
9.	*Phormidium* sp. 02	2.0 mg	17.66 ± 0.57	17.00 ± 1.73
10.	*Phormidium* sp. 03	2.0 mg	ND	ND
11.	*Phormidium tenue*	2.0 mg	15.00 ± 0.00	15.66 ± 1.52
12.	*Phormidium usterii*	2.0 mg	16.66 ± 0.57	17.66 ± 1.15
13.	*Symploca parietina*	2.0 mg	16.33 ± 0.57	16.66 ± 1.52

Note: The values represent the mean ± SD (n = 3). ND = Not detected.

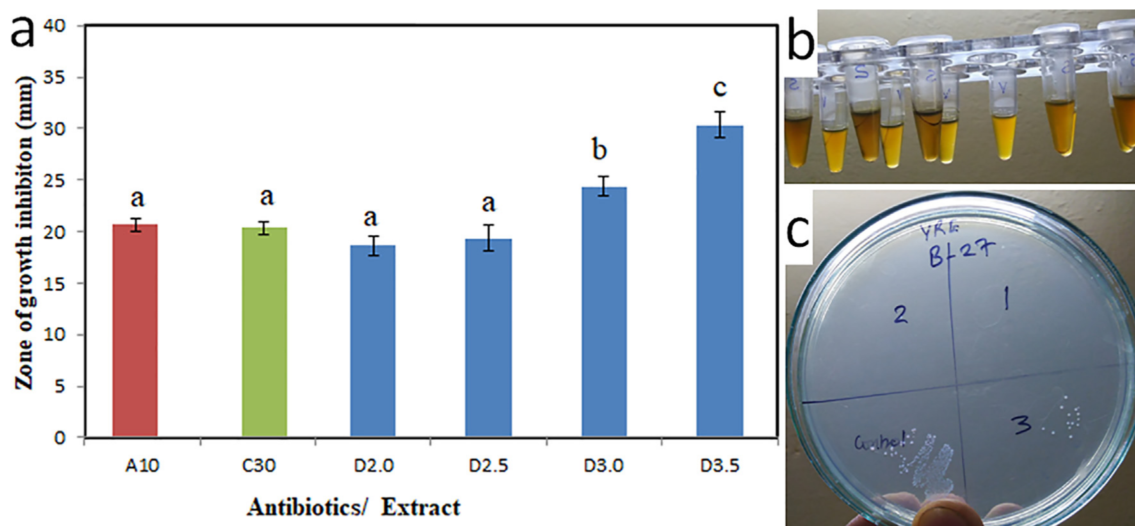

Fig. 2. Dose dependent anti-enterococcal activity of DEEL-3 (A10 = Amikacin; 10 µg, C30 = Chloramphenicol; 10 µg, D2.0 - D3.5 = DEEL-3; 2.0–3.5 mg/mL) (**a**), Macro broth dilution assay for the MIC determination of DEEL-3 (**b**), Subculture of VRE at MHA containing MIC of DEEL-3 (**c**). The values marked with the different letters are significantly different from each other ($p < 0.05$).

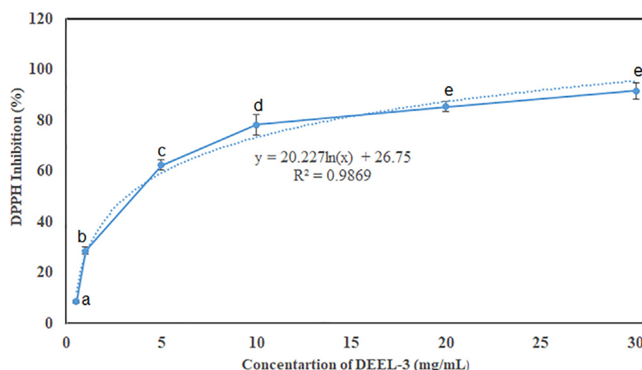

Fig. 3. DPPH scavenging activity of DEEL-3 at different concentration. The values marked with the different letters are significantly different from each other ($p < 0.05$).

3.5. GC–MS analysis

GC–MS analysis of the DEEL-3 was performed to determine its chemical profiling and the resulting chromatogram is shown in Fig. 5. The retention time, name of the corresponding compound, similarity index, molecular weight, and peak area of peaks observed in the chromatogram and having similarity index of > 80 with the compounds available in NIST17 mass spectral library, are mentioned in table 2. These peaks covered about 30% of the total peak area. The rest of the peaks covering about 70% of the total peak area were considered unidentified. Based on the peak area, the compounds identified in the tested extract belong to hydrocarbon (12.6%), phenolics (12.25%), hydrocarbon derivatives (4.1%), ester (2.1%), ether (0.05%), and alkaloid (0.66%) chemical classes. Among the phenolics, tris (2,4-di-*tert*-butylphenyl) phosphate with the peak area of 6.55% was recorded as the major compound followed by Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite with the peak area of 5.1% (Fig. 6).

4. Discussion

Cyanobacterial strains from different habitats have been reported to exhibit various bioactivities including anti-bacterial and antioxidant activities due to their diverse metabolite spectrum. However, the thermophilic cyanobacteria are minimally explored in this regard (Dobretsov et al., 2011). Several workers

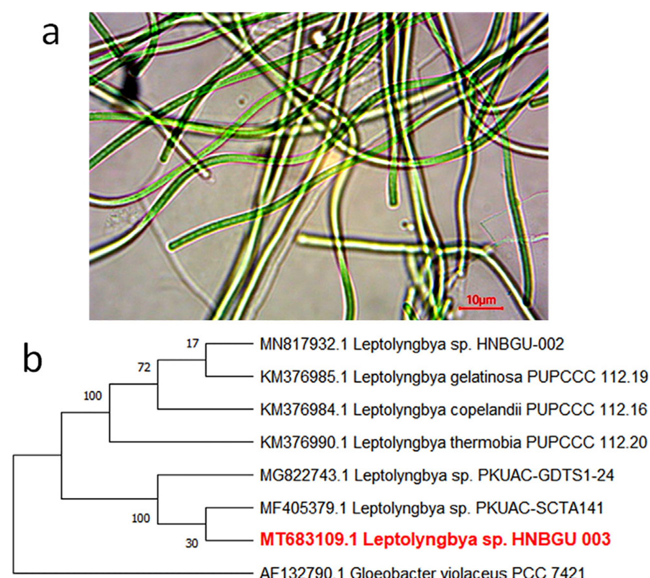

Fig. 4. Microscopic view (100x) of entangled *Leptolyngbya* sp. HNBGU 003 filaments growing in cultures (a), Phylogenetic tree of *Leptolyngbya* sp. HNBGU 003 based on partial 16S rDNA sequence (b).

have predicted the bioactive potential of thermophilic cyanobacteria due to their adaptation for survival in the hassled environment of hot springs (Badr et al., 2019). Though there are studies on the antibacterial potential of thermophilic cyanobacteria, only a few studies are available on their anti-enterococcal activity (Fish and Codd, 1994).

The present study initially investigated the anti-enterococcal capacity of diethyl ether extracts of eleven thermophilic cyanobacteria selected based on a previous study. Eight out of 11 (72%) cyanobacterial extracts exhibited anti-enterococcal activity. A similar finding on antibacterial activity of non-polar extracts prepared from thermophilic cyanobacteria against gram-positive bacteria has been reported by Dobretsov et al (2011). Thus, the result of the present study reconfirmed the anti-enterococcal potential of thermophilic cyanobacteria and strongly supported the idea of Badr et al (2019).

Unlike the previous studies, the present investigation also tested the anti-enterococcal potential of thermophilic cyanobacterial extracts against multidrug-resistant *E. faecium* (EF-DR). Moreover, all the extracts showing anti-enterococcal activity have almost similar potency against both multidrug-resistant and -sensitive strains of *E. faecium*. The results indicate that these extracts might have some anti-enterococcal compounds with an action mechanism different than that of standard antibiotics, in the active extracts. Thus, these extracts may be further tested against different drug-resistant enterococcal strains for the development of new anti-enterococcal agents.

Among the extracts with anti-enterococcal activity, only the diethyl ether extract of *Leptolyngbya* sp., DEEL-3, had shown > 18 mm zones of growth inhibition against both the enterococcal strains and hence was selected for further analysis. The dose-dependent response of the selected extract against vancomycin-resistant EF-DR strain confirmed the presence of an anti-enterococcal compound in it. Further, the MIC of DEEL-3 against EF-DR as determined by quantitative assay indicates the significant potential of the extract to be fractionated for isolation of anti-enterococcal compounds. The anti-enterococcal activity of diethyl ether extract of *Leptolyngbya* sp. HNBGU 002 (DEEL) against vancomycin-resistant *Enterococci* has been previously reported (Tyagi and Singh, 2020). However, the DEEL-3 has shown greater anti-enterococcal potential against EF-DR strain than DEEL as evidenced by the results of the quantitative anti-enterococcal assay.

As enterococci are food-borne pathogens, the anti-enterococcal compounds with antioxidant activity have been used as food additives in fermented and packaged food items to prevent enterococcal infections (Aziz and Karboune, 2018; Ouerghemmi et al., 2017). Keeping this fact in view, DEEL-3 was also tested for its antioxidant potential. The antioxidant activity of DEEL-3 in terms of DPPH free radical scavenging has shown significant antioxidant power of the extract with an IC_{50} of 3.16 mg/mL. Singh et al (2017) have shown the antioxidant activity in methanol extracts prepared from few strains of *Lyngbya* with somewhat similar IC_{50} values (2.72 mg/

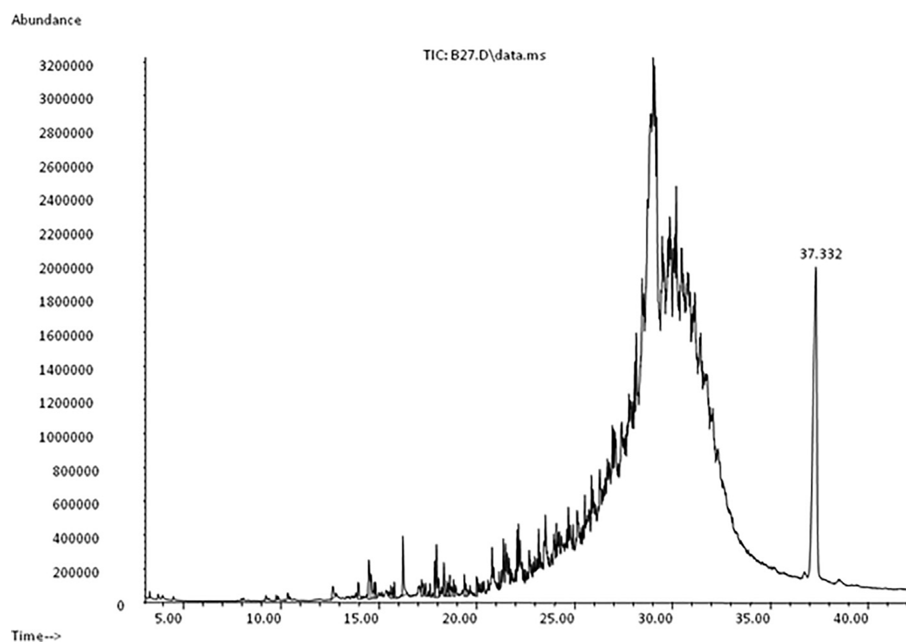

Fig. 5. GC–MS chromatogram of DEEL-3.

Table 2
Compounds identified from the GC–MS analysis of DEEL-3.

S. N	Retention Time (min)	Compounds	Quality	Mol Weight (amu)	Peak area (%)
1.	12.667	Dodecane	97	170.203	0.105
2.	13.967	Hexadecane, 2,6,11,15-tetramethyl-	90	282.329	0.123
3.	14.504	Tridecane	98	184.219	0.323
4.	16.241	Tetradecane	98	198.235	0.365
5.	17.205	2,6,10-Trimethyltridecane	96	226.266	0.078
6.	17.86	Pentadecane	97	212.25	0.169
7.	17.96	2,4-Di-*tert*-butylphenol	97	206.167	0.276
8.	18.346	Pentacosane	90	352.407	0.145
9.	19.378	Hexadecane	97	226.266	0.102
10.	19.462	Methoxyacetic acid, 2-tetradecyl ester	91	286.251	0.090
11.	20.292	Isopropyl tetradecyl ether	91	256.277	0.052
12.	20.804	Heptadecane	97	240.282	0.187
13.	21.375	Dodecane, 4,6-dimethyl-	92	198.235	0.306
14.	21.576	Tetradecane, 4-methyl-	86	212.25	0.219
15.	21.677	Carbonic acid, octadecyl vinyl ester	83	340.298	0.178
16.	22.079	1-Octadecene	99	252.282	0.200
17.	22.239	Tetracosane, 11-decyl-	83	478.548	0.324
18.	23.48	Tritetracontane	91	604.689	0.162
19.	23.531	7,9-Di-*tert*-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	95	276.173	0.310
20.	23.95	Sulfurous acid, dodecyl 2-propyl ester	91	292.207	0.108
21.	24.084	2-Methylhexacosane	87	380.438	0.133
22.	25.443	Nonadecane	90	268.313	0.105
23.	25.535	Hexadecane, 2,6,10,14-tetramethyl-	90	282.329	0.210
24.	25.963	Nonahexacontanoic acid	91	999.07	0.299
25.	26.014	1-Decanol, 2-hexyl-	91	242.261	0.166
26.	26.307	Nonadecane, 1-chloro-	93	302.274	0.663
27.	26.433	Octadecane	90	254.297	0.208
28.	26.517	Tetrapentacontane, 1,54-dibromo-	91	914.682	0.292
29.	26.945	Octacosyl trifluoroacetate	93	506.431	0.661
30.	27.003	Carbonic acid, eicosyl vinyl ester	91	368.329	0.550
31.	27.255	Octatriacontyl pentafluoropropionate	93	696.584	0.382
32.	27.901	Eicosane	95	282.329	0.741
33.	28.002	Valeramide, N-tetradecyl-	90	297.303	0.763
34.	28.086	1-Chloroeicosane	90	316.29	1.045
35.	28.253	Tetracosane	95	338.391	0.612
36.	29.21	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	94	646.451	5.106
37.	29.495	Hexacosane	95	366.423	1.211
38.	29.596	Docosane	96	310.36	1.196
39.	29.638	Heneicosane	94	296.344	0.592
40.	29.78	1-Tetracosene	96	336.376	1.987
41.	31.013	Ethanol, 2-(octadecyloxy)-	93	314.318	0.923
42.	30.208	Tricosane	95	324.376	3.243
43.	31.995	Octadecane, 1-iodo-	94	380.194	0.261
44.	37.332	Tris(2,4-di-*tert*-butylphenyl) phosphate	83	662.446	6.558

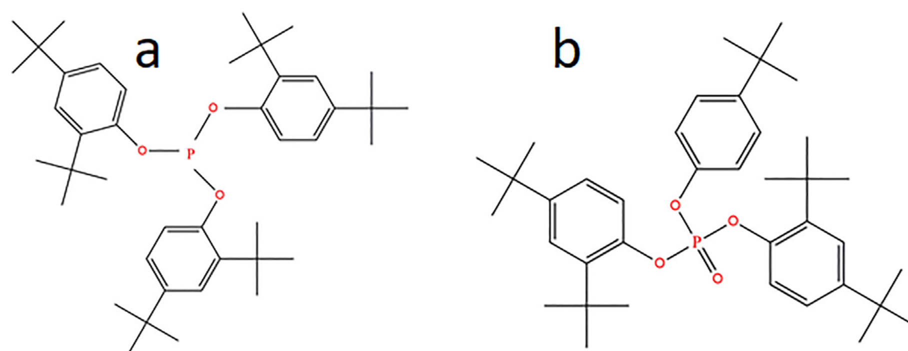

Fig. 6. Structure of two major compounds, phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) (a), and tris(2,4-di-*tert*-butylphenyl) phosphate (b) observed in GC–MS analysis of DEEL- 3.

mL) (Trabelsi et al., 2016). However, the antioxidant activity in the non-polar extract of cyanobacteria is not commonly reported.

The GC–MS analysis has been the very decent option in the past for the study of volatile compounds present in the biological extracts (Mu et al., 2019). In the present study, GC–MS chromatogram of DEEL-3 recorded two phenolic compounds; phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1), and tris(2,4-di-*tert*-butylphenyl) phosphate were reported as the major compounds based on its peak area percentage. These two phenolics have been previously isolated from few other cyanobacteria, actinomycetes, and *Lactococcus* sp. and are reported to exhibit selective antioxidant and antimicrobial activity against the broad range of pathogenic microbes in the previous studies (Sood et al., 2008; Trabelsi et al., 2016). A strong correlation between the antioxidant activity

and phenolic content in the cyanobacterial extract is well established by several workers (Abd El-Aty et al., 2014; Blagojević et al., 2018; Singh et al., 2017). Similarly, phenolic compounds of cyanobacterial origin have also been reported to exhibit antibacterial/ anti-enterococcal activity (Abd El-Aty et al., 2014; Singh et al., 2017). The phenolics may exhibit antibacterial activity by altering the permeability of bacterial cell membrane, and cell integrity (Ismail et al., 2020). Thus, the significant anti-enterococcal and antioxidant activity of DEEL-3 may be attributed to the synergistic effects of these two phenolic compounds. The high content of phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1), and tris(2,4-di-*tert*-butylphenyl) phosphate in the DEEL-3 make the extract, DEEL-3, an attractive source for the isolation of these phenolic compounds.

5. Conclusion

The diethyl ether extract of *Leptolyngbya* sp. HNBGU 003 (DEEL-3) may be used for the isolation of phenolic compounds, phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) and tris(2,4-di-*tert*-butylphenyl) phosphate, with anti-enterococcal and anti-oxidant activities. Such compounds may be developed as food additives which will reduce the transmission of food-borne enterococci.

Declaration of Competing Interest

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

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