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Research article

# Lipophilic bioactive compounds from thermophilic cyanobacterium *Leptolyngbya* sp. HNBGU-004: Implications for countering VRSA resistance

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ABSTRACT

Extremophiles thrive in extreme conditions, showcasing rich and unexplored diversity. This resilience hints at the existence of novel biochemical pathways and unique bioactive compounds. In contrast, the issue of drug resistance and excessive misuse of antibiotics in various settings, such as healthcare, agriculture, and veterinary medicine, has contributed to the emergence and spread of drug-resistant microorganisms.

In the present research, *Leptolyngbya* sp. HNBGU-004, was obtained from an extreme location, a hot water spring in the Garhwal Himalayan region of India. The lipophilic fraction derived from *Leptolyngbya* sp. HNBGU-004 exhibited significant inhibitory effects against vancomycin-resistant *Staphylococcus aureus* (VRSA), displaying a bactericidal concentration of 0.5 mg $mL^{-1}$. Furthermore, gas chromatography-mass spectrometry (GC-MS) analysis of the lipophilic extract unveiled the major constituents.

*Leptolyngbya* sp. HNBGU-004 holds significant promise as a primary source of potent anti-vancomycin-resistant *S. aureus* components. These findings emphasize the importance of *Leptolyngbya* sp. HNBGU-004 as a foundational source for use as both a synergistic and alternative agent against VRSA.

## 1. Introduction

Extremophiles, showcasing a rich and unexplored diversity, thrive in extreme conditions like high temperatures, acidic or alkaline environments, and high salinity. This resilience hints at the existence of novel biochemical pathways and unique bioactive compounds not typically found in organisms from milder environments [1–3]. Specifically, extremophiles, including those inhabiting hot water springs—an underexplored water source—offer a promising reservoir for bioactive compounds like antibiotics. The microorganisms in these springs have adapted to distinct nutritional, physiological, and metabolic conditions [4]. Moreover, extremophiles exhibit a remarkable trait of producing biomolecules that remain stable under extreme conditions, such as high temperatures or extreme pH levels. This stability becomes particularly advantageous in industrial processes and pharmaceutical applications, where maintaining the integrity of bioactive compounds is indispensable [1,5]. However, delving into the study of extremophiles is not without

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challenges. Cultivating these organisms in a laboratory setting proves difficult, and accessing their ecosystems poses unique hurdles. Nevertheless, overcoming these challenges necessitates innovative approaches and technologies, propelling scientific advancements in the process [6].

The importance of extremophiles in the context of the urgent need for new antibiotics arises from their unique ability to thrive in extreme environments. As antibiotic resistance becomes an increasingly critical global concern, the search for novel sources of antimicrobial compounds is paramount [3,7]. Extremophiles offer a promising frontier for the discovery of previously unknown bioactive molecules with the potential to combat antibiotic-resistant strains. Extremophiles yield the novel compounds or variations of known antibiotics, providing a valuable reservoir for combating drug-resistant microbial strains and revitalizing the arsenal of available antimicrobial agents [8]. The development of drug resistance in microorganisms is an observable and predictable phenomenon resulting from the principles of natural selection, governed by genetic plasticity, mutational adaptations, and the acquisition or alteration of gene expression [9]. The development of drug-resistant microflora has various consequences, in healthcare settings, it can lead to increased morbidity and mortality rates as infections become more difficult to treat. Drug-resistant microbial infections may require prolonged hospital stays, intensive treatments, and may even face higher risks of treatment failure. In a recent report, it was highlighted that bacterial antimicrobial resistance (AMR) was associated with approximately 4.95 million deaths in 2019 [10]. Among these deaths, around 1.27 million were directly attributable to bacterial AMR. If left unchecked, AMR has the potential to become a significant threat to public health going to be in next decade [11].

Furthermore, it is important to recognize that drug resistance extends phenomenally beyond pathogenic organisms, as common microflora and opportunistic pathogens have also evolved into formidable multidrug-resistant (MDR) pathogens [12]. It is also worth noting that drug-resistant microorganisms not only affect humans but also pose a significant threat to various species of wild, companion, and agricultural animals [13]. The utilization of antibiotics in animal husbandry and agricultural crop production can greatly contribute to the rise and dissemination of drug-resistant microflora. This practice establishes an environment in which selective pressure promotes the survival and propagation of resistant strains. The existence of these resistant microorganisms among farmland animals poses a significant threat to human health along the food chain. The potential exists for the transmission of resistant bacteria to humans through the consumption of contaminated food items, consequently undermining the efficacy of antibiotics in infection treatment and amplifying the prevalence of drug-resistant pathogens within the population [14]. Therefore, addressing the antibiotic use in agriculture and implementing robust measures to mitigate the transmission of resistant microorganisms through the food supply are vital for safeguarding both animal and human health [15,16].

*S. aureus,* a Gram-positive coccus, is an opportunistic pathogen capable of causing mild cutaneous infections but also severe and life-threatening conditions such as bacteremia and endocarditis [17]. It is a primary cause of infections in ICU patients, resulting in an estimated annual mortality of approximately 18,650 cases [11,17]. *S. aureus* commonly colonizes about 20 % of the human population and is also a significant source of infection and skin-related diseases in animals used for household and agricultural purposes [18]. These infections can lead to reduced animal efficiency and serve as a reservoir for multidrug-resistant *Staphylococcus* variants. The emergence of drug resistance in *S. aureus*, including methicillin-resistant *S. aureus* (MRSA), has become increasingly common, rendering β-lactam antibiotics ineffective [19]. Vancomycin has traditionally been the treatment of choice; however, the global concern has arisen due to the prevalence of VRSA in public health settings over the past decade [20]. Resistance to vancomycin, with a minimum inhibitory concentration (MIC) $\geq$ 16 μg $mL^{-1}$, has emerged in a significant population of VRSA in recent years [21,22]. VRSA infections are commonly observed in immunocompromised patients [23], and although teicoplanin is currently the only effective option, its susceptibility is rapidly decreasing, leaving limited treatment choices for VRSA [24]. Consequently, VRSA poses a serious threat to public health settings as well as animals used for household and agricultural purposes. Urgent efforts are required to develop potent anti-VRSA therapeutics [25]. However, existing therapeutic agents are being compromised at an alarming rate. If this trend persists, we may enter a pre-antibiotic era by 2040, therefore necessitating the exploration of new sources of drugs from unexplored entities [11,26].

In this study *Leptolyngbya* sp. HNBGU-004 has been selected to conduct an in-depth investigation for anti VRSA components. The selection has been made based on previous research findings of thermophilic *Leptolyngbya* strains [26,27]. Recognizing the potential therapeutic value, we sought to delve deeper into the chemical composition of the lipophilic extract. For a thorough examination, gas chromatography-mass spectrometry (GC-MS) analysis was employed, aiming to identify and characterize the diverse array of compounds present in the extract. Additionally, our aim is to achieve a more nuanced understanding of the potential pharmacological and antimicrobial properties associated with this cyanobacterial strain.

## 2. Materials and methods

### 2.1. Collection of thermophilic cyanobacteria

The thermophilic cyanobacterial sample (mats and water) were collected from the Taptkund hot water spring, Badrinath (30°74′48″ N and 79°49′18″ E; elevation 3250 m) situated in Garhwal Himalaya, Uttarakhand. The pure culture of *Leptolyngbya* sp. HNBGU-004 (LB4) was characterized and identified using molecular techniques, including 16S rRNA gene sequencing and phylogenetic analysis. Furthermore, the deposited strain was assigned the NAIMCC-C-00338 accession number by the 'National Agriculturally Important Microbial Culture Collection' center, INDIA.

### 2.2. Preparation of lipophilic fractions with LB4

The pure culture of LB4 was cultivated in Castenholz-D medium under controlled laboratory conditions. The culture was grown in an illuminated incubator, maintaining a 14:10 h light:dark cycle and a temperature of 50 ± 2 °C. The LB4 biomass was harvested during the late stationary phase of growth. To prepare the extract, the harvested biomass was crushed in the presence of liquid nitrogen, and the resulting material was dissolved in diethyl-ether solvent. The extract was obtained by centrifugation at 4500 rpm for 30 min, followed by air drying. The dried extract was then dissolved in DMSO (Di-methyl-sulfoxide) to achieve a concentration of 10 mg $mL^{-1}$ and stored at 4 °C until further use. This standardized procedure ensures the consistency and stability of the extracted compounds for subsequent experiments and analysis [28].

### 2.3. Tested bacterial strains

Drug-sensitive *S. aureus* (SA-DS) and multidrug-resistant *S. aureus* clinical strain (VRSA) were obtained from VCSGG Institute of Medical Sciences, Srinagar (Garhwal), Uttarakhand, India. The strains were further characterized in laboratory using standard biochemical tests, as well as determination of antibiotic resistance profiles (antibiogram) against the antibiotics commonly used for gram-positive bacteria treatment, following the guidelines provided by the Clinical and Laboratory Standards Institute.

### 2.4. Antibacterial activity of diethyl ether extract of LB4 (DELB4)

To assess the antibacterial activity of DELB4, standard agar-well diffusion assay was employed which is standardized in the previous findings [26,27]. First, bacterial lawns of both SA-DS and VRSA strain were prepared on Mueller Hinton Agar (MHA) plates. Using an agar borer, wells with a diameter of 8 mm were created on the agar surface. Subsequently, 250 μl of the DELB4 extract, corresponding to a concentration of 2.5 mg, was added to the test wells. As a positive control, 25 μl of chloramphenicol at a concentration of 30 μg was added to a separate well. The negative control well received an equivalent volume of DMSO to account for any solvent effects. After incubation under appropriate conditions, the plates were examined for the presence of clear zones of growth inhibition around the wells. The size of these inhibition zones was measured as an indicator of the antibacterial activity of DELB4 against the SA-DS and VRSA strains. Larger inhibition zones corresponded to greater antibacterial activity, suggesting the potential efficacy of DELB4 in inhibiting the growth of both drug-sensitive and multidrug-resistant *S. aureus* strains.

The minimum inhibitory concentration (MIC) was determined through in-vitro testing using the modified macro tube dilution method. In this procedure, tubes were inoculated with a standard bacterial culture, each containing 1 mL of Muller Hinton broth. In test tube, each receiving varying volumes of DELB4 extract concentration ranging from 0.1 to 2.0 μL. The test tubes were then incubated for 18–24 h at 37 °C. After incubation, each test tube was examined for visible growth, and further sub-culturing was performed from each tube to assess the biocidal activity.

### 2.5. Molecular characterization of cyanobacterium

The partial 16S rDNA region of LB4 was amplified using specific primers, CYA106F and CYA781R, procured from Eurofin, India. The resulting amplicon was subjected to Sanger sequencing, which provided a high-quality sequence for analysis. The obtained 16S rDNA sequence was compared with the sequences available in the National Center for Biotechnology Information (NCBI) database using the BLASTn algorithm. The closest matching sequences were retrieved and aligned using appropriate bioinformatics tools. A phylogenetic tree was constructed using the MEGA X software (version 10.1.6), employing the neighbor-joining or maximum likelihood method, along with bootstrap analysis to assess the robustness of the tree topology [29].

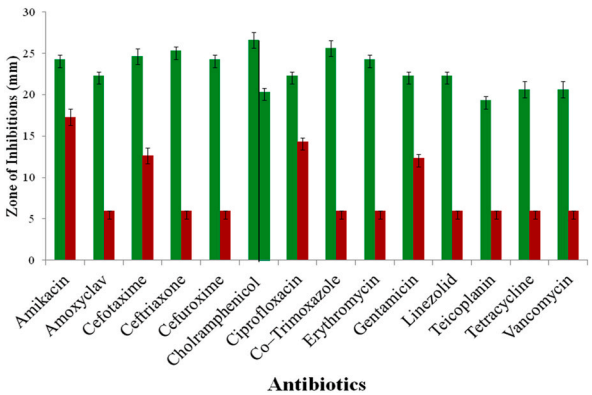


**Fig. 1.** Antibiogram of SA-DS (first bar) and VRSA strain (second bar). Red color of the bars shows resistance while green color represents the sensitivity to particular antibiotics. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### 2.6. GC-MS characterization

The selected cyanobacterial extract obtained from LB4 was subjected to comprehensive chemical characterization using gas chromatography-mass spectrometry (GC-MS) analysis. The use of GC-MS characterization in the current study is justified by previous findings, as the preparation of the extract from a DEE solvent is suitable for higher temperature extraction. Moreover, GC-MS is considered the most suitable technique for compound identification in the context of thermophilic origins [26,27]. The GC-MS analysis was performed on an Agilent GC 7890B coupled with an MS 5977B mass spectrometer from the United States. The slightly modified method employed for this analysis was based on the protocol [30]. The resulted mass spectra will be provided valuable information about the molecular structure and composition of the individual compounds. By comparing the obtained mass spectra with those available in databases, the compounds present in the extract could be tentatively identified. The chemical characterization of the extract revealed the presence of several bioactive compounds with potential pharmacological activities. Overall, the GC-MS analysis provided a comprehensive profile of the chemical composition of the LB4 extract.

## 3. Results

### 3.1. Antibiotic susceptibility assay

The antibiogram of two *S. aureus* strains: SA-DS (represented by the first bar) and VRSA (represented by the second bar) were shown (Fig. 1), SA-DS demonstrates susceptibility to all tested antibiotics, indicating that it is sensitive to their effects. In contrast, VRSA exhibits resistance to all antibiotics except for chloramphenicol (30 μg), implying that it is only susceptible to this specific antibiotic.

### 3.2. Anti-VRSA potential of DELB4

DELB4 demonstrated remarkable bacterial growth inhibition zones of greater than 18 mm for both SA-DS and VRSA (Table 1). The minimum inhibitory concentration (MIC) of DELB4 against VRSA was determined to be 0.5 mg $mL^{-1}$. Notably, when the VRSA pathogen was sub-cultured with the MIC of DELB4 (Fig. 2 (a), it exhibited a bacteriostatic response (Fig. 2 (b). These findings highlight the potent antimicrobial activity of DELB4 against VRSA, emphasizing its potential as a valuable therapeutic candidate for combating drug-resistant *S. aureus* infections.

### 3.3. Estimation of chemical compounds in DELB4 using GC-MS analysis

The GC-MS chromatogram of DELB4 revealed a total of 137 peaks (Fig. 3). Among these peaks, only 41 (30 %) exhibited a similarity index of ≥80 with the compounds listed in the MassHunter/NIST17 library (Table 2). The remaining 70 % of peaks were categorized as unidentified compounds. Within the identified compounds, the majority (40 %) belonged to the hydrocarbon class, followed by phenolics (38 %), hydrocarbon derivatives (6 %), fatty acids (4 %), esters (2 %), ethers (1 %), and other chemical classes (10 %) (Fig. 4).

One prominent phenolic compound, Tris (2,4-di-tert-butylphenyl) phosphate, displayed the highest peak area (6.316), representing approximately one-third of the total identified compounds. Additionally, a delta-lactam compound, 2-Piperidinone, N-[4-bromo-n-butyl], was reported for the first time from cyanobacteria (*Leptolyngbya*) (Fig. 5 (c). The presence of a diverse array of compounds, particularly phenolic, in the GC-MS analysis suggests the potential for bioactive properties in DELB4. Phenolic compounds are known for their antimicrobial and antioxidant activities, which could contribute to the observed antibacterial effects against SA-DS and VRSA. The identification of a novel delta-lactam compound highlights the discovery of previously unreported bioactive molecules within *Leptolyngbya*. This finding expands our understanding of the chemical diversity and potential therapeutic applications of cyanobacteria-derived compounds. Further investigations are warranted to elucidate the specific roles and mechanisms of action of the identified compounds in the antibacterial activity of DELB4. Additionally, efforts should be directed towards the identification and characterization of the remaining unidentified peaks to uncover potential novel bioactive molecules with therapeutic relevance.

### 3.4. Molecular characterization of Leptolyngbya sp. HNBGU004

To determine its genetic relatedness, a BLASTn analysis was performed using a 589 bp partial 16S rRNA sequence (MT887288) obtained from LB4. The analysis revealed that LB4 exhibited a similarity up to 98.47 % with *Leptolyngbya* sp. PKUAC GDTS1-28. Interestingly, the analysis demonstrated that LB4 forms a distinct clade, indicating its unique genetic lineage within the

**Table 1**
Antibacterial susceptibility of DELB4.

| S.N | Cyanobacterium/antibiotics | Dose | Size of growth Inhibition zone (mm) | |
|---|---|---|---|---|
| | | | VRSA | SA-DS |
| 1 | ***Leptolyngbya* sp. HHBGU004** | **0.5 mg $mL^{-1}$** | **18.66 ± 0.57** | **21.33 ± 0.57** |
| 2 | **Chloramphenicol** | 30 μg | 20.33 ± 0.47 | 28.66 ± 0.94 |

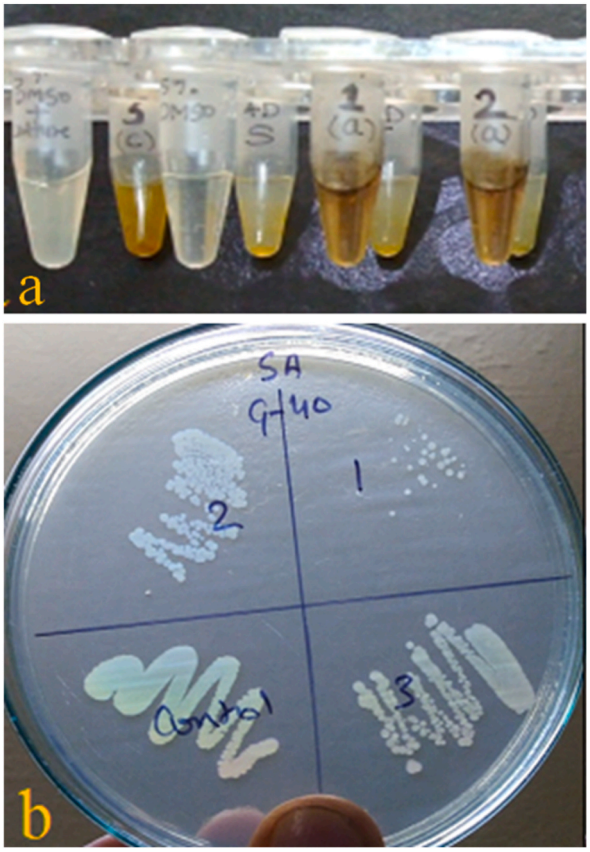


**Fig. 2.** **(a)** Determination of MIC and **(b)** bacteriostatic nature of DELB4 at their MIC and at lower concentration growth appear in subculture plate.

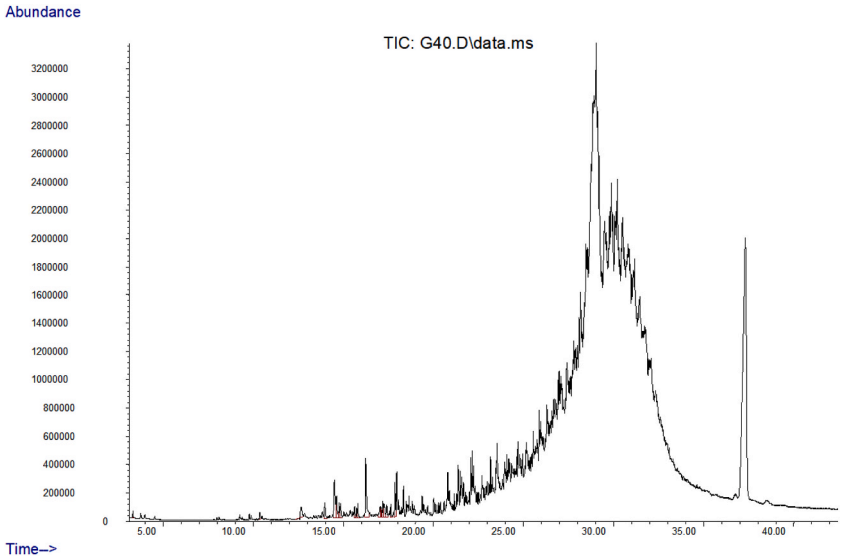


**Fig. 3.** Abundance of chemical compounds observed in chromatogram of DELB4.

**Table 2**
List of chemical compounds identified in DELB4.

| S.N | RT (min) | Compound Name | Quality | Mol Weight (amu) | Peak area percentage |
|---|---|---|---|---|---|
| 1. | 13.967 | Octadecane, 2,6-dimethyl- | 90 | 282.329 | 0.120 |
| 2. | 16.241 | Tetradecane | 98 | 198.235 | 0.447 |
| 3. | 15.637 | Eicosane, 2-methyl- | 87 | 296.344 | 0.064 |
| 4. | 17.86 | Pentadecane | 98 | 212.25 | 0.176 |
| 5. | 17.96 | **2,4-Di-tert-butylphenol** | 97 | 206.167 | 0.260 |
| 6. | 18.346 | Octacosane | 86 | 394.454 | 0.144 |
| 7. | 19.378 | Hexadecane | 96 | 226.266 | 0.102 |
| 8. | 19.462 | Tritetracontane | 91 | 604.689 | 0.089 |
| 9. | 20.804 | Heptadecane | 97 | 240.282 | 0.220 |
| 10. | 20.888 | Octatetracontane, 1-iodo- | 87 | 800.664 | 0.120 |
| 11. | 21.677 | Sulfurous acid, octadecyl 2-propyl ester | 87 | 376.301 | 0.127 |
| 12. | 22.071 | 1-Octadecene | 99 | 252.282 | 0.197 |
| 13. | 22.239 | Octacosane, 2-methyl- | 83 | 408.47 | 0.238 |
| 14. | 23.279 | Eicosyl isobutyl ether | 86 | 354.386 | 0.103 |
| 15. | 23.48 | 1-Bromodocosane | 91 | 388.27 | 0.156 |
| 16. | 23.531 | **7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione** | 94 | 276.173 | 0.315 |
| 17. | 24.084 | Dotriacontane, 1-iodo- | 87 | 576.413 | 0.134 |
| 18. | 24.344 | Carbonic acid, octadecyl vinyl ester | 81 | 340.298 | 0.210 |
| 19. | 24.621 | 1-Eicosene | 95 | 280.313 | 0.106 |
| 20. | 24.94 | 1-Docosene | 90 | 308.344 | 0.139 |
| 21. | 25.15 | Carbonic acid, eicosyl vinyl ester | 87 | 368.329 | 0.163 |
| 22. | 25.535 | Nonadecane | 95 | 268.313 | 0.217 |
| 23. | 25.754 | Octatriacontylpentafluoropropionate | 89 | 696.584 | 0.160 |
| 24. | 25.787 | **2-Piperidinone, N-[4-bromo-n-butyl]-** | 86 | 233.042 | 0.127 |
| 25. | 25.879 | Heneicosane | 95 | 296.344 | 0.320 |
| 26. | 25.963 | Eicosane | 93 | 282.329 | 0.295 |
| 27. | 26.022 | 1-Decanol, 2-hexyl- | 91 | 242.261 | 0.148 |
| 28. | 26.441 | Dodecane, 2,6,11-trimethyl- | 80 | 212.25 | 0.222 |
| 29. | 26.517 | Tetrapentacontane, 1,54-dibromo- | 93 | 914.682 | 0.312 |
| 30. | 26.945 | 1-Nonadecene | 97 | 266.297 | 0.702 |
| 31. | 27.003 | Nonadecane, 1-chloro- | 89 | 302.274 | 0.548 |
| 32. | 27.406 | Octadecane | 91 | 254.297 | 0.895 |
| 33. | 27.087 | Nonahexacontanoic acid | 94 | 999.07 | 1.081 |
| 34. | 28.765 | **Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)** | 99 | 646.451 | **4.454** |
| 35. | 29.21 | Ethanol, 2-(octadecyloxy)- | 93 | 314.318 | 2.707 |
| 36. | 29.503 | Tricosane | 96 | 324.376 | 3.445 |
| 37. | 29.596 | Docosane | 96 | 310.36 | 1.997 |
| 38. | 30.149 | Hexadecane, 1-iodo- | 90 | 352.163 | 0.804 |
| 39. | 30.711 | 1-Tetracosene | 95 | 336.376 | 1.017 |
| 40. | 31.617 | Hexacosane | 95 | 366.423 | 0.644 |
| 41. | 37.322 | **Tris(2,4-di-tert-butylphenyl) phosphate** | 99 | 662.446 | **6.316** |

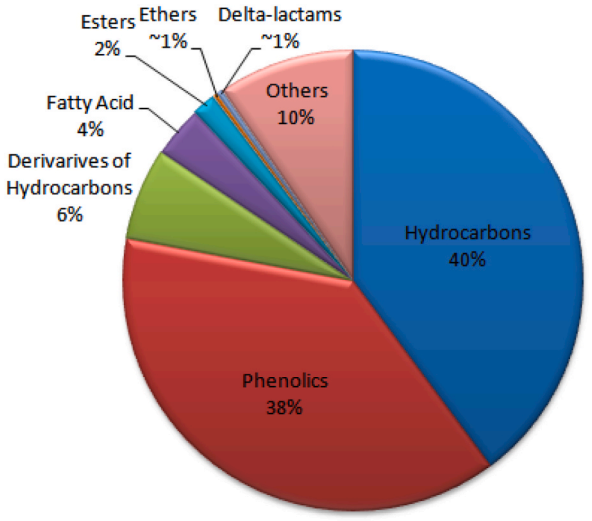


**Fig. 4.** Different chemical classes reported from the DELB4.

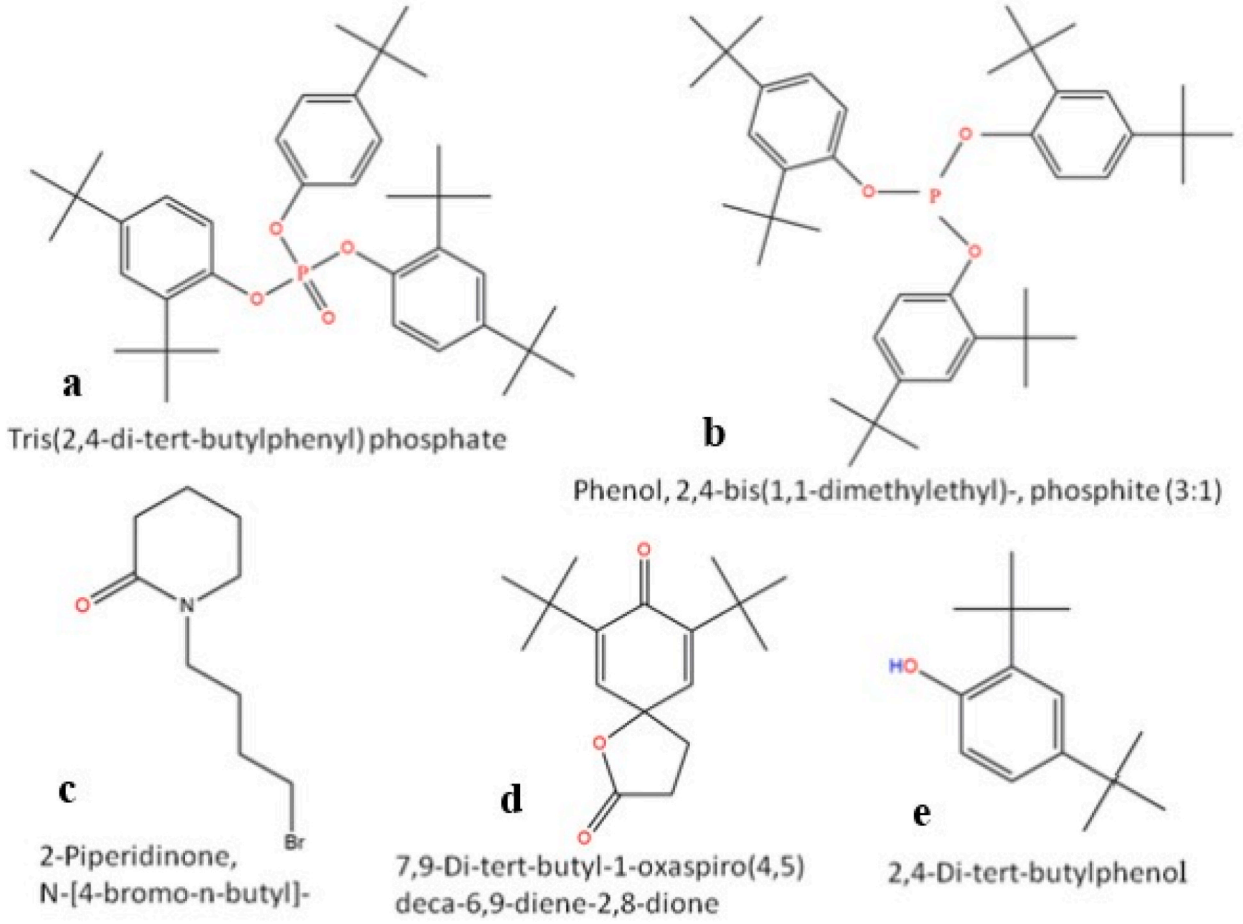


**Fig. 5.** Major compounds reported from the DELB4 (**a-e**).

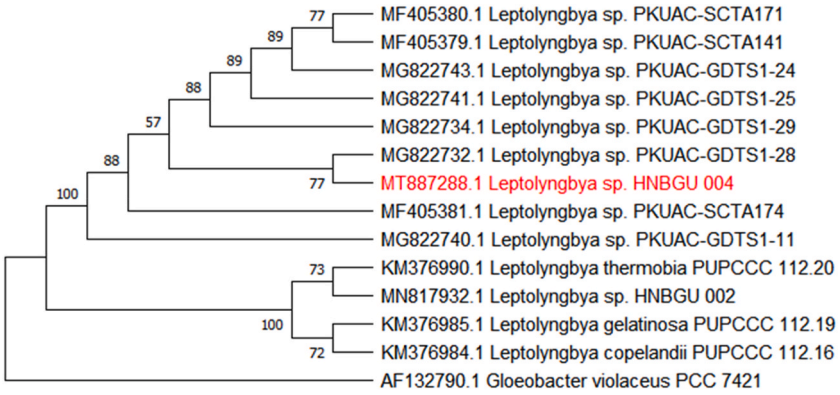


**Fig. 6.** Phylogenetic tree of LB4 based on their 16S rDNA sequence and their closest matches.

*Leptolyngbya* genus (Fig. 6). The LB4 strain was preserved in 50 % glycerol stock and subsequently deposited at the 'National Agriculturally Important Microbial Culture Collection' center under the accession number NAIMCC-C-00338.

## 4. Discussion

Extremophiles are regarded as promising sources for antibiotic production due to their unique adaptations to extreme environments [31]. These microorganisms have evolved distinctive biochemical pathways, producing bioactive compounds with potential

antimicrobial properties. The harsh conditions in which extremophiles thrive often result in the development of novel antibiotic structures, offering alternative modes of action against antibiotic-resistant strains. The biosynthetic gene clusters found in extremophiles harbor the potential for discovering new antibiotics. Additionally, the enzymatic stability of extremophile-produced compounds is advantageous for industrial-scale antibiotic production. The diverse range of extremophiles increases the chances of identifying antibiotics with varied mechanisms of action, and their ability to inhibit biofilm formation is crucial for preventing persistent infections. Exploring extremophiles also allows for the discovery of unique biotransformation abilities, enabling the synthesis of modified antibiotics. The reduced microbial competition in extreme environments, coupled with the largely unexplored niches, provides opportunities for mining new antibiotic-producing strains. Overall, the exploration of extremophiles holds immense potential in addressing the urgent need for novel antibiotics in the face of increasing antibiotic resistance [32].

The current study focuses on the characterization and antimicrobial potential of *Leptolyngbya* sp. HNBGU-004, a cyanobacterial strain isolated from hot water springs. *Leptolyngbya* is a genus of cyanobacteria known for its diverse secondary metabolites and pharmaceutical potential. The genus has been associated with various biological activities, including antibacterial activity against drug-resistant bacterial pathogens [26,33].

In our research studies, the antibiogram of VRSA reveals a concerning trend. VRSA is found to be resistant to all antibiotics tested, except for chloramphenicol (30 μg). The emergence of VRSA, with its high level of resistance to multiple antibiotics, poses a significant challenge in clinical settings. It highlights the urgent need for the development of new antimicrobial agents or therapeutic strategies to effectively combat VRSA infections. Additionally, it underscores the importance of implementing appropriate antibiotic stewardship practices to prevent the spread of drug-resistant bacteria and preserve the effectiveness of available antibiotics [19,24]. This indicates that VRSA has acquired resistance mechanisms that render it ineffective against the majority of antibiotics used in the study [34]. The resistance of VRSA to multiple antibiotics is a serious public health concern as it limits the available treatment options for infections caused by this strain.

*Leptolyngbya* strains inhabiting extreme thermal environments have attracted significant attention due to their unique characteristics. However, the lack of comprehensive morphological and molecular data has limited their taxonomic recognition. To overcome this limitation, molecular markers such as 16S rRNA and 16S–23S intergenic spacer (ITS) sequences have been employed for taxonomic identification. Current study reported the, *Leptolyngbya* sp. HNBGU004 have novel morphological and molecular characteristics belonging to the *Leptolyngbya* genera of the cyanobacteria. *Leptolyngbya* have been reported various secondary metabolites associated with various biological activities [33]. Like the similar findings, *Leptolyngbya* genera have been shown the extensive pharmaceutical potential included the antibacterial activity against the drug-resistant bacterial pathogens [26,35–37]. *Leptolyngbya* also reported the most polyphyletic genera inhabited extreme thermal environments. However, thermal *Leptolyngbya* strains lack the proper morphological and molecular data, therefore due limitation, 16SrRNA and 16S–23S intergenic spacer (ITS) play the significant molecular evidence for the taxonomic recognition [38–40]. Apparently, the lack of systematic and genomic characteristics most of *Leptolyngbya* submitted to NCBI with strain numbers only [38].

Moreover, as per our best knowledge, present study first time reported the anti-VRSA activity from any cyanobacteria (*Leptolyngbya*) isolated from the hot water springs. At the dose of 0.5 mg $mL^{-1}$ diethyl ether extract of the *Leptolyngbya* sp. HNBGU-004 (DELB4) exhibited maximum significant activity as compared to vancomycin MIC against VRSA. Further chemical characterization of the bioactive fraction of DELB4 was performed using GC-MS analysis. The results revealed a high content of phenolic compounds, which have been previously reported in *Leptolyngbya* strains isolated from hot water springs. Among the identified compounds, Tris (2,4-di-tert-butylphenyl) phosphate exhibited the highest peak area percentage (6.316 %). *Tert*-butyl compounds, including analogs of terfenadine, have demonstrated activity against drug-resistant *S. aureus* strains in previous studies [41–43]. The parental *tert*-butyl compounds showed low MIC values (2 μg/mL) against MRSA, vancomycin-intermediate *S. aureus* (VISA), and VRSA [41]. Another significant phenolic compound identified in DELB4 was phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1), which exhibited antibacterial activity in previous research [43]. Additionally, DELB4 contained minor peaks of a delta-lactam compound, 2-Piperidinone, N-[4-bromo-n-butyl], which has been widely reported and isolated from various sources for its antimicrobial activities [44]. The findings of this study highlight the pharmaceutical potential of *Leptolyngbya* sp. HNBGU004 and its ability to produce bioactive compounds with significant antibacterial properties. The presence of phenolic compounds, including *tert*-butyl compounds and phosphite, further enhances its potential as a source of novel antimicrobial agents.

Future avenues will involve optimizing the extraction and purification of DELB4. Innovative technologies, such as metabolomics, will be employed to identify and quantify the range of bioactive compounds present in the extract. The research will also explore potential synergistic effects by combining DELB4 with different antibiotics and assessing their combined impact on vancomycin-resistant *S. aureus*. Additionally, in vivo studies will be designed and conducted using relevant animal models of VRSA infection to evaluate the efficacy and safety of DELB4.

## 5. Conclusion

*Leptolyngbya* sp. HNBGU004 exhibits unique morphological and molecular characteristics, and its distinctive mode of action represents an intriguing avenue of research with several promising aspects. The strain demonstrates notable antibacterial activity against VRSA, highlighting its potential as a source of new therapeutics. Combining cyanobacterial extracts with existing antibiotics may result in synergistic effects, enhancing the overall efficacy of treatment and potentially overcoming the resistance mechanisms observed in VRSA. Exploring cyanobacterial extracts as part of combinatorial therapeutic approaches, by combining them with existing antibiotics or other therapeutic agents, may offer a multi-faceted strategy to combat VRSA and minimize the risk of developing further resistance. These findings significantly contribute to the understanding of the pharmaceutical potential of *Leptolyngbya*

cyanobacteria and open avenues for further research in the field of antimicrobial drug discovery.

## Data availability

*Leptolyngbya* sp. HNBGU004 strain with NAIMCC-C-00338 accession number could be available from 'National Agriculturally Important Microbial Culture Collection' center, INDIA.

## CRediT authorship contribution statement

**Sachin Tyagi:** Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rahul Kunwar Singh:** Supervision, Funding acquisition, Formal analysis. **Ashok Kumar:** Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rahul Kunwar Singh reports financial support and equipment, drugs, or supplies were provided by University Grants Commission. If there are other authors, they 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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