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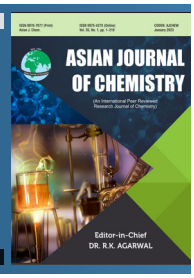

Phytochemicals Profiling of Blue-Green Alga *Nostoc* sp. HANL07: Antioxidant, Antibacterial Activity and GC-MS Analysis

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In recent years, studies on phytochemicals, antioxidants, and antibiotics using blue green algae from harsh environments has become increasingly prevalent. The search for novel bioactive molecules exhibiting diverse biological activity is one of the most important strategies for addressing the issue of rising concerns regarding bacterial antibiotic resistance. In present study, the phytochemicals, antioxidant potentiality and antibacterial assessment of organic solvent extracts of blue-green alga isolated from Naini lake water were characterized. From the mass culture of alga, extracts were prepared in MeOH, EtOH, acetone, AcOEt, hexane and DCM and antioxidant potential and antibacterial properties were studied against multiple antibiotic-resistant *E. coli*, *P. aeruginosa*, *P. fluorescence*, *S. enteric*, *S. typhimurium* and *Sh. dysenteriae*. The 16S rRNA gene sequence analysis and phylogenetic tree revealed that blue-green algal isolate belongs to *Nostoc* sp. A higher extraction yield (5.3%) was recorded for MeOH extract as compared to other solvent extracts. Alkaloids, flavonoids and phenols were present in all extracts. DPPH free radical scavenging activity was determined using various concentrations of extracts (1 to 2048 µg mL^{-1}). Maximum % inhibition (free radical scavenging activity) was observed in MeOH extract (35.23 ± 0.12). The methanolic extract was found to be inhibitory against *P. aeruginosa* and *P. fluorescence.* 2048 µg mL^{-1} MIC value was recorded against *P. aeruginosa*. In GC-MS profiling, a total of 8 significant peaks showing various compounds were identified among the 21 peaks. Oxirane hexadecyl was reported as the main components in this compound. The results showed that *Nostoc* sp. HANL07 is a good source of antioxidant and antibacterial compounds and could be used for further study in the field of drug discovery.

Keywords: Phytochemicals, Phenolic compounds, Flavonoids, Antibiotic resistance, Blue-green algae, GC-MS analysis.

INTRODUCTION

Blue-green algae (BGA) are prokaryotic, oxygenic, photosynthetic, microscopic organisms that have evolved and developed the capacity to endure a variety of light intensities, pH and salinity, such endurance is brought about by producing specific bioactive compounds in adverse conditions [1]. The worldwide distribution of blue-green algae demonstrates the tremendous ecological diversity of environments, including freshwater. There are thought to be roughly 2000 different freshwater and marine blue-green algae strains. They are a suitable option to develop in lakes, ponds and oceans that are poor in nutrients, represent a major hazard to water and cause eutrophication due to their ability to thrive in challenging settings and their autotrophic nature. Due to their ability to develop swiftly under challenging environmental conditions, these photosynthesis capable bacteria are referred to as major biological resources with a variety of biotechnological applications in the modern world [2].

WHO published its first-ever list of critically, high and medium priority resistant bacteria for which new bioactive compounds are urgently needed [3,4]. The prospect of antimicrobial resistance, which has an impact on public health and raises questions about the efficacy of present medicines for life-threatening bacterial diseases, is a recent development

[5]. Terrestrial, marine, freshwater and brackish water habitats all exist and their existence is generally known. They can be in relationship with plants or animals in different forms like symbiotic, commensal or parasitic.

In the highly competitive atmosphere of contemporary pharmaceutical research and the creation of new compounds, the BGA offers a distinct feature of molecular diversity and biological activity that is essential for drug discovery [6,7]. The studies on bioactive compounds of BGA have evolved in the past few years and have been proven to be extremely helpful in the development of drugs with diverse biological activities such as antioxidant [8], antibacterial [9], antifungal [10], antiviral [11], anticancer [12], *etc*. Several novel bioactives from BGA have already been commercialized and scientists are putting their efforts into novel BGA from different/extreme environments to harness their potential for pharmaceutical and biotechnological applications. Algal-based bioactive compounds like hapalindole A, oscillapeptin A, minutissamide A, anabaenopeptin E, caylobolide B and lyngbic acid are reported by Demay *et al.* [13]. According to Jaspars & Lawton [14], 40% of the BGA-based compounds can be used as potent antimicrobial and anticancer agents. Secondary metabolites of BGA like lipopeptides, depsipeptides, fatty acid amides, macrolactones, swinholides, *etc*. are novel and pharmacologically important bioactive compounds that show a broad spectrum of biological activities [15]. The most studied genera of cyanobacteria that have a history of being associated with antimicrobial activity reports and are being considered as potential therapeutic targets include *Oscillatoria* sp., *Microcystis* sp. and *Lyngbya* sp., *Anabaena variabilis*, *Anabaena circinalis*, *Nostoc* sp. and *Scytonema* sp. [16]. Recently, nearly 1630 unique molecules reported from BGA are grouped into 260 families of metabolites [13]. Cyanobacterial orders such as Synechococcales produce 9.4% metabolites from 31 families, Chroococcales 10.3% from 34 families, Nostocales 29.7% from 98 families, however, order Oscillatoriales produces the largest number of metabolites *i.e*. 46.5% from 153 families [17]. Bioactives include fatty acids, alkaloids,terpenoids, phenolic compounds, N-glycosides, pigments and their derivatives, linear and cyclic peptides and pigments [18].

The extreme climate of high-altitude Naini lake, has favoured the evolution and biochemical adaptations of indigenous species of blue-green algae (BGA). The lake harbors BGA, which may represent a reservoir of undiscovered microalgal biodiversity and their ecological successes in coping with harsh conditions. Such adaptation is mainly supported by the synthesis of a broad range of potentially valuable bioactive compounds. Keeping this in view, *Nostoc* sp. was isolated from Naini lake, Nainital, India and explored for the characterization of pharmaceutically important compounds. The study was designed to explore the potentials of different BGA extracts for phytochemicals, antioxidant potential and antibacterial activity against multiple antibiotic resistant bacteria.

EXPERIMENTAL

Sample collection and isolation: Lake water samples were collected in 1 L Amber coloured sterilized bottles in the month of December 2021 from various points of high altitude central Himalayan lakes in Nainital, India (29º23′27.2″N, 79º27′20.4″E). During the sampling, several physico-chemical characteristics of the lake water were noted. All samples were moved aseptically to the lab to cultivate and separate different blue-green algae species. Using BG-11 agar plates, pure culture isolation was performed by applying the streak plate technique. The morphological identification of algal strain was done based on cell size measurement and morphological characteristics using a bright field microscope. The blue-green algae species were identified taxonomically according to the method as described by Nowruzi *et al*. [19]. The purity of the observed colony was examined and the isolated purified sample was stored for further research.

Molecular identification: The identified specie was subsequently subjected to DNA extraction and 16S rRNA gene PCR amplification. The previously reported approach was used to extract DNA [20]. Briefly, the mortar and pestle were used to grind the BGA biomass to disrupt the cell wall mechanically and the cell content was extracted in the cell lysate. The material was placed in 2 mL Eppendorf tube with CTAB buffer and the tube was vortexed to mix the contents evenly. The mixture was then incubated at 65 ºC for 1 h in a water bath with 10 µL of mercaptoethanol. After 15 min of 13,000 rpm centrifugation, the supernatant was collected in another cleaned and sterilized tube. It was mixed with chloroform and isoamyl alcohol in an equal ratio (24:1). The DNA precipitation process was initiated by adding 10 mL of cold isopropanol. After 20 min of incubation at room temperature and 15 min of centrifuging at 13,000 rpm, the DNA was precipitated. After discarding the supernatant, 500 mL of 70% ethanol was used to wash the settled DNA. The solution was centrifuged for 5 min at 8000 rpm, remove the supernatant followed by the addition of Tris-EDTA buffer. The samples were stored at –20 ºC until used. The forward and reverse primers were 27F and 1492R with 5′-AGAGTTTGATCMTGGCTCAG-3′ and 5′-TACGGYTACCTTGTTACGACTT-3′ sequences, respectively for amplification of 16s RNA gene. A 0.5 mM $MgCl_2$ was used to prepare 50 µL PCR mixture. A 1 µL of isolated cyanobacterial genomic DNA, 2.5 U of Taq DNA polymerase (Merck), 0.25 mM dNTP, 0.5 mM of each primer and 1 × PCR buffer. An initial denaturation phase at 95 ºC for 3 min was followed by 30 cycles, each lasting 1 min. A 58 ºC for 50 s, 72 ºC for 1 min and 72 ºC for a final extension of 7 min were the annealing temperatures. The amplified PCR products were run on 1.2% agarose gel to resolve the bands and suitable bands were cut from the gel got eluted using spin gel extraction and purification kit Bangalore Genei, India. The extracted PCR amplicon was cloned using an *E. coli* vector and the further cloned gene was isolated and purified and the sequence using was sequenced in both directions using applied biosystems. The obtained sequence was analyzed against the NCBI database and phylogenetic tree analysis was prepared using MEGA 11 tool.

Growth pattern: The isolated alga was continuously rotated while being cultivated and maintained in BG-11 growth medium at pH 7 and 25 ºC. The culture was continuously illuminated at a 3500 LUX light intensity, with a 12 h cycle of

light and dark. The UV-Vis spectrophotometer (Shimadzu, UV-1700 Pharmaspec), which can generate monochromatic light in the range of 200-800 nm for measuring the absorbance, was used to record the algal absorption spectrum.

Mass culturing and extraction: Axenic culture of alga was mass cultured in 2 L flasks. Different extracts were prepared as per the reported method [21] with some modifications. Briefly, the culture was harvested after 25 days of stationary growth. Biomass and used medium were separated using a filtration technique. For 1 h, biomass was dried in a hot-air oven at 60 ºC. Solvent extraction of 10 g dried biomass was done by soaking it well in the organic solvents individually with 1 L of methanol (MeOH), ethanol (EtOH), acetone (Ace), ethyl acetate (AcOEt), hexane (Hex), dichloromethane (DCM). The mixture was left for a few hours at room temperature and then sonicated for 10 min. For 10 min, the mixture was centrifuged at 4000 rpm. Following centrifugation, the supernatant was collected and dried fully using a rotary evaporator. The extraction yield was calculated using the following formula:

$$\text{Yield } (\%) = \frac{E}{R} \times 100$$

where E = wt. of extracted residue and R = wt. of dried biomass.

The dried extracts were collected for further analysis after being dissolved in dimethyl sulphoxide (DMSO).

Phytochemicals analysis: To determine the presence of significant bioactives in the BGA isolate, the phytochemical screening experiments were conducted. According to the reported method, the analysis on terpenoids, alkaloids, steroids, saponins, tannins, flavonoids, coumarins, phenols, quinones, glycosides, protein and carbohydrates were conducted [22].

Quantification of total phenolic content (TPC) and total flavonoid content (TFC): According to the procedure outlined by Farasat *et al.* [23], the TPC of extracts was determined using the Folin-Ciocalteu reagent with a slight modification. The standard reference was gallic acid. TPC was quantified as mg gallic acid equivalents per gram of dried extract and absorbance was measured at 600 nm (mg GAE g^{-1}). Using the colorimetric approach, TFC was estimated by obtaining the absorbance at 415 nm. The standard reference was rutin. The amount of TFC was calculated as mg Rutin equivalents per gram of dry extract (mg RE g^{-1}).

Antioxidant properties of different extracts: The method previously developed by Nainangu *et al.* [17] was used to determine the 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. By measuring the absorbance at 492 nm using a UV/visible spectrophotometer, the DPPH free radical was quantified. The standard reference was ascorbic acid. The DPPH free radical scavenging % was estimated using the formula:

$$\text{Inhibition (Free radical scavenging, \%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Antibacterial potential of extracts

Multiple antibiotic resistant bacteria: The MAR bacterial strains used for the assessment of antibacterial activity were isolated from the sewage water samples [24] and maintained in the laboratory conditions. *Escherichia coli* (NCBI Accession No. NR_112558.1 resistant to doxycycline and metronidazole); *Escherichia albertii* (NCBI Accession No. NR_025569 resistant to amoxicillin, ampicillin, doxycycline, vancomycin, metronidazole and cloxacillin); *Pseudomonas aeruginosa* (NCBI Accession No. NR_117678.1 resistant to tetracycline, doxycycline, metronidazole); *Pseudomonas fluorescence* (NCBI Accession No. NR_113647 resistant to doxycycline, metronidazole); *Salmonella typhimurium* (NCBI Accession No. AE006468.2 resistant to tetracycline, doxycycline and metronidazole); and *Shigella dysenteriae* (NCBI accession No. NR_026332.1 resistant to gentamycin, doxycycline, vancomycin and metronidazole) were used in the study.

Antibacterial activity: At 37 ºC, selected bacterial strains were inoculated on trypticase soy agar (TSA) media and afterwards incubated for 24 h. All the strains were then suspended in a saline solution of 0.85% NaCl and adjusted to produce approximately $1.0 \times 10^7 - 1.0 \times 10^8$ CFU mL^{-1} by using a spectrophotometer (25% transmittance at 530 nm). Using the agar well diffusion experiment, antibacterial activity of various crude extracts was assessed. Muller Hinton Agar (100 mL) and adjusted culture (100 µL) were added. Once the media had solidified in a sterile Petri dish (90 mm in diameter, Hi-Media, India), it was divided up into individual plates and inoculated with the test organisms. Using a sterile borer, the well on the culture plates was punched to a diameter of 6 mm. Each extracted sample was pipetted into the well of the test plates at a volume of 100 µL before being incubated at 37 ºC for 24 h. In sterile conditions, three repetitions of each test were conducted [25].

Assessment of minimum inhibitory concentration: According to NCCLS guidelines, the macro broth tube dilution method was used to calculate the MIC of the most active extracts [25]. In a nutshell, MeOH extract concentration from 1 to 2048 µg mL^{-1} was prepared using Mueller Hinton broth (M-H Broth) medium in a series of two-fold dilutions. A tube of the growth medium without MeOH extract served as growth control and 100 µL of the standard inoculum of the appropriate MAR strain was added to equal volumes (5 mL) of each concentration. A tube of MHB that had not been inoculated was incubated as a negative growth control. As solvent control, methanol was also added to the MHB medium. At 37 ºC, all tubes were incubated before being checked for growth. The MIC was defined as the concentration of extract that prevented the growth of MAR as indicated by the absence of optical turbidity (corresponding to the negative growth control).

GC-MS analysis: The qualitative analysis of compounds present in the column-purified MeOH fraction was conducted on a GC coupled with MS (GC-MS-TQ8040 Shimadzu) instrument. A fused silica capillary column DB-5 ms (30 m, 0.25 mm ID) with a 0.25 m film thickness was installed in the GC. As carrier gas, helium was employed at a flow rate of 1.0 mL min^{-1}. The electron energy was 70 eV, the ion source for electron impact (EI) was 220 ºC and the transmission line was 300 ºC. The GC ran for a total of 37 min. The sample was prepared by taking 0.1 mL of MeOH fraction extract into a polypropylene tube and diluted with 1 mL of ethyl acetate. Vortexed

and transferred to GC vial and injected 1 µL of sample into GC-MS. Based on a comparison of mass spectra with those found in the National Institute of Standards and Technology (NIST) library, certain volatile constituents were identified. According to the sample's GC-MS analysis's overall peak area, each compound's concentration was reported as a percent peak [26].

Statistical analysis: Results were shown as the mean and standard deviation of three different values. One-way ANOVA was used for the statistical analysis with 95% confidence intervals ($p < 0.05$).

RESULTS AND DISCUSSION

The physico-chemical parameters of the lake water were recorded during the sampling. The temperature of water samples ranged between (12 ± 2 to 18 ± 2 ºC), alkalinity (220 to 255 mg L^{-1}), dissolved oxygen (7.9 to 8.6 mg L^{-1}), turbidity (7 to 17 NTU), total hardness (190 to 280 mg L^{-1}), electrical conductivity (510 to 570 mg L^{-1}), total dissolved solid (369 to 411 mg L^{-1}), pH was recorded in the range of 6.7 to 8.8. All samples were aseptically transferred to the laboratory for cultivation and isolation of blue-green algal species. *Nostoc* sp. has been identified based on cultural, microscopic and molecular characteristics. *Nostoc* sp. has spherical, barrel-shaped or oval cells that form unbranched filaments. Both heterocysts and akinetes are present in the filaments. The 16S rRNA gene sequence analysis and phylogenetic tree (Fig. 1) also revealed that the BGA isolate belongs to *Nostoc sp.* The organism is well grown

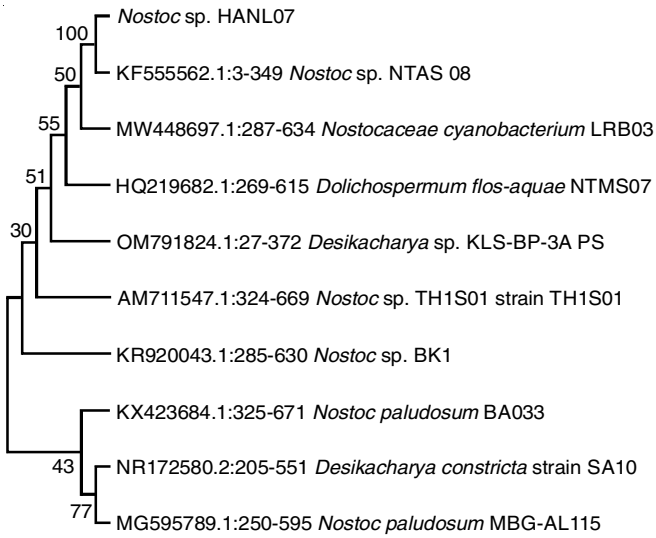

Fig. 1. Phylogenetic study of the *Nostoc* Sp. HANL07 strain's 16S rRNA sequencing using NCBI reference sequences

in BG-11 medium at 25 ± 2 ºC at 12 h of light and dark cycle. The organisms achieved a stationary phase in around 16-18 days of incubation (Fig. 2). It exhibited an increasing growth pattern that was studied up to 26 days of the incubation period.

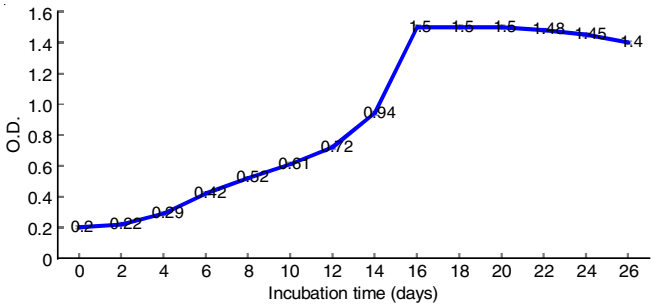

Fig. 2. Growth pattern of *Nostoc* sp. in BG-11 medium

Extraction and phytochemicals profiling: A higher extraction yield (5.3%) was recorded for MeOH extract as compared to other solvent extracts (Fig. 3). Alkaloids, flavonoids and phenols were present in all extracts. Phytochemicals such as steroids, tannins, saponins, coumarins, quinones were found to be absent in all organic solvent extracts tested (Table-1). Results of the quantitative estimation of total phenolic and flavonoid content are summarized in Table-2. Higher phenolic content (32.22 ± 2.3 mg GAE g^{-1}) was observed in MeOH extract followed by EtOH, Ace, AcOEt, DCM and Hex extracts.

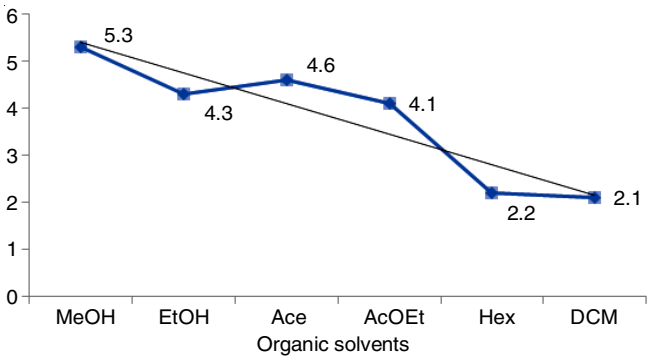

Fig. 3. Extraction yield (%) of biomass of *Nostoc* sp. of various organic solvents

Antioxidant activity: The DPPH free radical scavenging activity was determined using various concentrations of extracts from 1 to 2048 µg mL^{-1}. Maximum % inhibition (free radical scavenging activity) was observed in MeOH extract (35.23 ± 0.12). Decreasing order of activity was observed in all extracts when diluted from higher concentration to lower concentration. The results of % inhibition (free radical scavenging activity)

TABLE-1
QUALITATIVE ESTIMATION OF PHYTOCHEMICALS IN DIFFERENT EXTRACTS OF *Nostoc* sp. HANL07

Extracts	Alkaloids	Steroids	Tannins	Saponins	Flavonoids	Phenols	Coumarins	Quinones
Methanol	Present	Absent	Absent	Absent	Present	Present	Absent	Absent
Ethanol	Present	Absent	Absent	Absent	Present	Present	Absent	Absent
Acetone	Present	Absent	Absent	Absent	Present	Present	Absent	Absent
Ethyl acetate	Present	Absent	Absent	Absent	Present	Present	Absent	Absent
Hexane	Present	Absent	Absent	Absent	Present	Present	Absent	Absent
Dichloromethane	Present	Absent	Absent	Absent	Present	Present	Absent	Absent

TABLE-2
QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT

Phytochemical constituents	Organic solvent extract					
	Methanol	Ethanol	Acetone	Ethyl acetate	Hexane	DCM
Total phenolic content (mg GAE g^{-1})	32.22 ± 2.3	28.12 ± 3.2	15.12 ± 4.5	11.32 ± 3.4	10.08 ± 4.2	13.22 ± 4.9
Total flavonoid content (mg RE g^{-1})	17.20 ± 3.2	11.32 ± 4.6	9.23 ± 1.6	8.05 ± 3.2	7.23 ± 5.2	6.34 ± 1.9

All tests were carried out in triplicate. Data are means of ± SD and variations between both the values in each column are significant ($p < 0.05$).

observed for each extract are summarized in Table-3. No free radical scavenging activity was observed when extracts Ace, AcOEt and Hex extracts were diluted at 1 and 2 μg mL^{-1}) and the DCM extract did not exhibit any activity at 2 μg mL^{-1} of concentration.

Antibacterial activity: The antibacterial activity of all extracts was determined against six multiple antibiotic resistant bacteria using the agar well diffusion method. The MeOH extract was found to be inhibitory against *P. aeruginosa* and *P. fluorescence* (Table-4). However, this extract was found inactive against *E. coli, E. albertii, S. typhimurium* and *S. Dysenteriae.* The EtOH extracts exhibited less antibacterial activity against *P. aeruginosa* and *P. fluorescence* as a little zone of inhibition was observed against these bacteria and extract did not exhibit any activity against *E. coli, E. albertii, S. typhimurium* and *S. Dysenteriae.* In other extracts Ace, AcOEt, Hex and DCM were found completely inactive as no zone of inhibition was observed against all six bacterial strains tested. Thus, the MeOH extract was selected for the determination of minimum inhibitory concentration as this extract exhibits maximum antibacterial activity against *P. aeruginosa* and *P. fluorescence*. Data of MIC value calculated are shown in Table-5. It was observed that 2048 μg mL^{-1} MIC value was recorded against *P. aeruginosa* but did not inhibit *P. fluorescence* up to 2048 μg mL^{-1}.

GC-MS analysis of MeOH extracts of *Nostoc* sp.: Based on a comparison of mass spectra with those contained in the NIST collection, the specific compounds were identified (Fig. 4). According to the GC-MS analysis, the concentration of

TABLE-3
DPPH FREE RADICAL SCAVENGING ACTIVITY (%) OF DIFFERENT EXTRACTS OF *Nostoc* sp.

Extracts	Concentrations of extracts (μg mL^{-1})											
	1	2	4	8	16	32	64	128	256	512	1024	2048
Methanol	1.04 ± 0.43	1.98 ± 0.43	2.34 ± 0.23	3.43 ± 0.23	4.34 ± 0.43	6.43 ± 0.43	8.43 ± 0.43	12.42 ± 0.32	15.23 ± 0.42	20.22 ± 0.23	28.22 ± 0.32	35.23 ± 0.12
Ethanol	0.98 ± 0.54	1.22 ± 0.76	1.98 ± 0.55	2.98 ± 0.43	3.23 ± 0.43	6.42. ± 0.43	9.43 ± 0.45	11.32 ± 0.54	13.43 ± 0.43	18.23 ± 0.32	22.54 ± 0.12	30.43 ± 0.23
Acetone	0.0	0.0	0.98 ± 0.33	1.22 ± 0.44	2.98 ± 0.65	5.34 ± 0.54	8.43 ± 0.54	10.54 ± 0.45	11.54 ± 0.54	14.54 ± 0.43	18.32 ± 0.98	28.22 ± 0.43
Ethyl acetate	0.0	0.0	0.91 ± 0.30	1.11 ± 0.54	2.11 ± 0.54	4.98. ± 0.65	8.11 ± 0.43	9.45 ± 0.54	11.11 ± 0.87	14.65 ± 0.65	17.45 ± 0.54	27.45 ± 0.54
Hexane	0.0	0.0	0.89 ± 0.22	1.02 ± 0.54	2.22 ± 0.54	4.34 ± 0.54	7.45 ± 0.54	8.45 ± 0.33	10.54 ± 3.4	14.34 ± 0.43	17.56 ± 0.23	26.54 ± 0.56
Dichloromethane	0.0	0.91 ± 0.32	1.54 ± 0.65	2.45 ± 0.54	3.12 ± 0.65	7.09 ± 0.55	9.98 ± 0.43	11.66 ± 0.34	13.87 ± 0.65	19.23 ± 0.09	22.65 ± 0.65	29.45 ± 0.23

All tests were carried out in triplicate. Data are means of ± SD and variations between both the values in each column are significant ($p < 0.05$).

TABLE-4
ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS AGAINST MULTIPLE ANTIBIOTIC-RESISTANT BACTERIA

MAR bacteria	Zone of inhibition (antibacterial activity in mm)					
	Methanol	Ethanol	Acetone	Ethyl acetate	Hexane	Dichloromethane
E. coli	0.0	0.0	0.0	0.0	0.0	0.0
E. albertii	0.0	0.0	0.0	0.0	0.0	0.0
P. aeruginosa	14.04 ± 0.22	9.33 ± 0.22	0.0	0.0	0.0	0.0
P. fluorescence	11.22 ± 3.4	8.45 ± 0.43	0.0	0.0	0.0	0.0
S. typhimurium	0.0	0.0	0.0	0.0	0.0	0.0
Sh. Dysenteriae	0.0	0.0	0.0	0.0	0.0	0.0

All tests were carried out in triplicate. Data are means of ± SD,

TABLE-5
MIC OF MeOH EXTRACT OF *Nostoc* sp. AGAINST *P. aeruginosa* and *P. fluorescence*

MAR bacteria	MeOH extract concentration (μg mL^{-1})												MIC (μg mL^{-1})
	1	2	4	8	16	32	64	128	256	512	1024	2048	
P. aeruginosa	+	+	+	+	+	+	+	+	+	+	+	-	2048
P. fluorescence	+	+	+	+	+	+	+	+	+	+	+	+	> 2048

+ (Growth observed); – (No growth observed)

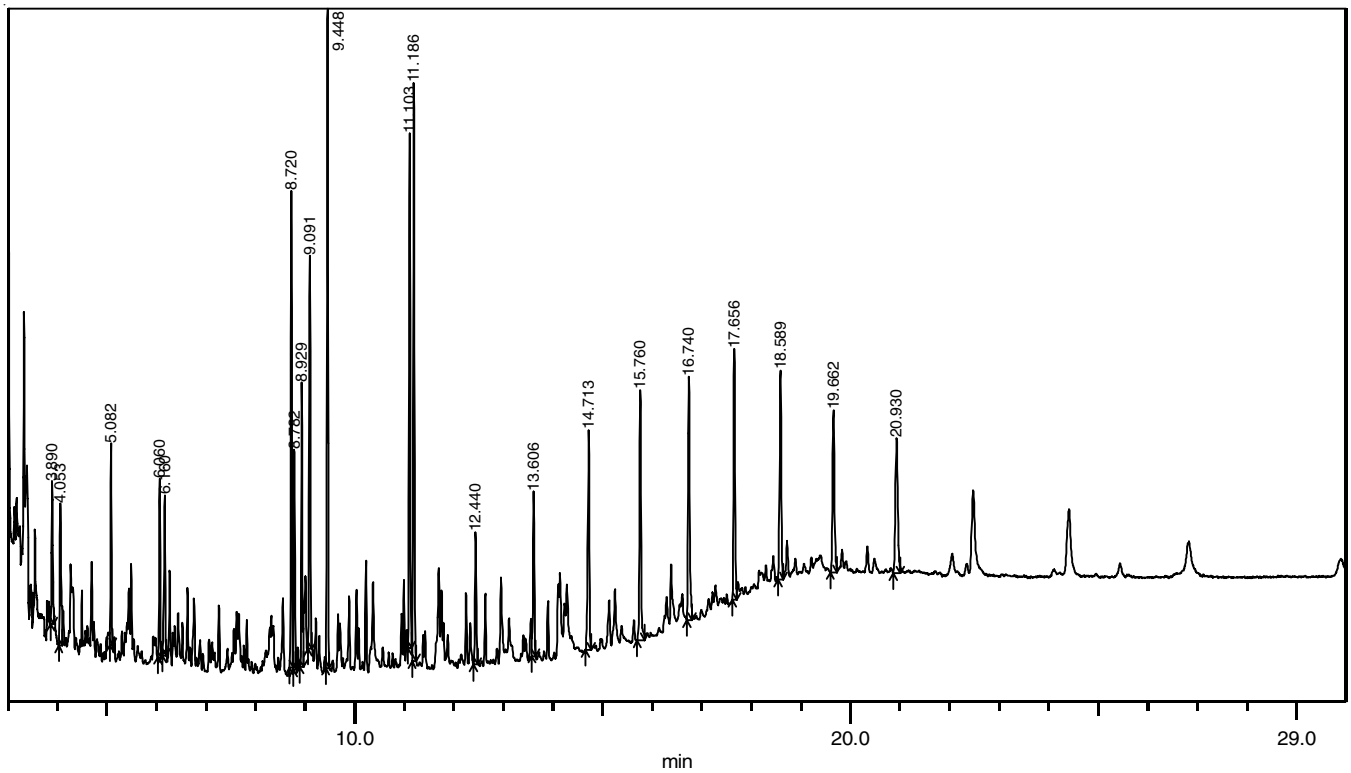

Fig. 4. GC-MS chromatogram of MeOH fraction of *Nostoc sp.* HANL07

each chemical was reported as a percentage of the sample's overall peak area. In GC-MS profiling (Fig. 5), a total of 8 significant peaks displaying various compounds were found among the 21 peaks (Table-6). Based on the results, compounds identified were cycloheptasiloxane, tetradecamethyl, cyclohexasiloxane dodecamethyl, 1-(3-aminopropyl)-2-pipecoline, oxirane tetradecyl, 2-pentadecanone, 6,10,14-trimethyl, 6-octen-1-ol, 3,7-dimethyl-, propanoate, oxirane tetradecyl, oxirane hexadecyl. As the spectra of this molecule (9.00%) were seen as the highest peaks with a maximum hit (11.10), it was identified that oxirane hexadecyl was the primary compound.

Conclusion

Blue green alga *Nostoc* sp. HANL07 was isolated and identified with significant antioxidant and antibacterial activity, which is attributed due to the presence of phytochemicals. In most of the studied different organic extracts, alkaloids, flavonoids and phenols were present in all extracts. Among all the extracts tested, MeOH extract showed significant % inhibition free radical scavenging activity. This extract was found to be inhibitory against *P. aeruginosa* and *P. fluorescence*. The antioxidant and antibacterial activities of the MeOH extract is attributed because of the presence of various compounds in the extract, oxirane hexadecyl was reported as main compound. The results of the study showed that *Nostoc* sp. HANL07 is a good source of antioxidant and antibacterial compounds and could be used for further research in the field of drug discovery.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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TABLE-6
LIST OF MAJOR COMPOUNDS IDENTIFIED IN MeOH FRACTION OF *Nostoc* STRAIN

Compound name	m.f.	Retention time (min)	Start time (min)	End time (min)	Area (%)	Height (%)
Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	6.06	6.025	6.085	2.37	3.11
Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	5.082	5.055	5.115	2.89	3.52
1-(3-Aminopropyl)-2-pipecoline	$C_9H_{20}N_2$	6.16	6.115	6.19	2.84	2.81
Oxirane, tetradecyl-	$C_{16}H_{32}O$	8.72	8.69	8.755	7.44	8.33
2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	8.782	8.69	8.815	3.12	3.85
6-Octen-1-ol, 3,7-dimethyl-, propanoate	$C_{13}H_{24}O_2$	8.929	8.895	8.96	4.2	4.97
Oxirane, tetradecyl-	$C_{16}H_{32}O$	9.091	9.05	9.125	6.19	6.87
Oxirane, hexadecyl-	$C_{18}H_{36}O$	11.103	11.07	11.14	8.43	9.0

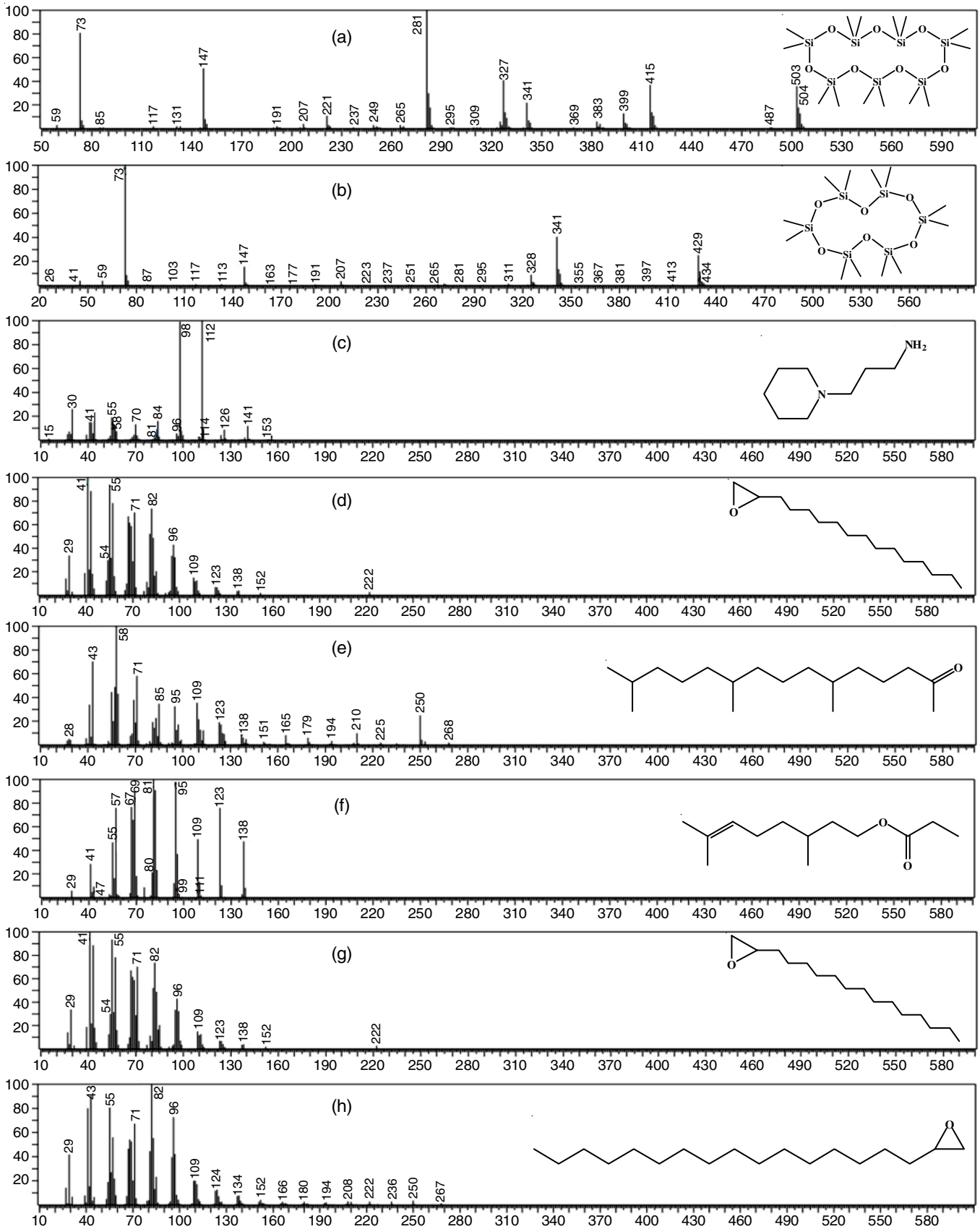

Fig. 5. GC-MS profiling displaying mass spectra with the structure of the main compound identified in the purified MeOH fraction of *Nostoc sp.* HANL07 (a) cycloheptasiloxane, tetradecamethyl (b) cyclohexasiloxane dodecamethyl; (c) 1-(3-aminopropyl)-2-pipecoline; (d) oxirane tetradecyl (e) 2-pentadecanone, 6,10,14-trimethyl; (f) 6-octen-1-ol, 3,7-dimethyl-, propanoate (g) oxirane tetradecyl (h) oxirane hexadecyl

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