Plant Archives Vol. 22, No. 1, 2022 pp. 431-436 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2022.v22.no1.067

ANTIBACTERIAL POTENTIALS OF *CALOTHRIX* SP. NDOP009

J.P. Singh, Om Prakash and N. Dwivedi*
Dept. of Botany, U.P. College (Autonomous), Varanasi, India
*Corresponding Author: drnagendra.dwivedi@gmail.com
Phone No.: 91-9452303984
ORCID: https://orcid.org/0000-0002-6724-5075

(Date of Receiving : 26-10-2021; Date of Acceptance : 09-01-2022)

ABSTRACT

Calothrix sp. NDOP009 was isolated from the agriculture fields of Azamgarh, U.P., India, and characterized by morphological means. Biomass of *Calothrix* sp. NDOP009 was extracted in seven solvents i.e., ethanol, methanol, acetone, petroleum ether, chloroform, n-hexane, autoclaved double distilled water, and screened for antibacterial potential. Crude extract in ethanol produced maximum inhibition zone of 10.31±0.58 mm against *S. aureus*. Active compounds in cyanobacterial extract were separated by two rounds of TLC. Purified extract of **a** band of second-round TLC showed antibacterial activity against Gram-positive bacteria *S. aureus* with an inhibition zone of 15±0.52 mm. MIC of crude extract was 0.0625 mg/ml which is less than the standard antibiotic. Compounds of **a** band were analyzed by GC-MS. Total twenty-four compounds were reported. 1, 2-Benzenedicarboxylic acid (61.8%) and Dehydroabietic acid (9.72%) are major compounds in extract and might be responsible for the antibacterial properties.

Keywords: Cyanobacteria, *Calothrix* sp. NDOP009, antibacterial compounds

Introduction

Cyanobacteria are one of the ancient organisms on earth (Sergeev *et al*., 2002). The existence of cyanobacteria has been traced up to 2.8 billion years ago (Olson, 2006). They are photosynthetic prokaryotes with wide occurrence including stressful habitats of the earth. Cyanobacteria possess characters of both prokaryotes as well as eukaryotes. Two types of classification systems i.e., botanical system and bacteriological system are followed in the classification of cyanobacteria. Cyanobacteria are identified mainly based on morphological features. Molecular parameters are also used for the confirmation of cyanobacterial strains. The role of cyanobacteria infertility in agricultural fields is well established. Cyanobacteria are a rich source of bioactive compounds i.e., mycosporine-like amino acids (MAAs) and scytonemin, toxic metabolites (anatoxin, microcystin and saxitoxin), pharmaceutical compounds (alkaloids, peptides, depsipeptides, and polyketides, etc.), iron chelators (anachelin, schizokinen and synechobactin), protease inhibitors (oscillapeptin, cyanopeptolins and micropeptin) (Rastogi and Sinha, 2009). A score of cyanobacteria has been searched for pharmaceutical compounds. Several antibacterial compounds have been isolated and characterized from cyanobacteria i.e., abietane (diterpenoid) from *Microcoleous lacustris*, Ambigol B (Aromatic), Ambiguine derivatives, Fischambiguine B and Hapalindole G (Indole alkaloid) from *Fischerella ambigua*, Borophycin (Peptide) from *Nostoc linckia* and *Nostoc spongiaeforme*, Calothrixin A (Indolophenanthridine) from *Calothrix* sp., Carbamidocyclophane (Cyclophane) from *Nostoc* sp. CAVN, Comnostins (Diterpenoid) from *Nostoc commune*, Cyanobacterin (Aromatic) from *Scytonema hofmanni*, Muscoride A (Linear peptide) *Nostoc muscorum*, Nostocine A (Extracellular pigment) from *Nostoc spongiaeforme*, Scyptolin A (Cyclic depsipeptides) from *Scytonema hofmanni* PCC 7110, etc (Dwivedi, 2019). Reports on antibacterial potentials of cyanobacteria isolated from Azamgarh, U.P., India are lacking. Hence, this experiment was designed to study the antibacterial potential of cyanobacterium *Calothrix* sp. NDOP009 isolated from district Azamgarh of U.P., India.

Material and Methods

Isolation, Purification, and cultivation of cyanobacteria

Cyanobacterium *Calothrix* sp. NDOP009 was isolated from soil samples of the agricultural field of Azamgarh, U.P., India following procedure as described by Singh *et al*. (2017). The cyanobacterial strain was purified by repeated streaking. The purified strain was grown in nitrogen-free liquid BG-11 medium (Stanier *et al*., 1971) in a culture room maintained at 28 ± 20 C and illuminated with fluorescent light of 12 Wm^{-2}.

Identification of cyanobacteria

The cyanobacterium was viewed at 400x and 1000x using Olympus 21Xi microscope. Morphological parameters

i.e., nature of filament, shape and size of vegetative cells, akinetes and heterocyst of cyanobacteria were analyzed and assigned to species following descriptions of Desikachary (1959).

Mass cultivation of cyanobacteria and preparation of extract

The cyanobacterial strain was inoculated in five liters round bottom flask having three liters of autoclaved nitrogen-free BG-11 medium. Culture was incubated for 25 days in the culture room. Biomass was harvested by centrifugation at 5000 rpm. Biomass was dried at 50^0c for 24 hrs in a hot-air oven. Two hundred mg of the dried pellet of each strain was dissolved in 10 ml of solvents i.e. ethanol, methanol, acetone, petroleum ether, chloroform, and autoclaved double distilled water and placed in freeze for overnight. Centrifuged the content and collected filtrate. The filtrate was evaporated and the residue was dissolved in 500 µl of respective solvents.

Antibacterial test

Disk diffusion assay (Bauer *et al*., 1966) of the cyanobacterial extract was performed following the protocol of Singh *et al*., (2017). Test organisms i.e., *E. coli* and *S. aureus* were obtained from Dept. of Endocrinology and Metabolism, IMS, BHU, Varanasi, India. Inhibition zone produced was noted. The mean and standard error of the inhibition zone was calculated.

Minimum inhibitory concentration (MIC) of purified extract

MIC of the crude purified extract was determined as described by Andrews (2001). A range of serial dilutions of cyanobacterial extract stock (10 mg/ml) was prepared by using Mueller-Hinton broth and broth without extract was control. Each tube was inoculated by 100 µl (10^7 colony forming units (CFU)/ ml) of *S. aureus* and incubated at 37^0C for 24 hrs. MIC was the lowest concentration which produced a clear tube after 24 hrs incubation. All determinations were performed thrice.

Isolation of antibacterial compounds

Two rounds of thin-layer chromatography (TLC) of cyanobacterial extract were performed for the isolation of antibacterial compounds. Both rounds of TLC were performed on a silica gel plate. The mobile phase for 1st round was carbon tetrachloride: methanol (9:1) and hexane: ethyl acetate (1:1) for the second round TLC. Antibacterial compounds of the potent band of 1st round TLC were eluted in the minimum amount of solvent and run for the 2nd round of TLC.

Characterization of antibacterial compounds

Antibacterial compounds of the potent band of the 2nd round of TLC were analyzed by GC-MS on a GCMS-QP 2010 Plus Shimadzu system equipped with an auto-injector and RTX-5 column (Restek, USA, 60 m, ID 0.25 mm, film thickness 0.25 µm) of JNU, New Delhi.

Results and Discussion

The cyanobacterial strain was isolated from agriculture fields of Azamgarh, U.P., India, purified and characterized. Morphological parameters were noted and characterized following keys of Desikachary (Desikachary, 1959). Cyanobacterium is filamentous, unbranched, heterocystous, and polar. Filaments are short and entangled. Vegetative cells are barrel to cylindrical-shaped constricted at cross walls, 3.93 µm to 6.57 µm long and 3.31 µm-4.25 µm broad. Heterocyst only on the base of filament, spherical to sub-spherical in shape, 4.28-5.67 µm long and 3.77-4.49 µm broad (Fig. 1). Morphological features closely resemble genera *Calothrix* (Desikachary, 1959). Cyanobacterium does not resemble any known species of *Calothrix*. Hence, cyanobacterium is identified as *Calothrix* sp. with strain as NDOP009.

Biomass of *calothrix* sp. NDOP009 was extracted in seven solvents i.e. ethanol, methanol, acetone, petroleum ether, chloroform, n-hexane and autoclaved double distilled water and screened for antibacterial potentials. *S. aureus* and *E. coli* were test organisms. Extract in ethanol produced a maximum inhibition zone of 10.31±0.58 mm against *S. aureus* (Tab.-1) and no inhibition against *E. coli*. Hence, ethanol extract was further analyzed for antibacterial compounds. Active compounds in cyanobacterial extract were separated by two rounds of TLC (Fig.-2). **A** band of first-round TLC showed antibacterial activity. It was isolated and dissolved in the minimum amount of ethanol. Second round TLC of A band produced band **a** with antibacterial potential (Fig.-2). Crude purified extract of **a** band showed antibacterial activity against Gram-positive bacteria *S. aureus* with an inhibition zone of 15±0.52 mm (Tab.-2, Fig.-3). MIC of crude extract was 0.0625 mg/ ml (Tab.-I).

Compounds of **a** band were analyzed by GC-MS. Total twenty-four compounds were reported (Fig-4). Two compounds i.e., 1, 2-Benzenedicarboxylic acid (61.8%) and Dehydroabietic acid (9.72%) are in maximum proportions (Fig. 4, Tab.-II). 1, 2-Benzenedicarboxylic acid and derivatives of Dehydroabietic acid are reported to possess antibacterial properties. Hence, these compounds might be responsible for the antibacterial activity of the cyanobacterial extract.

Several cyanobacteria isolated from different regions of the world have shown antibacterial potentials (Dwivedi, 2019). Some cyanobacteria i.e., *Cylindrospermum* sp. NDUPC005 and *Cylindrospermum* sp. NDUPC006 (Singh, 2017), *Nostoc ellipsosporum* NDUPC002 and *Nostoc* sp. NDUPC002 (Singh, 2017), *Nostoc polludosum* and *Cylindrospermum licheniforme* (Singh, 2017), *Fischerella muscicola* NDUPC001 (Singh, 2017), *Phormidium* CCC727, *Geitlerinema* CCC728, *Arthrospira* CCC729, *Leptolyngbya* CCC732, *Phormidium* CCC730, *Phormidium* CCC731 (Shrivastava *et al*., 2016) isolated from Varanasi, India have shown antibacterial potentials. The *Calothrix* sp. NDOP009 was isolated from Azamgarh, U.P., India and ethanol extract of this cyanobacterium have antibacterial potentials against *S. aureus*. A number of secondary metabolites from cyanobacteria i.e., Cyclic peptides, Polyphenyl ether, Cyclic undecapeptide, Terpenoid, Diterpenoid, Lipopeptides (Dwivedi, 2019) have shown antibacterial potentials. Hydroxyl derivatives of dehydroabietic acid showed considerable antibacterial activity to Gram-positive bacteria i.e., *S. aureus*, *S. marcescens* and *P. aeruginosa* (Feio *et al*., 2002). Butyldecyl ester of 1, 2-benzenedicarboxylic acid isolated from seeds and pods of *Acacia nilotica* Linn have shown antibacterial activity against Gram-positive bacteria (Shoge *et al*., 2016). In this investigation, major compounds in crude purified ethanol extract of cyanobacterium are 1, 2-Benzenedicarboxylic acid (61.8%) and Dehydroabietic acid (9.72%). Hence, these two compounds might be responsible

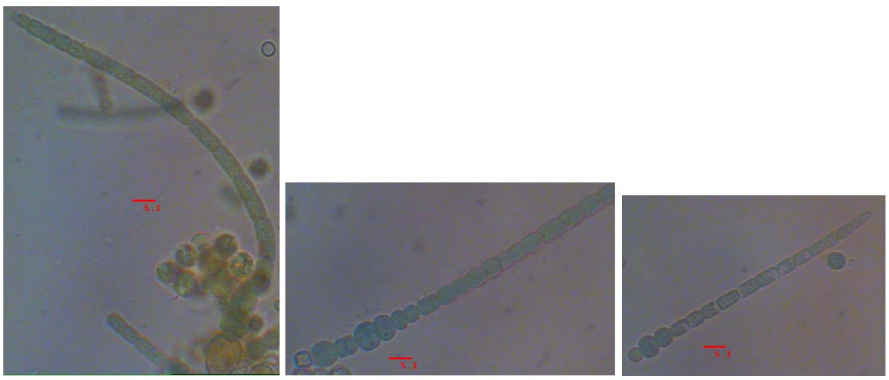
for the antibacterial potential purified extract of cyanobacterium. MIC of some commercial antibiotics vancomycin and ampicillin is 0.8 mg/ ml against gram-positive bacteria (Arvino *et al*., 2020). MIC of crude purified extract of the cyanobacterium is less than some commercial antibiotic compounds. A considerable proportion of ethanol extract of *calothrix* sp. NDOP009 is 1, 2-Benzenedicarboxylic acid. Hence, this cyanobacterium may be searched for alternative antibiotic compounds for Gram-positive bacteria.

Table 1 : Antibacterial activity of extract of *Calothrix* sp. NDOP009 on *S. aureus*. ± Represent standard deviation

S.N.	Parameters	Effective zone of inhibition (mm)	MIC (mg/ml)
1	Biomass extract in ethanol	10.31±0.58	
2	TLC purified extract (**a** band dissolved in ethanol)	15±0.52	less than 0.0625

Table 2 : List of compounds analyzed by GC-MS from the TLC purified extracts of *Calothrix* sp. NDOP009

Peak#	R.Time	Area	Area%	Name
1	10.568	86359	0.47	Heptadecane
2	11.014	921134	5.02	PHENOL,2,4-BIS(1,1-DIMETHYLETHYL)-
3	13.077	94061	0.51	HXADECANE,1-IODO-
4	15.318	48666	0.27	Eicosane,1-iodo-
5	16.970	106108	0.58	13-Hexyloxacyclotridec-10-en-2-one
6	17.122	277334	1.51	Phthalic acid, ethyl pentadecyl ester
7	19.669	102255	0.56	3-(6,6-DIMETHYL-2-METHYLENE-3-CYCLOHEXEN-1
8	19.803	94318	0.51	1-Phenanthrene methanol,1,2,3,4,4a,9,10,10a-Octahydro-1,4
9	19.886	172631	0.94	Methyldehydroabietate
10	20.047	100908	0.55	(1R,4aR,4bR,10aR)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4
11	20.251	251266	1.37	Methylabietate
12	20.556	123308	0.67	Methyl dehydroabietate
13	20.916	11329390	61.80	1,2 BENZENEDICARBOXYLIC ACID
14	21.343	32895	0.18	BICYCLO[2.2.1]HEPTAN-2-OL,1,7,7-TRIMETHYL-3-(R
15	21.406	155172	0.85	Androst-5-ene-3-ol,Trifluoroacetate,(3.beta)-
16	22.209	805444	4.39	1-Phenanethrenecarboxylic acid,7-ethenyl-1,2,3,4,4a,4b,5,6
17	22.339	91347	0.50	Androst-7-ene,(5.alpha)-
18	22.460	1782638	9.72	DEHYDROABIETIC ACID
19	22.837	1011525	5.52	Methylabietate
20	22.964	107595	0.59	Squalene
21	23.483	37919	0.21	Methyldehydroabietate
22	25.128	227470	1.24	Stigmost-5-en-3-ol,oleate
23	26.911	112812	0.62	Cholestanol
24	27.326	260294	1.42	.gamma-sitosterol
		18332849	100	



A B C

Fig. 1 : Photomicrograph of *Calothrix* sp. NDOP009 (Scale bar=5.1 µm).
A, Mature filament. B & C, Young filament.

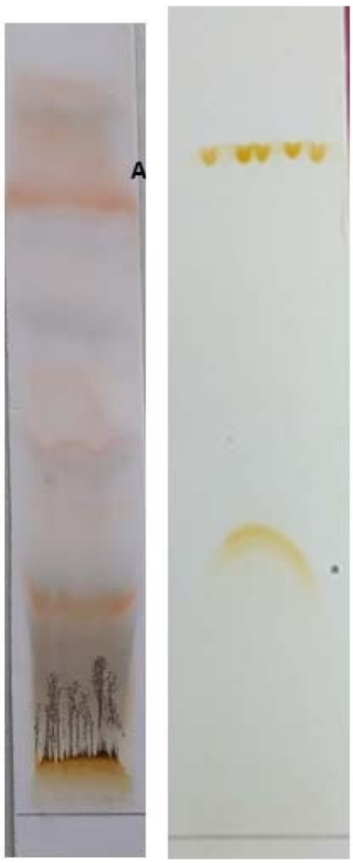


Fig. 2 : Thin-layer chromatography (TLC) of ethanol extract of *Calothrix* sp. NDOP009. (1) First round TLC, (2) Second round TLC

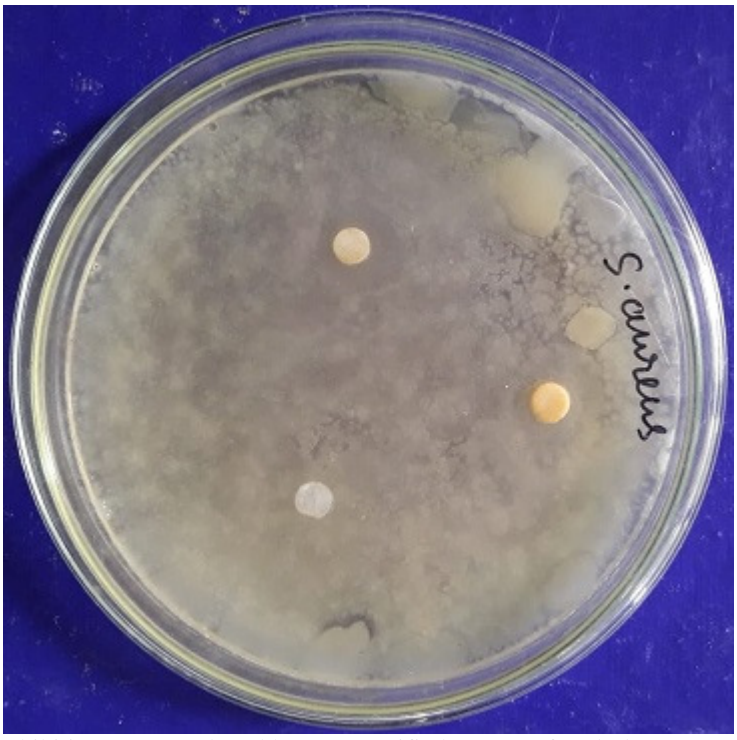


Fig. 3 : Inhibition zone produced by TLC purified extract of *Calothrix* sp. NDOP009.

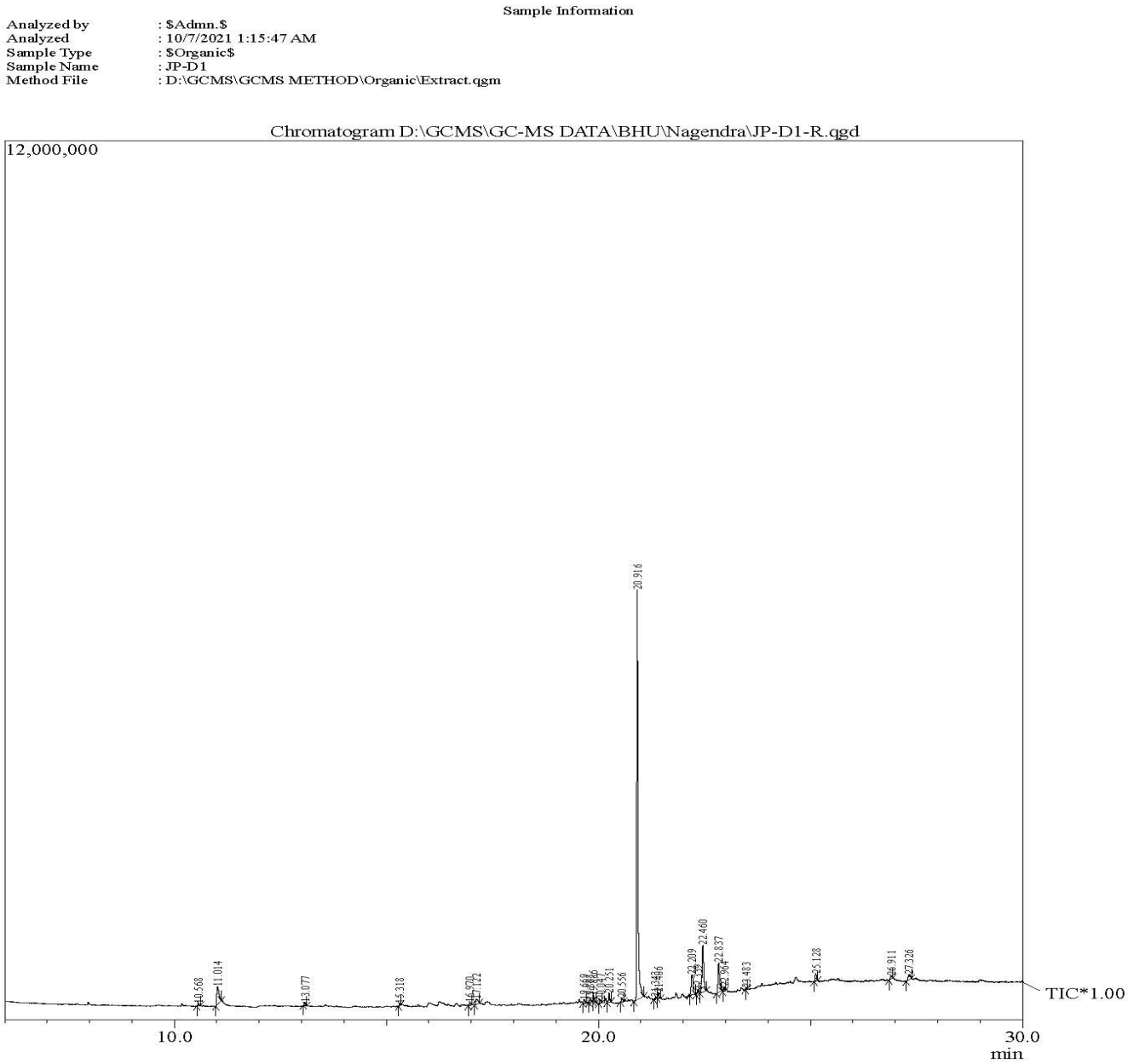


Fig. 4 : GC-MS analysis of TLC purified extract of *Calothrix* sp. NDOP009.

Acknowledgment

The authors are thankful to the Head, Department, of Botany and Principal, Udai Pratap College, Varanasi, Uttar Pradesh, India for providing necessary laboratory facilities. We also thank Prof. Neeraj Kumar Agrawal, Department of Endocrinology and Metabolism, IMS, BHU, Varanasi, India for providing bacterial strains. The authors do not have any conflict of interest.

References

Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* 48: 5-16.

Arvino, M.; Wasiu, B.A.; Mugera, W.G. and Amidou, S. (2020). Functionalization and antimicrobial evaluation of ampicillin, penicillin and vancomycin with *Pyrenacantha grandiflora* Baill and silver nanoparticles. Scientific Reports. https://doi.org/ 10.1038/ s41598-020-68290-x

Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 45(4): 493-496.

Desikachary, T.V. (1959). Cyanophyta. *Indian Council of Agriculture Research, New Delhi, India.*

Dwivedi, N. (2019). Antibacterial Compounds from Heterocystous Cyanobacteria: A Review. *IJSRR* 8(2): 4916-4924.

Feio, S.S.; Silva, A.M.; Reis, L.; Gigante, B.; Roseiro, J.C. and Marcelo-Curto, M.J. (2002). Antimicrobial Activity of Dehydroabietic Acid Derivatives (II); Rauter, AP; Palma, FB; Justino, J; Araújo, ME; dos Santos, SP. (Eds.), Natural Products in the New Millennium: Prospects and Industrial Application, Vol. 47, Proceedings of the Phytochemical Society of Europe; Springer, Dordrecht. https://doi.org/10.1007/ 978-94-015-9876-7_25.

Olson, J.M. (2006). Photosynthesis in the Archean era. *Photosyn Res* 88: 109–17.

Rastogi, R.P. and Sinha, R.P. (2009). Biotechnological and industrial significance of cyanobacterial secondary metabolites. *Biotechnology Advances* 27: 521–539.

Sergeev, V.N.; Gerasimenko, L.M. and Zavarzin, G.A. (2002). The proterozoic history and present state of cyanobacteria. *Microbiology* 71: 623–37.

Shoge, M.; Garba, S. and Labaran, S. (2016). Antibacterial activity of 1, 2-benzenedicarboxylic acid, butyldecyl ester isolated from the seeds and pods of *Acacia nilotica* Linn. *Basic Research Journal of Microbiology* 3(2): 8-11.

Shrivastava, A., Singh, V.K.; Patnaik, S.; Tripathi, J.; Singh, P.; Nath, G. and Asthana, R.K. (2016). Antimicrobial assay and genetic screening of selected freshwater cyanobacteria and identification of a biomolecule dihydro-2H-pyran-2-one derivative. *Journal of Applied Microbiology* 122: 881-892.

Singh, J.; Mishra, S.K.; Agrawal, N.K. and Dwivedi, N. (2017). Antibacterial activity of *Fischerella muscicola* NDUPC001. *Journal of Biotechnology and Biochemistry* 3(4): 87-90.

Stanier, R.Y.; Kunisawa, R.; Mandel, M. and Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (Order chroococcales). *Bacteriol Rev* 35(2): 171-205.