Antibacterial Activity of Cyanobacteria from Wetland Ecosystems

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Antibacterial activity of Cyanobacteria from Wetland ecosystems**" is based on the results of investigations carried out by me in the Microbiology Programme at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr Judith M. Noronha and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Antibacterial Activity of Cyanobacteria from Wetland Ecosystems"** is a bona fide work carried out by **Ms. Varsha Vasudev Naik** under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of Master of Science in Microbiology, in the Discipline Microbiology, at the School of Biological Sciences and Biotechnology, Goa University.

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PREFACE

Wetland ecosystems are unique environments which harbour many microorganisms. These habitats with their abundant biodiversity, play an important role in preserving nature and provide essential benefits to humans. Microorganisms have become multidrug resistant and this is a great challenge today. Therefore, this study focuses on the importance of various cyanobacteria thriving in such wetland ecosystems and their antibacterial properties against pathogens found in it. Cyanobacteria contain secondary metabolites which are effective against these ‘superbugs’. The antibacterial properties of cyanobacteria hold huge potential for dealing with major challenges in medicine and industry.

In the following pages, we delve further into the wetland ecosystems, and the mechanisms used by cyanobacteria and pathogens for their survival and the different compounds which have antibacterial activity. Further, we study the antibacterial activity of cyanobacteria by screening their crude extracts against identified gram negative pathogens.

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TABLES & FIGURES

ABBREVIATIONS USED

Abbreviation	Expansion
BLAST	Basic local alignment search tool
DIZ	Diameter of inhibition zone
DNA	Deoxyribonucleic acid
EMB agar	Eosin methylene blue agar
MDR	Multidrug resistance
MEGA	Molecular Evolutionary Genetics Analysis
MH agar	Mueller-Hinton agar
MRSA	Methicillin-resistant *Staphylococcus aureus*
PABA	Para-aminobenzoic acid
PCR	Polymerase chain reaction
PKS	Polyketide synthases
RNA	Ribonucleic acid
SS agar	Salmonella-Shigella agar
TCBS	Thiosulfate citrate bile salts sucrose agar
TSI	Triple Sugar Iron
WHO	World Health Organization

ABSTRACT

Cyanobacteria can produce various secondary metabolites which can inhibit the growth of bacterial pathogens. In the present study, three cyanobacterial consortia were isolated from wetland ecosystems and were maintained along with three previously isolated pure cultures of *Synechococcus sp.*, *Sunechocystis sp*. and *Limnothrix sp.* and screened for their antimicrobial properties. Test pathoogens were isolated and identified by biochemical and molecular identification. Crude extracts of cyanobacteria were prepared and the antibacterial activity was tested against *E.coli*, *Klebsiella sp.*, *Shigella sp*., and *Vibrio cholerae* using swab method on Mueller-Hinton agar and overlay method on nutrient agar. No zones of inhibition were observed aginst any of the test organisms. Therefore, further studies are required to screen cyanobacterial extracts for antibacterial compounds and test their activity.

INTRODUCTION

1. Wetland ecosystems

Wetlands are unique habitats that are found on earth which provide various benefits to human beings. Therefore, they are ecologically adaptive and sensitive systems. Wetlands significantly differ based on their geographic location, genesis, water regime and chemistry, dominating species, and soil and sediment features. Wetlands are locations where water plays a significant role in controlling the environment, including plant and animal life. They are viewed as a resource because they provide useful goods, like peat, and provide important services, such as carbon storage and water purification. One of the first widely used wetland classification systems divides wetlands into estuarine (including deltas, tidal marshes, marine (coastal wetlands), mangrove (swamps), riverine (along rivers and streams), lacustrine (lakes), and palustrine ('marshy'-marshes, swamps, and bogs) based on their ecological, hydrological, and geological characteristics (Jayakumar, 2019). Nanda Lake was also one of the first six official wetlands recognized for Goa on October 1, 2021 (Malkarnekar, 2022). Nanda Lake consists of intermittent freshwater wetlands along one of the Zuari River's major tributaries. A sluice gate links them to the adjacent river channel and, when closed, it makes available to the marshes to be flooded. This wetland is a habitat for various migrating waterbirds, along with several other flora and animals. The local people are allowed to use the water during the off-monsoon season to grow rice paddies downstream of the lake and promote fishing and recreation. The lake also protects the downstream region from monsoon flooding. Invasive non-native species, rubbish and solid waste, overfishing, and the exploitation of aquatic resources are the major threats to this habitat. (Ramsar Sites Information Service., 2022)

Wetland ecosystems include various microbial communities with an essential part in nutrient recycling, and biogeochemical cycles (including the carbon cycle, nitrogen cycle,

phosphorus cycle, sulfur cycle, and iron cycle), and are important for several wetland processes (Bodelier & Dedysh, 2013). There are two methods for determining the diversity of microorganisms in wetlands: culture-dependent and culture-independent. Pure culture techniques for detecting and enumerating microbial species were as reliable as traditional techniques. Culture-based approaches provide useful knowledge about a small part of microorganisms. Also, Culture-dependent techniques provide physiological information (De Mandal et al., 2020).

2. Multidrug resistance of pathogens

The ability of a microorganism to resist the action of various antimicrobials is called multidrug resistance (MDR). Multiple types of resistance mechanisms are observed in microbes, including natural resistance in particular microbes against a specific antimicrobial, genetic mutation or acquired resistance from other species (Catalano et al., 2022). Drug resistance is increasing due to the indiscriminate use of antimicrobial drugs, and these resistant microorganisms are very difficult to tackle, as they require different or higher dosages of antimicrobials, or there is a lack of potent antimicrobials. According to the World Health Organization (WHO), MDR infections known as 'superbugs' are one of the most severe public health risks, accounting for several million fatalities worldwide each year (Parmanik et al., 2022).

2.1.Mechanisms of Bacterial resistance

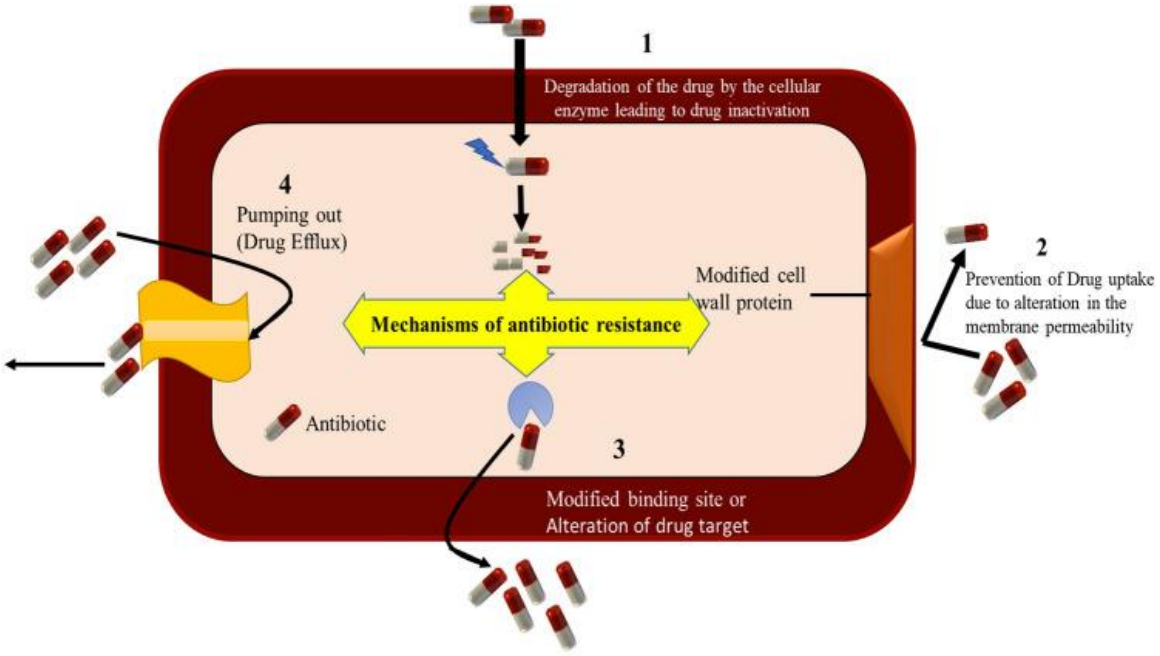

Fig.1.Mechanisms of antimicrobial resistance. (1) Drug inactivation by bacterial enzyme; (2) drug absorption decreases by reducing bacterial cell permeability; (3) changes in the structure of drug target by gene mutation; and (4) drug efflux outside the bacterial cell membrane (Gaglio et al. 2016).

Antibiotic resistance in microorganisms is primarily caused by four different mechanisms (Fig. 1). Penicillin G inactivation by β-lactamase enzyme produced by penicillin-resistant bacteria is an example of how the protective enzymes of the bacterial cell wall deactivate antibiotics throughout the drug inactivation process (Gaglio et al. 2016). Similarly, a protective protein attached to the bacterial cell's ribosomes inactivates the antibiotics by "target altering" their structural confirmation. Their ability to synthesize proteins is subsequently inhibited. Methicillin-resistant *Staphylococcus aureus* (MRSA) exhibits this kind of resistance, in which protective proteins attached to the bacterial cell's ribosomes render antibiotics inactive. As a result, bacterial protein synthesis is inhibited and antibiotic binding to the ribosome is prevented (Lambert, 2005). As bacteria alter a metabolic

pathway, they often protect or modify the target site and restrict the capacity of antibiotics to attach to it, which lowers the affinity of antibiotic molecules. With the precursor of para-aminobenzoic acid (PABA), bacteria produce nucleic acid and necessary folic acid (Lade et al., 2021). However, when an antibiotic targets this mechanism, the bacteria use folic acid that has already been prepared to adapt. This limits the effectiveness of medicines that reduce bacterial growth by blocking the PABA pathway, such as sulphonamide. Additionally, bacteria use a drug efflux mechanism to either restrict drug permeability from the cell surface or enhance the expression of active efflux pumps, which leads to low-level antibiotic susceptibility. This particular type of resistance to fluoroquinolones has been demonstrated by some bacteria before the drug is absorbed through the bacterial cellular membrane's integrated pump (Davin-Regli et al., 2008). Gram-negative bacteria employ all four drug resistance mechanisms; however, gram-positive bacteria do not have a drug efflux mechanism due to the absence of lipopolysaccharides in their outer membranes. This shows that the shape of bacteria's outer membrane is essential to drug resistance to a variety of antibiotics. (Pang et al., 2019).

3. Cyanobacteria

Cyanobacteria are ubiquitous photosynthetic bacteria, that are abundant in in all kinds of ecosystems, including extreme environments (Whitton and Potts, 2000). Cyanobacteria were the first photosynthetic microorganisms to live in aquatic environments 3.5 billion years ago. They possessed prokaryotic cell structures and were able to carry out photosynthesis (Yalcin, 2020). They are found in a wide range of environments because of their vast evolutionary histories and physiological adaptations (Weis & Pang, 2010). By changing their metabolic pathways in response to the environment, these organisms may

multiply in a variety of habitats (such as lakes, oceans, mountains, deserts, and even Antarctica and the Arctic) (Stal, 1995). They exhibit substantial morphological variations across species and between filamentous and single-celled forms. Cyanobacteria cells range in size from 0.5-1 μm to 40 μm. It can produce pigments such as chlorophyll-a and at least one phycobilin. Most cyanobacteria that include chlorophyll-a have a blue-green colour due to the presence of phycocyanin, a blue pigment that belongs to the phycobilin group (Madigan, Martinko, Stahl, & Clark, 2012). The capacity of specific cyanobacteria species to fix atmospheric nitrogen (N2) is a significant characteristic. Nitrogen-fixing cyanobacteria species are typically filamentous, with a limited number of specialized cells known as "Heterocysts" being responsible for N2 fixation (Whitton, 2000). Cyanobacteria have developed defence mechanisms to survive in a highly competitive environment, which led to the production of a huge diversity of chemicals from several metabolic pathways. The majority of these metabolites are physiologically active compounds with a range of chemical structures, methods of action, and bioactive targets (Skočibušić et al., 2019).

Cyanobacteria produce many secondary metabolites with unique compositions and functions, involving chemical defense, preservation, and quorum sensing (Brilisauer et al., 2019). Cyanobacterial metabolites consist of antiviral, antibacterial, antifungal, and herbicidal properties, thus being potentially useful for human health, agriculture, and industry. Secondary metabolites produced by cyanobacteria are frequently utilized as pigments and dyes considering they have antibacterial properties and may prevent the growth of infections in marsh water. Natural bioactive compounds found in cyanobacteria are hard to synthesize chemically. Numerous biological actions, including antibacterial, antioxidant, and anticancer properties, are exhibited by these compounds (Estela et.al., 2013; Shishido et al., 2020)

4. Antibacterial compounds of Cyanobacteria

The excessive use of antibiotics is the main reason for the rise in multidrug-resistant organisms. Most antibiotics have been shown to cause microbial resistance so far, and the situation has been made worse by antibiotic adverse reactions (Alwathnani et al., 2017). Therefore, it's essential to identify a new class of antibacterial drugs with little to no adverse reactions. A focus on aquatic species, particularly cyanobacteria, has been made.

Cyanobacteria-derived bioactive compounds have been observed to have antibacterial (Heidari et al. 2012, Malathi et al. 2014), antifungal (Najdenski et al. 2013), antiviral, anticoagulant, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antitumor properties (Abd El Sadek et al. 2017). Cyanobacteria synthesise a variety of potent antimicrobial secondary metabolites, including fatty acids (Gheda et al. 2013), acrylic acid, halogenated aliphatic compounds, terpenes, sulfur-containing heterocyclic compounds, polysaccharides, and phenols (Pandy 2015). Cyanobacteria contain biologically active molecules with antioxidant activity, including polyunsaturated fatty acids (PUFA), phycobiliproteins, β-carotene, pro-vitamins, and phenolic compounds (Shanab et al. 2012). These molecules could function collectively to induce antimicrobial and cytotoxic activities (Bharat et al. 2013).

4.1. Cyanobacterial metabolites as novel antibacterial agents

i. Alkaloids

Alkaloids are naturally occurring nitrogen-containing molecules with diverse structures. Cyanobacteria-derived alkaloids, particularly indole alkaloids, are effective antimicrobials. Fischerella sp. contains numerous alkaloid compounds that are effective against pathogens such as *Staphylococcus aureus,*

Mycobacterium tuberculosis, Mycobacterium smegmatis, Bacillus anthracis, Bacillus subtilis, Staphylococcus aureus, and Staphylococcus epidermis (Mo et al., 2010).

ii. Polyketides

Polyketides are common secondary metabolites found in plants, fungi, bacteria, insects, and some marine organisms. Polyketide synthases (PKS) can synthesize diverse structures from distinct building blocks. Additionally, they have been shown to have substantial bioactivity and to hold promise for the development of novel natural products as drugs (Ma et al., 2020; Yuzawa and Kuzuyama, 2020). Polyketides such as anaephenes A-C and cylindrofridins are efficient against infections such as Methicillin Resistant *Staphylococcus aureus, Bacillus cereus, Mycobacterium TB, and Streptococcus pneumoniae* (Brumley et al., 2018)

iii. Peptides

Cyanobacteria metabolites include numerous peptides, which are effective antimicrobials. Cyclic peptides isolated from cyanobacteria include AK-3, calophycin, hormothamnin A, lobocyclamide B, nostocyclamide, and tolybyssidin A and B. Several compounds isolated from cyanobacteria include Laxaphycin A, Tiahuramide A-C, Hormothamnin A, and [D-Leu1] MC-LR (Levert et al., 2018).

iv. Other classes of metabolites

Terpenes and polyphenols class of chemicals has also been isolated from cyanobacteria with a significant antibacterial action (Carpine and Sieber, 2021). Cyanobacteria produce polyhalogenated compounds (PHCs), including Ambigols A, B, C, D, and E, which are effective against MRSA. (Choi et al. 2010).

4.2.Mechanism of action of cyanobacteria metabolites as antibacterial agents

Quorum sensing inhibition is a prevalent method of action for metabolites. Bacteria have an intercellular communication system called quorum sensing, which is crucial for their pathogenicity and ability to form biofilms. Another mechanism of action involves damaging the target bacteria's cell membrane. discovered that lipids and peptides have antibacterial activity by compromising membrane integrity, causing cell lysis, altering electron transport chains, and blocking key bacterial enzymes (Yoon et al.,2018). Several biological mechanisms in bacteria, including the Shikimate pathway, the electron transport chain, and cell wall production, are also disrupted by various bioactive substances. By collapsing the proton motive force, alkylphenols cause bacteriostasis by preventing ATP production and active transport (Denyer et al., 2011). Furthermore, cyanobacteria's secondary metabolites can influence the activity of many enzymes, including RNA polymerase and DNA polymerase, which have an impact on the synthesis of proteins, RNA, and DNA (Doan et al., 2000).

AIM

This study aims to determine the potential of Cyanobacteria from wetland ecosystems to produce antimicrobial compounds that can act against human pathogens and can be used in the medical field.

OBJECTIVES

1. To isolate and culture cyanobacteria from wetland ecosystems.
2. To screen cyanobacterial isolates to produce antimicrobial compounds against human pathogens.

HYPOTHESIS

Cyanobacteria in natural ecosystems are in competition with bacteria for limited resources and thus produce various antibacterial compounds as a survival mechanism. Isolation of pathogenic bacterial cultures and concurrent isolation of cyanobacterial cultures from wetland ecosystems, followed by screening of crude cyanobacterial extracts against pathogenic bacteria, is expected to yield isolates with potential antibacterial activity, which may be further tested.

LITERATURE REVIEW

1. Cyanobacteria and their Antibacterial activity

Thummajitsakul et al. (2012) studied eighteen cyanobacteria from habitats and evaluated their antimicrobial activity. Two taxa of cyanobacteria (NCI1 and NCI4) with positive antibacterial activity were isolated from two separate freshwater ponds and recognized mostly through external morphology, it showed that the two isolates belonged to the *Oscillatoriaceae* family. In 2014, Shaieb et al. conducted an experiment on *Nostoc commune* with different nitrogen concentrations. It found that the maximum growth rate and lowest generation time for *Nostoc* were recorded in the culture supplemented with +4 Nitrogen. Also, the dry mass and chlorophyll a level increased notably as the nitrogen concentration increased.

A study was done on *Synechococcus* sp. by do Amaral et al, (2020) concluded that lipophilic extracts exhibit higher antibacterial effects than hydrophilic compounds as the extracts did not show any inhibitory zone against any of the gram-positive test organisms.

Cyanobacteria produce bioactive substances with antibacterial, antifungal, antiviral, and anti-inflammatory properties having industrial, medicinal, and agricultural applications (Sethubathi & Prabu, 2010). According to Thajuddin & Subramanian, 2005, Cyanobacterial extracts have antibacterial action, making them an important source of unique bioactive substances in pharmaceuticals. Abd El-Aty et al. (2014) found that *Anabaena sphaerica* and *Oscillatoria agardhii* exhibit antibacterial efficacy against both Gram-negative and Gram-positive bacteria.

Yalcin D (2020) studied *D. affine* extracts using three solvents: methanol, chloroform, and aqueous extracts. *D. affine* indicated antibacterial activity against four out of five pathogenic strains. Methanol extracts showed higher antibacterial activity than chloroform extracts. DIZ data showed that *Bacillus subtilis* and *Escherichia coli* were more sensitive,

whereas *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed intermediate values. The antibacterial effect of *D. affine* extracts was compared to commercial antibiotics. The chloroform and methanol extracts from *D. affine* were found to be 63% and 66% effective against *E. coli*, respectively, similar to the efficacy of currently used antibiotics. These findings suggest that *D. affine* may be a viable alternative to commercial antibiotics in phytotherapy.

Halder (2015) used the agar well diffusion method to test the antibacterial effects of *Anabaena variabilis* extracts in various solvents against eight pathogenic bacterial strains, including three Gram-positive (*Bacillus subtilis, Micrococcus luteus, and Staphylococcus aureus*) and five Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Shigella flexneri*, and *Vibrio cholerae*).

Najdenski et al, (2013) did research using the samples of all investigated cyanobacterial strains that were initially evaluated for antimicrobial activity by agar diffusion test. Extracts were made in two methods: water extracts and culture liquids. The culture liquid of *Anabaena sp*. showed the highest activity against Gram-positive bacteria. Ethanol extracts and fatty acids from all cyanobacteria were active against *Streptococcus pyogenes* and/or *Staphylococcus aureus*. The fatty acids of *Synechocystis* sp. inhibited the growth of *Bacillus cereus, Escherichia coli* and *Candida albicans*.

In the study conducted by Safari et al., (2019), the antibacterial activity of two strains of cyanobacteria was investigated by the disc diffusion method. The results indicate that the aqueous and methanolic extracts of *F. ambigua* have a substantial antimicrobial action, whereas the studied extracts of *S. vaginata* do not. The results also clearly demonstrated that the aqueous extract of this cyanobacterial strain exhibited strong antibacterial activity against most pathogenic bacteria, with the highest activity against Staphylococcus aureus

(PTCC 1112). The results showed that aqueous extracts had a greater antibacterial impact against gram-positive bacteria than gram-negative bacteria.

Soil cyanobacteria for the present research by Soltanieta et al., (2005) were chosen from various geographical locations due to the high concentration of rice fields in these areas. Seventy-six strains were isolated, and the collected cells were extracted with solvents such as petroleum ether and methanol. The supernatant was removed separately. Seventeen isolated species showed antimicrobial activity. According to a study done by Shaieb et al. (2014), *Anabaena circinalis* extracts are effective against *S. marcescens* and *E. coli*, but not against *K. pneumoniae*. The Nostoc community is effective against a variety of bacteria, including *E. coli, S. marcescens, B. cereus, K. pneumoniae,* and *M. luteus*. *Nostoc muscorum* has a broad range of activity against both Gram-positive (*S. aureus, M. luteus, and B. cereus*) and Gram-negative (*P. aeruginosa, K. pneumoniae, and S. marcescens*). Dash et al.,(2022), studied using the agar well diffusion method which is used to evaluate the antibacterial activity of two marine cyanobacteria, *Oscillatoria boryana* and *Oscillatoria pseudogeminata*, against four pathogenic bacteria strains (*S. aureus, B. Subtilis, V. cholerae, and E. coli*). Different solvent extracts showed different antimicrobial activity against each pathogen. The highest zone of inhibition against *V. cholerae* measured 20mm.

Noaman et al. (2004) found that the species of *Synechococcus* produces an antibacterial compound which is effective against Gram-positive *Staphylococcus aureus* and factors such as media, temperature, pH and carbon source for their optimum growth and antimicrobial agent production were studied. BG-11 medium is reported to be a potential media for their growth.

MATERIALS AND METHODS

1. Sample collection and Isolation of Cyanobacteria

Two wetland ecosystems were chosen as sample sites in Goa. The samples were collected from these wetlands, and carried to the laboratory and physico-chemical parameters were checked. The samples were preserved at 4°C until used.

Table 1: Details of the sample site

Location	Date	Coordinates	Season
Nanda lake	7/07/2023	15.241323, 74.105460	Monsoon
	28/11/2023		Dry
Sarzora lake	7/07/2023	15.218624, 74.004720	Monsoon
	28/11/2023		Dry

The 500ml of water sample was pre-filtered through a 1.6µm filter membrane to eliminate the large particles and mud. Then this sample was again filtered through a 0.22µm filter membrane to collect Cyanobacteria. The filter membrane was collected and flushed using BG-11 medium in a sterile flask of 250ml containing 200ml of BG-11 medium and kept in direct sunlight at room temperature for growth. The flasks were shaken once a day to provide oxygen and growth was seen after 2-3 weeks. The culture was then subcultured for purification.

For the purification of culture, two different methods were used. In first, 0.5ml of grown culture was taken and inoculated in 4.5ml of BG-11 medium and diluted in a sterile six-well plate by serial dilution method and in the second method, a loopful of grown culture was directly streaked on BG-11 agar plates and incubated at room temperature under direct sunlight.

2. Isolation of Test organisms

Pathogenic bacteria were isolated by spread plating 0.1ml of sample water taken from different wetlands onto a differential medium (MacConkey's Agar, EMB agar, TCBS agar, and SS Agar) to obtain pathogenic bacteria that include *E.coli*, *Vibrio*, Enteric bacteria *Salmonella*, and *Shigella*. The plates were incubated at 37°C for 24 hours. Colonies with selective characteristics on the media were selected and subcultured on the same media for purification and tentatively identified. Then the colonies were picked and subcultured to purify them and to provide a preliminary identification.

2.1 Identification of test organism by Biochemical Tests

Biochemical tests were done for the test organisms which include, Indole, Methyl red, Voges Proskauer, Citrate, Triple Sugar Iron and sugar fermentation. The test pathogens were inoculated in the media for Indole, Methyl red, and Voges Proskauer and incubated at 37°C for 24 hours and results were tested. For sugar fermentation, test pathogens were inoculated in the media containing inverted Durham's tube and incubated at 37°C for 24 hours. Slants of Simmon's citrate agar were streaked and TSI slants were stabbed and streaked and kept for incubation at 37°C for 24 hours and results were obtained.

2.2.1. Identification of test organism by Molecular identification

The genomic DNA of the test organisms was extracted using a standard phenol-chloroform method (Green and Sambrook., 2017). 4ml of overnight grown test pathogenic culture was centrifuged and the pellet was suspended in lysis buffer and incubated for 45 minutes in the water bath. After incubation, Proteinase K and 10%

SDS were added and incubated for an hour. NaCl and CTAB/ NaCl were added and again incubated. Then equal amount of Phenol: Chloroform: isoamyl alcohol was added in the ratio of 25:24:1 and centrifuged. The aqueous layer was collected and an equal amount of Chloroform: isoamyl alcohol was added in the ratio 24:1 and again centrifuged. The aqueous phase was collected and 0.7 volume of Isopropanol was added and kept for incubation at room temperature for 1 hour and then centrifuged and the supernatant was discarded. Pellet was rinsed with 70% ethanol and tubes were allowed to dry. The pellet was then resuspended in 10mM TrisCl and stored at -20°C. The isolated DNA was run on a 0.8% agarose gel and visualised under a UV- transilluminator.

2.2.2. PCR amplification of the isolated DNA sample

The 16S rRNA genes were amplified using specific primers 27F and 1492R and other required reagents listed in Table no.2. and PCR was carried out as per the parameters mentioned in Table 3.

Table 2: PCR reagents

Sr. No	Reagents	Vol in µl
1.	MilliQ water	**35.5**
2.	PCR buffer	**5.0**
3.	dNTP mix	**1.0**
4.	Forward primer	**2.5**
5.	Reverse primer	**2.5**
6.	Template DNA	**3.0**
7.	Taq polymerase	**0.8**
	Total	**50.0**

Table 3: PCR reaction conditions

PCR parameters	Temperature	Time	
Initial denaturation	94°C	5 min	
Denaturation	94°C	30 sec	
Annealing	56°C	45 sec	15 cycles
Extension	72°C	1 min	
Denaturation	94°C	30 sec	
Annealing	59°C	45 sec	15 cycles
Extension	72°C	1min	
Final extension	72°C	5 min	

PCR products were checked on a 0.8% agarose gel and submitted for Sanger sequencing. The forward and reverse sequencing reads were combined to obtain a contig using Bioedit (7.7.1). The contig sequences were analysed using BLAST and the Phylogenetic tree was constructed using MEGA (11.0.13).

3. Preparation of Cyanobacterial crude extract

Cyanobacterial cells were harvested by centrifuging and the pellets were dried at 60°C and then ground in sterile tubes. The cells were mixed with 1 ml of 80% ethanol and were shaken for 5 minutes and kept at room temperature for 8 hours. After that, the solvent was dried by incubation at 60°C and redissolved in water (ratio 0.2, 0.06, 0.03, and 0.015 g/ml) and maintained at 4°C till use for further testing (Thummajitsakul et al., 2012).

4. Antibacterial test of cyanobacterial extracts by agar well diffusion

The antibacterial activity test was done using the agar well diffusion method (Perez, Pauli, & Bazerque, 1990). 0.1 ml of diluted inoculum (105 CFU ml-1) of the bacterial strains were swabbed on MH agar plates. A pour plate using Nutrient agar was also made to which 1 ml of test culture suspension was added to molten nutrient agar and overlayed on Nutrient agar plate. 6.0 mm size diameter wells on agar plates were punched with a sterile cork borer (6.0 mm) and 100µl of algal extract was added to the wells made on each plate and allowed to pre-diffuse for 30 mins at 4°C. And then incubated at 37±2°C for 24 to 48 h. Antibacterial activity was observed by measuring the zone of inhibitions (mm) against the bacterial strains. Negative controls were prepared using sterile distilled water.

ANALYSIS AND CONCLUSIONS

Fig 2. Sample collection sites and locations A) Nanda Lake B) Sarzora Lake

Two sample sites were chosen as given in Figure 1. A) Nanda Lake and B) Sarzora Lake and samples were collected during the wet season and dry season. Nanda Lake was found to have a salinity 0 during both seasons and the pH was 6.48 in the wet season and 6.38 in the dry season. Also, for the Sarzora Lake sample, Salinity was 0 in both seasons and pH was 6.7 and 6.64 in the wet and dry seasons respectively.

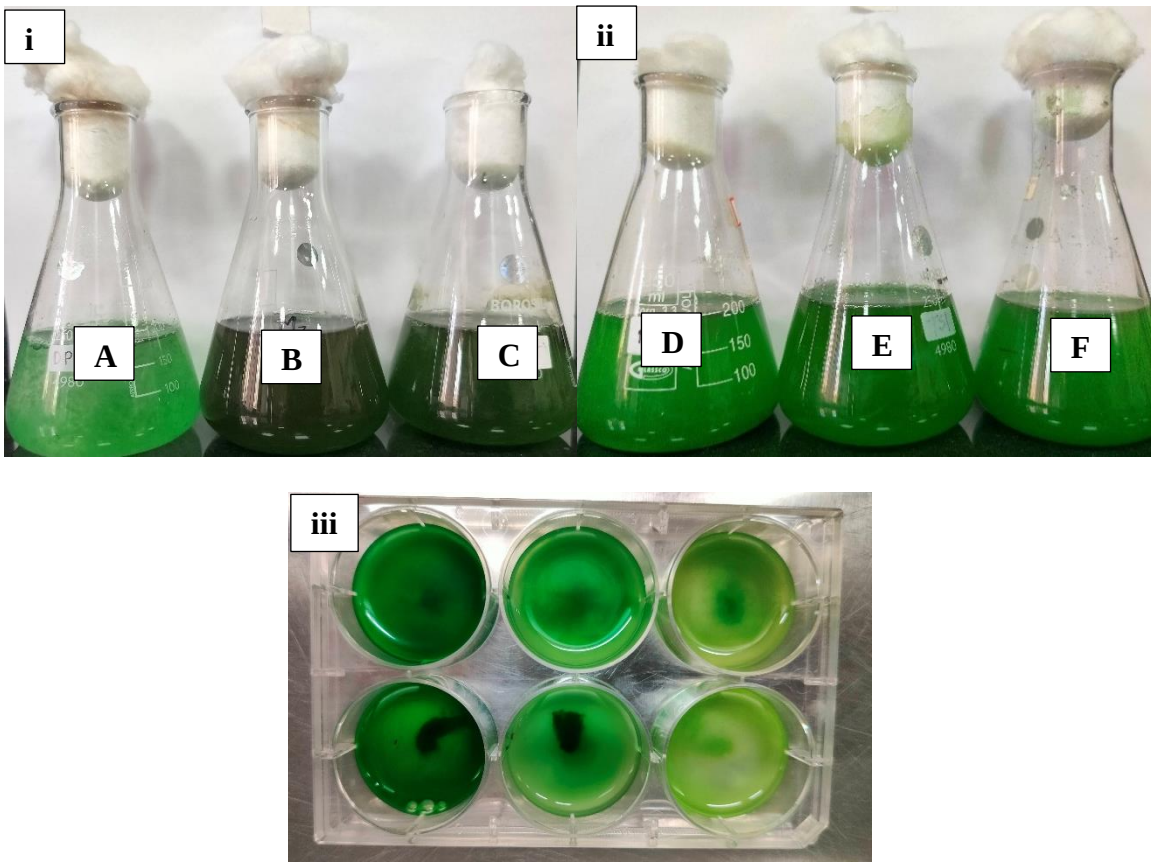

Fig 3. Isolation and purification of Cyanobacterial cultures:

i) Previously purified cultures; A) *Synechococcus sp.*, B) *Synechocystis sp.*, C) *Limnothrix sp*.

ii) Cultures isolated from sites; D) Nanda dry season consortia, E) Nanda wet season consortia, F) Sarzora overflow consortia

iii) Purification of Cyanobacterial culture by six-well plate serial dilution method

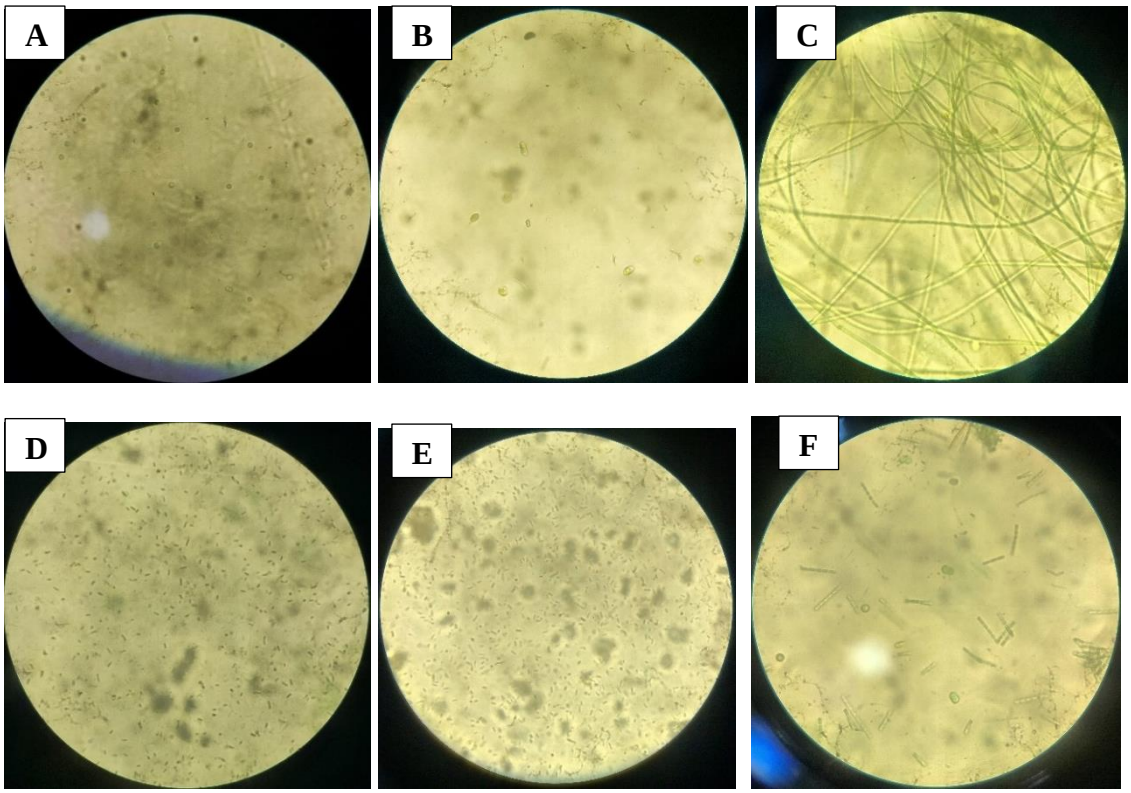

Fig 4. Microscopic images of Cyanobacteria: A) *Synechococcus sp.*, B) *Synechocystis sp*., C) *Limnothrix sp*., D) Nanda wet season consortia, E) Nanda dry season consortia, F) Sarzora overflow consortia

In total, 3 newly isolated cyanobacterial consortia were maintained and subcultured in BG-11 medium. The "Sarzora overflow" consortium was isolated from the overflow site at the lake. This is a section separate from the main body of the lake, which is maintained to balance the lake water levels, according to the opening and closing of the sluice gate.

All the grown cultures were visualised under a microscope to observe their morphology. Cultures A, B, and C were previously isolated and purified and cultures D, E, and F were isolated from sample sites. Culture A is *Synechococcus sp*, a unicellular cyanobacterium, culture B is *Synechocystis sp.*, also unicellular, and culture C is *Limnothrix sp*., which is filamentous in morphology. Cultures D, E and F are Nanda wet season consortia, Nanda dry season consortia and Sarzora overflow consortia, which are a mixture of various cyanobacterial forms.

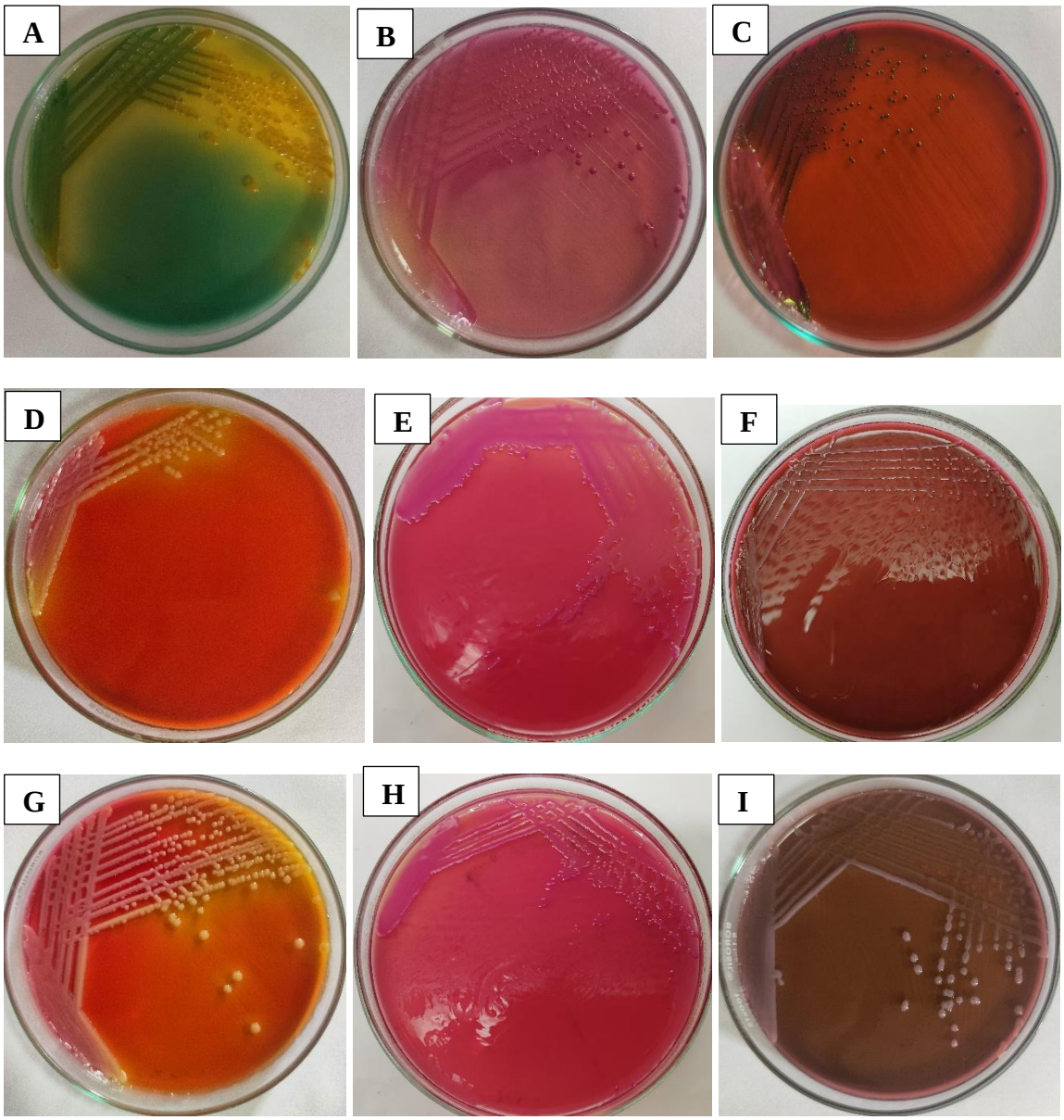

Fig 5. Isolation of pure bacterial cultures A) *Aeromonas* sp. on TCBS agar, B) *Klebsiella sp*. On Mac Conkey Agar, C) *E. coli* on EMB agar, D) *Shigella sp*. on SS agar, E) *E.coli* on MacConkey agar, F) *Salmonella/ Shigella sp.* on EMB agar, G) *Enterobacter* sp. on SS agar, H) *E.coli* on MacConkey agar, I) *Salmonella/ Shigella sp*. on EMB agar

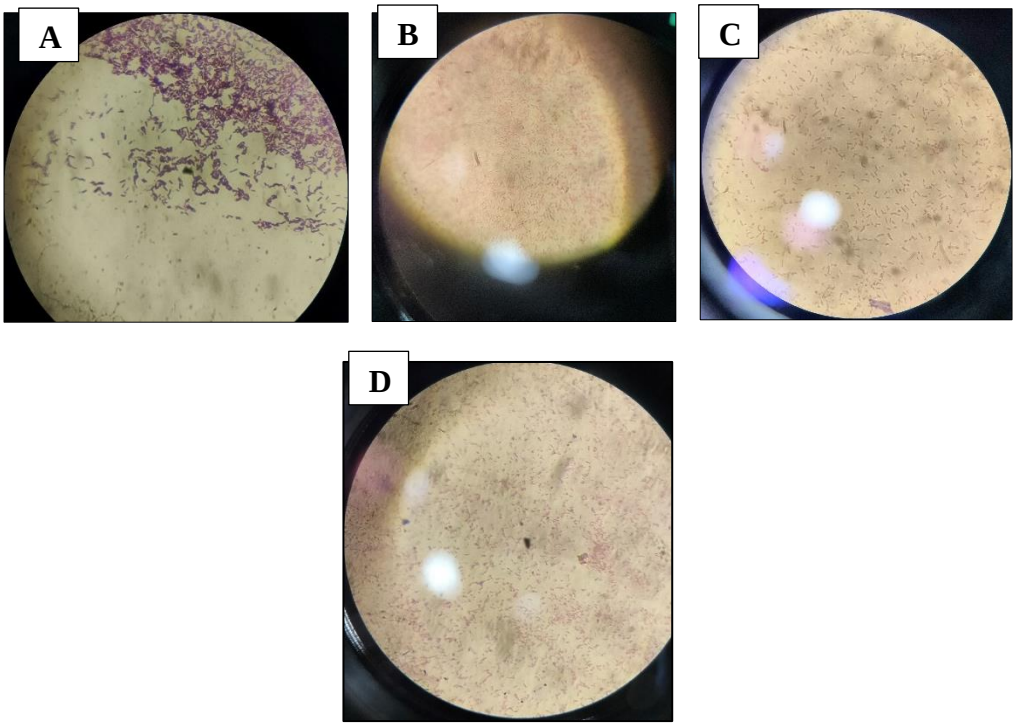

Fig 6. Gram staining of test organisms A) *Klebsiella sp*., B) *Vibrio cholerae*, C) *E.coli*, D) *Shigella sp.*

Table 4: Colony characteristics of test organism isolated from Nanda Lake

Media	TCBS	MacConkey	EMB
Time	24 hr	24 hr	24 hr
Temperature	37 °C	37 °C	37 °C
Size	0.3mm	0.2mm	0.2mm
Shape	Circular	Circular	Circular
Colour	Yellow	Dark pink	Green metallic sheen
Margin	Entire	Entire	Entire
Elevation	Raised	Convex	Convex

Surface texture	Smooth	Smooth	Smooth
Consistency	Viscid	Mucoid	Viscid
Opacity	Opaque	Opaque	Opaque
Gram character	Gram -ve curved rods	Gram -ve rods	Gram -ve curved rods

Table 5: Colony characteristics of test organism isolated from Sarzora lake

Media	SS	MacConkey	EMB
Time	24 hr	24 hr	24 hr
Temperature	37 ℃	37 ℃	37 ℃
Size	0.3 mm	0.2mm	0.3 mm
Shape	Round	Circular	Circular
Colour	Yellow	Pink	Colourless
Margin	Smooth	Smooth	Smooth
Elevation	Raised	Convex	Convex
Surface texture	Dry	Shiny	Viscous
Consistency	Mucoid	Mucoid	Mucoid
Opacity	Opaque	Opaque	Translucent
Gram character	Gram -ve rods	Gram -ve rods	Gram -ve rods

Table 6: Colony characteristics of test organism isolated from the Overflow region of Sarzora Lake

Media	SS	MacConkey	EMB
Time	24 hr	24 hr	24 hr
Temperature	37 °C	37 °C	37 °C
Size	0.3mm	0.2 mm	0.3 mm
Shape	Round	Round	Round
Colour	Yellow	Pink	Colourless
Margin	Entire	Entire	Entire
Elevation	Raised	Convex	Convex
Surface texture	Dry	Shiny	Viscous
Consistency	Mucoid	Mucoid	Mucoid
Opacity	Opaque	Opaque	Translucent
Gram character	Gram -ve rods	Gram -ve rods	Gram -ve rods

The isolates obtained on selective media were further characterized. On TCBS agar, colonies appearing in yellow colour are identified as *Vibrio cholerae* or *Aeromonas* sp., *E. coli* shows a green metallic sheen on EMB agar whereas non-lactose fermenters such as *Shigella* or *Salmonella sp.* appear as translucent colonies. Dark pink or red colonies on MacConkey agar are known to be *E. coli* and *Klebsiella sp.* appears as light pink in colour. *Shigella sp*. appears as a pink or yellow colonies producing a yellow halo around it and *Enterobacter* sp.forms yellow colonies on SS agar.

The gram character of each of the colonies was visualised under a microscope of which *Vibrio cholerae* is gram-negative and has curved rods. *E. coli*, and *Shigella sp*. are gram-negative short rods whereas *Klebsiella sp*. are encapsulated gram-negative short rods.

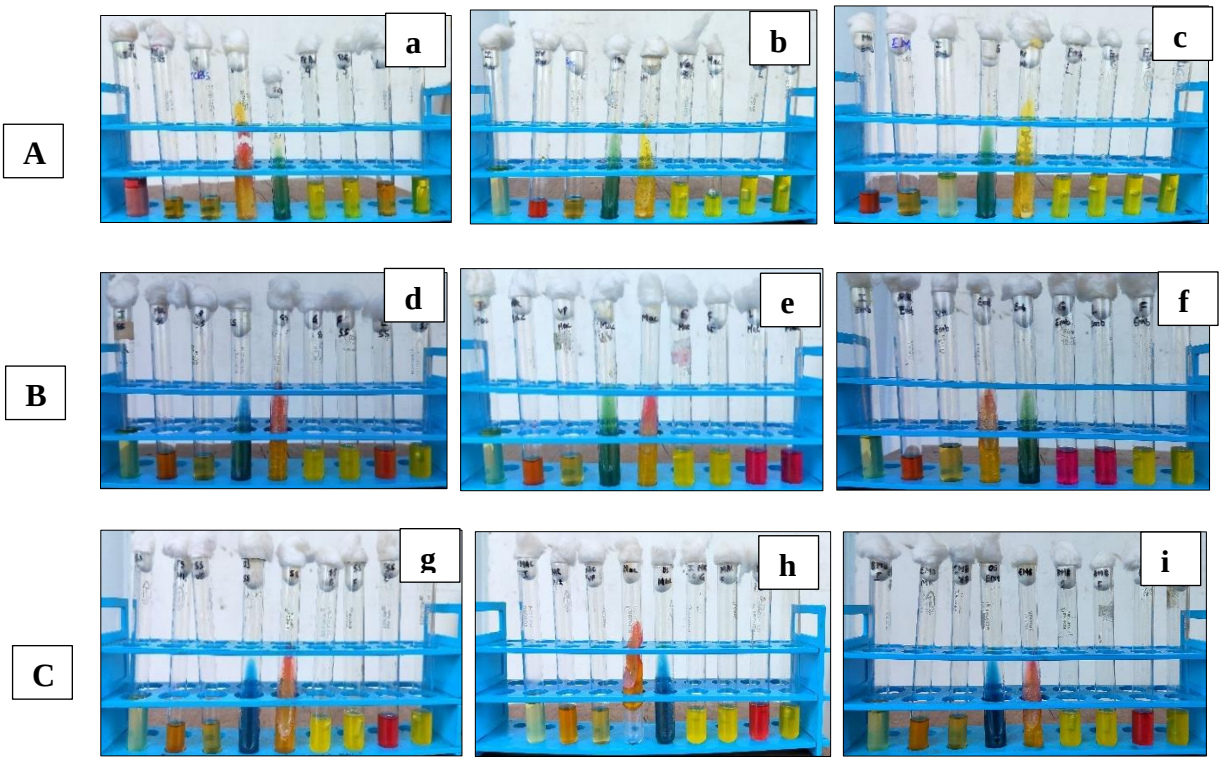

Fig 7. Identification of test organism by Biochemical tests:

A. Nanda Test pathogens; a) Isolate from TCBS agar, b) Isolate from MacConkey agar, c) Isolate from EMB agar

B. Sarzora test pathogens; d) Isolate from SS agar, e) Isolate from MacConkey agar, f) Isolate from EMB agar

C. Sarzora overflow test pathogens; g) Isolate from SS agar, h) Isolate from MacConkey agar, i) Isolate from EMB agar

Table 7: Biochemical test results of test organism isolated from Nanda Lake

Biochemical tests	**Nanda**		
Media	**EMB**	**MAC**	**TCBS**
Indole	-	-	+
Methyl red	+	+	-
Voges Proskauer	-	-	-
Citrate	-	-	-
TSI	A/A gas+, H2S-	A/A gas+, H2S-	K/A gas-, H2S-
Fermentation from sugars:			
Glucose	+gas	+gas	+
Sucrose	+gas	+gas	+
Fructose	+gas	+gas	+
Lactose	+gas	+gas	-

Table 8: Biochemical test results of test organism isolated from Sarzora Lake

Biochemical tests	**Sarzora**		
Media	EMB	MAC	SS
Indole	-	-	-
Methyl red	+	+	+
Voges Prouskeur	-	-	-
Citrate	-	-	-
TSI	K/A gas-, H2S-	K/A gas-, H2S-	A/A gas+, H2S-

Fermentation from sugars:			
Glucose	-	+gas	+ gas
Sucrose	+	-	+ gas
Fructose	+	-	+ gas
Lactose	-	-	-

Table 9: Biochemical test results of test organism isolated from the Overflow region of Sarzora Lake

Biochemical tests	**Sarzora overflow**		
Media	EMB	MAC	SS
Indole	-	-	-
Methyl red	+	+	+
Citrate	+	+	+
TSI	A/A gas+, H2S-	K/A gas+, H2S-	K/A gas+, H2S-
Fermentation from sugars:			
Glucose	+gas	+gas	+gas
Sucrose	+	-	+gas
Fructose	+gas	+gas	+gas
Lactose	+	+	+

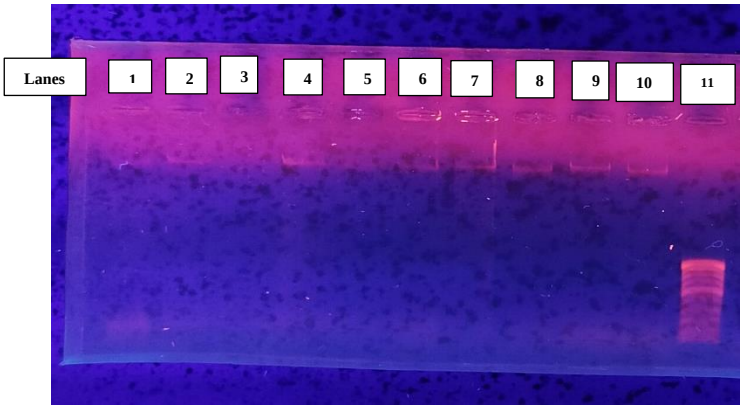

Fig 8. Gel picture of Genomic DNA:

Lane 1-Sarzora EMB, 2- Nanda MAC, 3- Nanda TCBS, 4- Nanda SS, 5- Nanda EMB, 6- Sarzora MAC, 7- Sarzora EMB, 8- Sarzora SS, 9- O. Sarzora MAC, 10-O. Sarzora SS

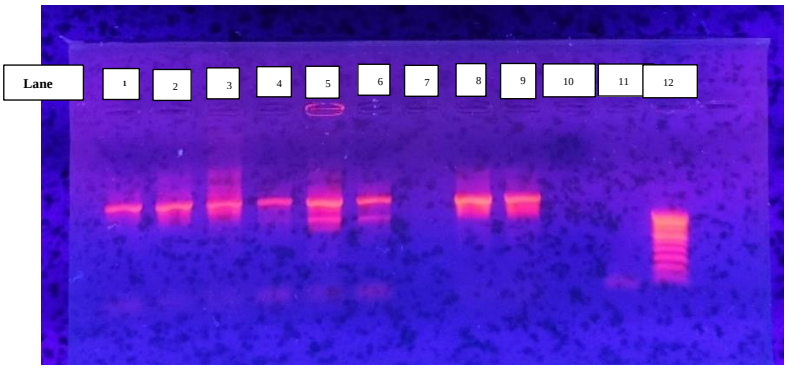

Fig 9. Gel image of the PCR Products:

Lane 1- Nanda TCBS, 2- Nanda EMB, 3- Nanda SS, 4- O.Sarzora MAC, 5- Sarzora SS, 6- O.sarzora SS, 12- DNA ladder

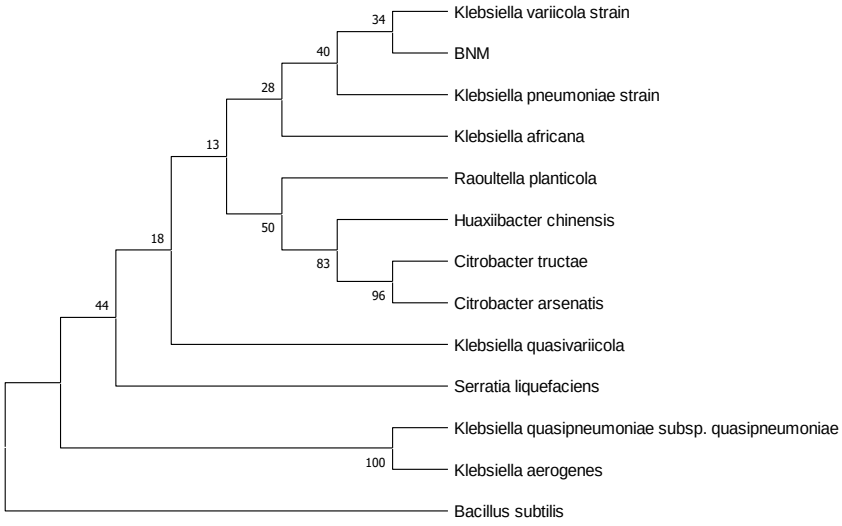

Fig 10: Phylogenetic tree constructed using 16S rDNA sequence of BNM (isolated from Nanda lake and grown on MacConkey agar) with sequences showing identity >90% and with *Bacillus subtilis* as the outgroup

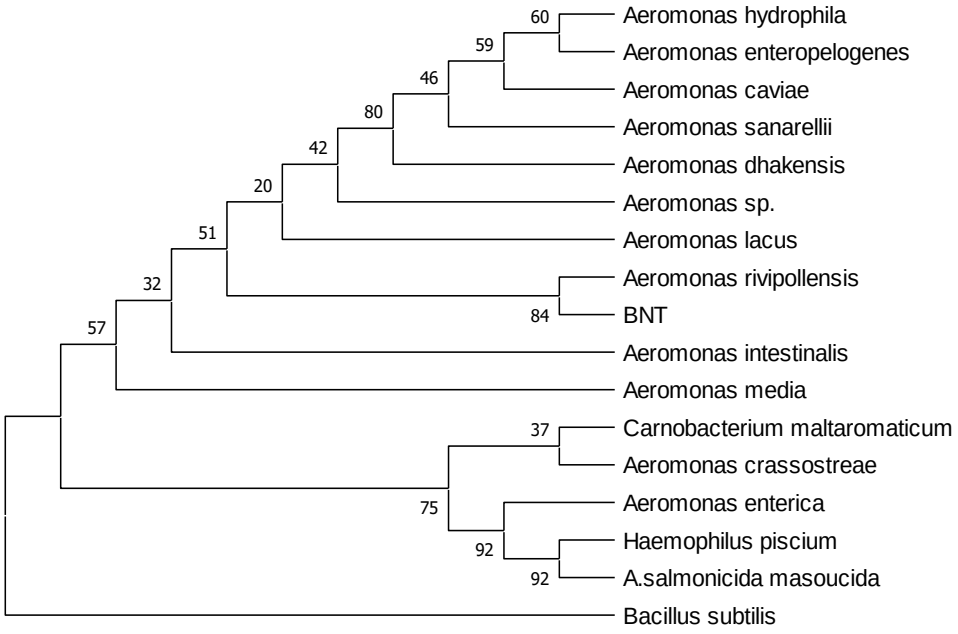

Fig 11: Phylogenetic tree constructed using 16S rDNA sequence of BNT (isolated from Nanda lake and grown on TCBS agar) with sequences showing identity >90% and with *Bacillus subtilis* as the outgroup

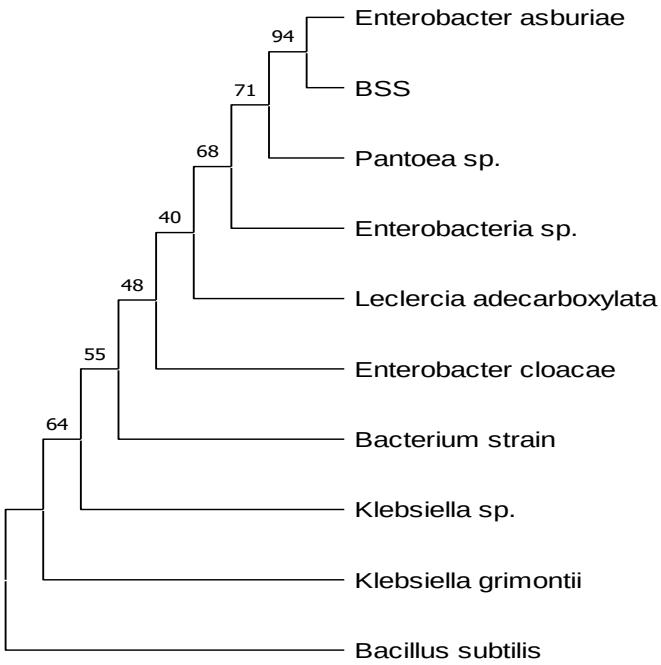

Fig 12: Phylogenetic tree constructed using 16S rDNA sequence of BSS (isolated from Sarzora lake and grown on SS agar) with sequences showing identity >90% and with *Bacillus subtilis* as the outgroup

The genomic DNA successfully isolated were of Sarzora EMB, Nanda MAC, Nanda TCBS, Nanda SS, Nanda EMB, Sarzora MAC, Sarzora EMB, Sarzora SS, O. Sarzora MAC, and O. Sarzora SS.

The PCR amplification of the 16S rRNA gene was successful for Nanda TCBS, Nanda EMB, Nanda SS, O.Sarzora MAC, Sarzora SS, and O.sarzora SS. The sequencing reads of Nanda MAC (here referred to as BNM for simplicity), Nanda TCBS (referred as BNT) and Sarzora SS (referred as BSS) were processed for the construction of a phylogenetic tree. The tree of BNM revealed the closest match to be *Klebsiella variicola*, thus the culture Nanda MAC is identified as *Klebsiella* sp.,the tree of Nanda TCBS matched closest to *Aeromonas riviopollensis* , so the culture is identified as *Aeromonas* sp.

The tree of BSS showed more similarity to *Enterobacter asburiae*,thus it is identified *Enterobacter* sp.

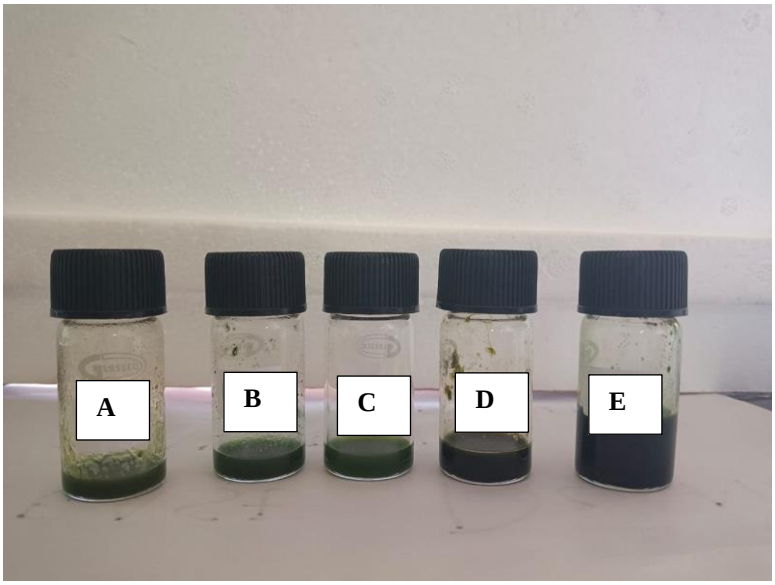

Fig 13. Cyanobacterial Crude extracts prepared from :

A) *Synechococcus sp*., B) *Synechocystis sp*., C) *Limnothrix sp*., D) Nanda consortia, E) Sarzora overflow consortia

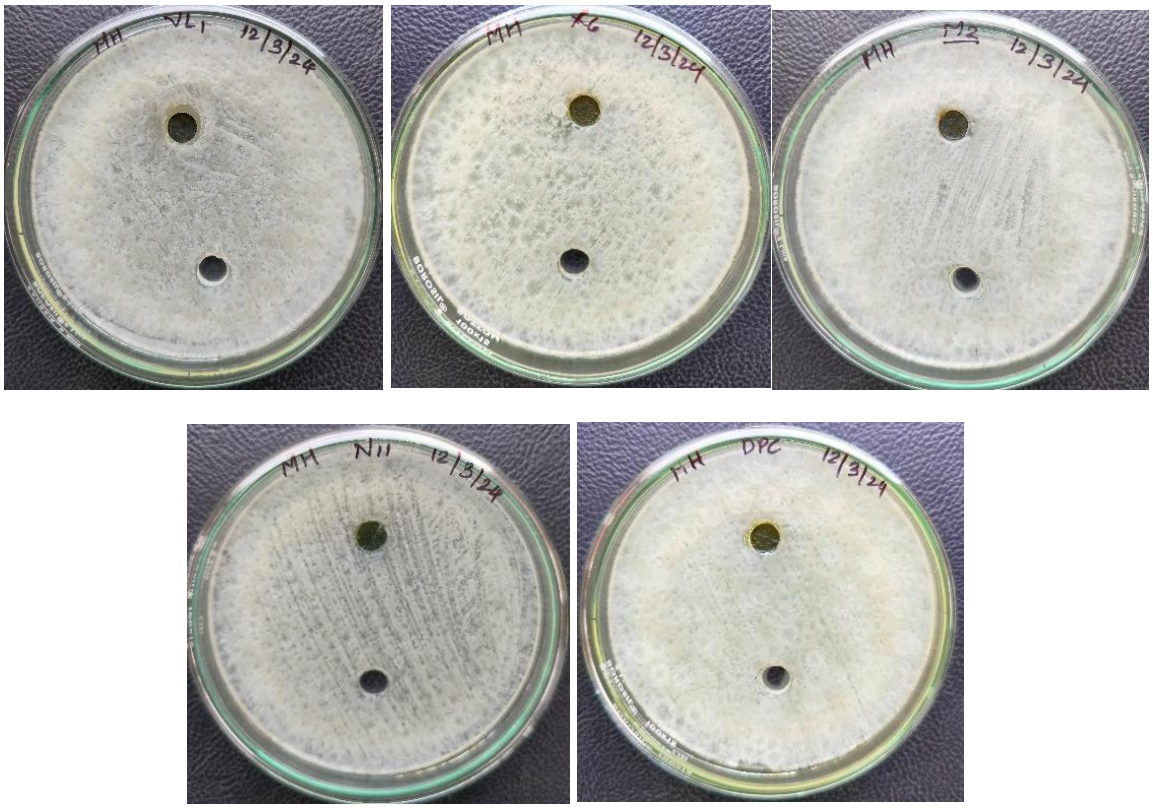

Fig 14. Antibacterial activity of Cyanobacteria against *E. coli* on MH agar

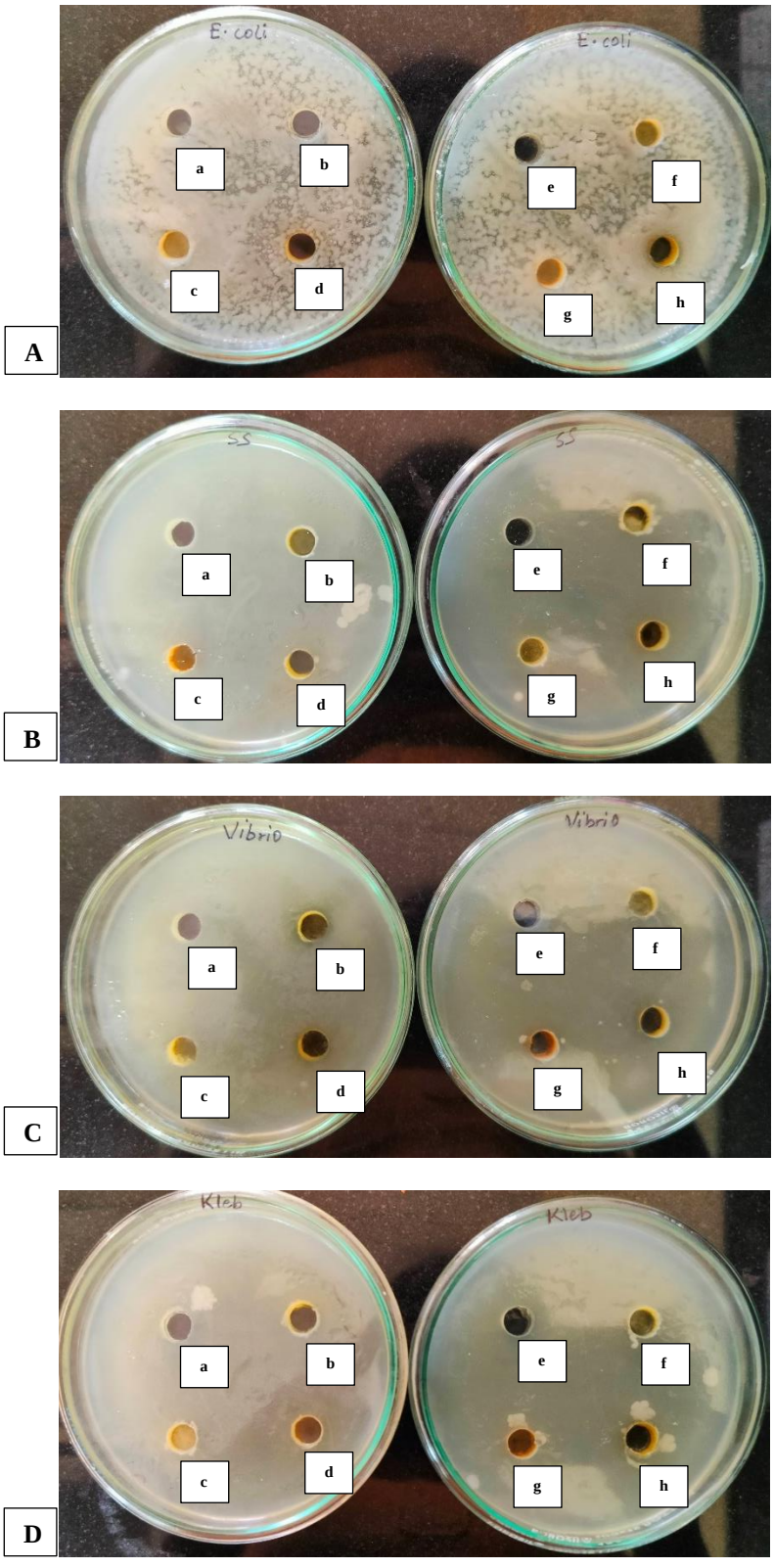

Fig 15: Antibacterial activity of Cyanobacteria against test pathogens

A) *E.coli*, B) *Shigella sp*. C) *Vibrio cholerae*, D) *Klebsiella sp*.

a) Control, b) *Synechocystis sp*., c) Overflow Sarzora consortia, d) *Synechococcus sp*.,

e) Control, f) Nanda monsoon season consortia, g) Nanda dry season consortia,

h) *Limnothrix sp.*

All four of the isolated test pathogens from sample sites were tested against Cyanobacterial consortia isolated from the same wetlands and previously maintained cultures. The cyanobacterial crude extracts did not appear to inhibit the growth of any of the tested pathogenic cultures (Fig. 12 and 13).

Najdenski et al, (2013) reported that the screening of extracellular and intracellular substances of Cyanobacteria was done using the agar diffusion assay method and highly concentrated extracts did not show inhibitory zones or minimal zones were observed, which might be due to low diffusion ability of the active metabolite substance into the agar. Also, water extracts of *Synechocystis sp.* and *Gloeocapsa sp.* were active against a limited range of susceptible pathogens. Rao, (2015), worked on freshwater Cyanobacteria and found that there were no inhibition zones observed in the water extracts and methanol extracts of *Nodularia spumigena* but acetone culture crude extract was effective against it. In the work of do Amaral et al (2020) did cyanobacterial extracts effectively inhibited the growth of *Salmonella typhimurium* ATCC 14021, but not of Gram positive bacterial pathogens It was concluded that lipophilic extracts exhibit greater effects in comparison to hydrophilic extract for antibacterial activity.

CONCLUSIONS

In this study, it was found that extracts prepared of *Synechococcus* sp, *Synechocystis* sp, *Limnothrix* sp. and different Cyanobacterial consortia isolated from wetlands have bioactive compound which are unable to show inhibition zone against gram negative bacteria isolated from same wetlands. Therefore, it concludes that the antibacterial activity is highly depend on solvent used and diffusion methods,

FUTURE PROSPECTS

Cyanobacteria contain various secondary metabolites which are known to possess antibacterial, antifungal, and anticancer properties. The cyanobacterial consortium isolated from wetlands in the present study, can be for further isolated and purified and antibacterial activity against diverse gram positive and gram negative pathogens can be tested. Different extraction methods using different solvents could be used for separating its bioactive compound and identifying its properties.

BIBLIOGRAPHY

1. Abd El Sadek Da, Hamouda Ra, Bassiouny K & Elharoun H. 2017. In vitro antioxidant and anticancer activity of cyanobacteria. Asian J Med Heal 6(3): 1-9.
2. Abd El-Aty, A. M., Mohamed, A. A. & Samhan, F. A. (2014). In vitro antioxidant and antibacterial activities of two fresh water Cyanobacterial species, Oscillatoria agardhii and Anabaena sphaerica. Journal of Applied Pharmaceutical Science, 4(7), 69-75.
3. Alwathnani H., and Perveen, K. Antibacterial activity and morphological changes in human pathogenic bacteria caused by Chlorella vulgaris extracts. Biomedical Research, 28 (4): 2017, pp.1610-1614.
4. Baquero, F., Martínez, J. L., and Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 19, 260–265. Doi: 10.1016/j.copbio.2008.05.006
5. BHARAT N, IRSHAD MD, RIZVI MMA & FATMA T. 2013. Antimicrobial and cytotoxic activities of cyanobacteria. Int J Innov Res Sci Eng Technol 2(9): 4328-4343.
6. Bodelier, P., & Dedysh, S. N. (2013). Microbiology of wetlands. *Frontiers in microbiology*, *4*, 50588.
7. Brilisauer, K., Rapp, J., Rath, P., Schöllhorn, A., Bleul, L., Weiß, E., et al. (2019). Cyanobacterial antimetabolite 7-deoxy-Sedoheptulose blocks the shikimate pathway to inhibit the growth of prototrophic organisms. Nat. Commun. 10:476. Doi: 10.1038/s41467-019-08476-8
8. Brumley, D., Spencer, K. A., Gunasekera, S. P., Sauvage, T., Biggs, J., Paul, V. J., et al. (2018). “Isolation and characterization of Anaephenes a−C, Alkylphenols from a filamentous cyanobacterium (Hormoscilla Sp., Oscillatoriales).”

9. Carpine, R., and Sieber, S. (2021). Antibacterial and antiviral metabolites from cyanobacteria: their application and their impact on human health. Curr. Res. Biotechnol. 3, 65–81. Doi: 10.1016/j.crbiot.2021.03.001

10. Catalano A, Iacopetta D, Ceramella J, Scumaci D, Giuzio F, Saturnino C, Aquaro S, Rosano C, Sinicropi MS. Multidrug resistance (MDR): a widespread phenomenon in pharmacological therapies. *Molecules.* 2022;27(3):616.doi: 10.3390/molecules2703616

11. Choi, H., Engene, N., Smith, J. E., Preskitt, L. B., and Gerwick, W. H. (2010). Crossbyanols A–D, toxic brominated polyphenyl ethers from the Hawai'ian bloom forming cyanobacterium Leptolyngbya crossbyana. J. Nat. Prod. 73, 517–522. Doi: 10.1021/np900661g

12. Colson AR, Morton A, Årdal C, Chalkidou K, Davies SC, Garrison LP, Jit M, Laxminarayan R, Megiddo I, Morel C, Nonvignon J. Antimicrobial resistance: is health technology assessment part of the solution or part of the problem? *Value Health.* 2021;24(12):1828–1834. Doi: 10.1016/j.jval.2021.06.002.

13. Davin-Regli A, Bolla JM, James CE, Lavigne JP, Chevalier J, Garnotel E, Molitor A. Membrane permeability and regulation of drug "influx and efflux" in enterobacterial pathogens. *Curr Drug Targets.* 2008;9(9):750–759. Doi: 10.2174/138945008785747824.

14. De Mandal, Surajit (2020). *Recent Advancements in Microbial Diversity || Microbial diversity and functional potential in wetland ecosystems., (), 289–314.* Doi:10.1016/B978-0-12-821265-3.00012-8

15. Denyer, S. P., Hugo, W. B., and Witham, R. F. (2011). The antibacterial action of a series of 4-N-Alkylphenols. J. Pharm. Pharmacol. 32:27P. Doi: 10.1111/j.2042-7158. 1980.tb10830.x

16. Do Amaral, S. C., Santos, A. V., da Cruz Schneider, M. P., da Silva, J. K. R., & Xavier, L. P. (2020, October 16). Determination of Volatile Organic Compounds and Antibacterial Activity of the Amazonian Cyanobacterium Synechococcus sp. Strain GFB01. *Molecules*, *25*(20), 4744. Https://doi.org/10.3390/molecules25204744

17. Doan, N. T., Rickards, R. W., Rothschild, J. M., and Smith, G. D. (2000). Allelopathic actions of the alkaloid 12-epi-Hapalindole E Isonitrile and Calothrixin a from cyanobacteria of the genera Fischerella and Calothrix. J. Appl. Phycol. 12, 409–416. Doi: 10.1023/A:1008170007044

18. Estela Silva-Stenico, Maria, Ramon Kaneno, Fabiana Albani Zambuzi, Marcelo GMV Vaz, Danillo O Alvarenga, and Marli Fatima Fiore. “Natural products from cyanobacteria with antimicrobial and antitumor activity.” Current pharmaceutical biotechnology, 2013;14(9): 820 828.

19. Fay, P. 1983. The blue green (Cyanophyta- Cyanobacteria), (Arnold-Heinemann, London).

20. Gaglio R, Couto N, Marques C, Lopes MD, Moschetti G, Pomba C, Settanni L. Evaluation of antimicrobial resistance and virulence of enterococci from equipment surfaces, raw materials, and traditional cheeses. *Int J Food Microbiol.* 2016;236:107–114. Doi: 10.1016/j.ijfoodmicro.2016.07.020.

21. Gheda Sf, Khalil Ma & Gheida Sf. 2013. In vitro and in vivo preliminary results on Spirulina platensis for treatment of impetigo: Topical cream application. Afri J Biotechnol 12(18): 2498-2509

22. Green, M.R., and Sambrook, J. (2017). Isolation of high -molecular- weight DNA using organic solvents. Cold Spring Harbor Protocols, 2017(4), pdb-prot093450

23. Halder, N. (2015). Phytoconstituents composition and in vitro antibacterial activity of a blue green alga *Anabaena variabilis* Kütz. Ex Born. Et Flah. Tropical Plant Research, 2(3), 288-291.

24. Jayakumar, D. R. (Ed.). (2019, January 1). *Research Trends in Multidisciplinary Research*. Https://doi.org/10.22271/ed.book.431 Journal, 144, 28–39. Https://doi.org/10.1016/j.bej.2019.01.007

25. Knoll, A., (2008). Cyanobacterial and earth history, In: Herrero A, floree, Eds. The cynobacteria molecular biology, UK, Casiter Academic Press pp.1-20.

26. Lade H, Kim JS. Bacterial targets of antibiotics in methicillin-resistant *Staphylococcus aureus*. *Antibiotics.* 2021;10(4):398. Doi: 10.3390/antibiotics10040398.

27. Lambert PA. Bacterial resistance to antibiotics: modified target sites. *Adv Drug Deliv Rev.* 2005;57(10):1471–1485. Doi 10.1016/j.addr.2005.04.003.

28. Levert, A., Alvariño, R., Bornancin, L., Mansour, E. A., Burja, A. M., Genevière, A. M., et al. (2018). Structures and activities of tiahuramides A–C, cyclic depsipeptides from a Tahitian collection of the marine cyanobacterium Lyngbya majuscula. J. Nat. Prod. 81, 1301–1310. Doi: 10.1021/acs.jnatprod.7b00751

29. Ma, L., Zhang, Q., Yang, C., Zhu, Y., Zhang, L., Wang, L., et al. (2020). “1.06-assembly line and post-PKS modifications in the biosynthesis of marine polyketide

natural products," in Comprehensive natural products III. Eds. Liuh.-W. (. B.). And T. P. Begley (Oxford: Elsevier), 139–197.

30. Madigan, T. M., Martinko, J. M., Stahl, D. A. & Clark, D. P. (2012). Brock biology of microorganisms. Thirteen edition, ISBN-13: 978 0321649638.
31. Malathi, T., Ramesh Babu, M., Mounika, T., Snehalatha, D. & Digamber Rao, B. (2014). Screening of cyanobacterial strains for antibacterial activity. Phykos: Journal of the Phycological Society, 44(2),
32. Malkarnekar, G. (2022, August 4). *Nanda lake in Curchorem is Goa's first Ramsar site*. The Times of India. Https://timesofindia.indiatimes.com/city/goa/nanda-lake-in-curchorem-is-states-first-ramsar-site/articleshow/93332412.cms
33. Mo, S., Aleksej, K., Bernard, D. S., Scott, G. F., and Jimmy, O. (2010). Hapalindole Related Alkaloids from the Cultured Cyanobacterium Fischerella Ambigua. Phytochemistry 71, 2116–23. Doi: 10.1016/j.phytochem.2010.09.004
34. NAJDENSKI HM ET AL. 2013. Antibacterial and antifungal activities of selected microalgae and cyanobacteria. Inter J Food Sci Technol 48(7): 1533-1540
35. Najdenski, H. M., Gigova, L. G., Iliev, I. I., Pilarski, P. S., Lukavský, J., Tsvetkova, I. V., Ninova, M. S., & Kussovski, V. K. (2013, March 25). Antibacterial and antifungal activities of selected microalgae and cyanobacteria. *International Journal of Food Science & Technology*, *48*(7), 1533–1540. Https://doi.org/10.1111/ijfs.12122
36. *Nanda Lake | Ramsar Sites Information Service*. (2022). Https://rsis.ramsar.org/ris/2471

37. Noaman, N. H., Fattah, A., Khaleafa, M., & Zaky, S. H. (2004, December). Factors affecting antimicrobial activity of Synechococcus leopoliensis. *Microbiological Research*, *159*(4), 395–402. Https://doi.org/10.1016/j.micres.2004.09.001

38. PANDY VD. 2015. Cyanobacterial natural products as antimicrobial agents. Inter J Curr Microbiol Appl Sci 4(1): 310-317.

39. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnol Adv*. 2019;37(1):177–192. Doi: 10.1016/j.biotechadv.2018.11.013.

40. Parmanik, A., Das, S., Kar, B., Bose, A., Dwivedi, G. R., & Pandey, M. M. (2022). Current Treatment Strategies Against Multidrug-Resistant Bacteria: A Review. *Current Microbiology*, *79*(12). Https://doi.org/10.1007/s00284-022-03061-7

41. Perez, C., Pauli, M. & Bazerque, P. (1990). An antibiotic assay by agar-well diffusion method. Acta Biologiae et Medecine Experimentaalis, 15, 113-115

42. Pratt, R., Daniels, T.C., Eiler, J.B., Gunnison, J.B., Kumler, W.D. et al. (1944). Chlorella is an antibacterial substance from Chlorella. Science, 99, 351–352.

43. Richmond A (1986). Handbook of Microalgal Mass Culture. CRC, Boca Raton, FL, USA., pp. 528.

44. Roger, P.A., Zimmerman, W.J., & Lumpkin T.A., (1993). Microbiological management of wetland rice fields. Fots meeting Jr. (ed), Soil microbial ecology. Application in agricultural & environmental management, M.Deleker New York, 417-455.

45. Safari, M., Ahmady-Asbchin, S., & Zamanifar, P. (2019). In vitro evaluation of antimicrobial activities from aqueous and methanolic extracts of cyanobacteria.

European Journal of Biological Research, 9(3), 184–192. Https://doi.org/10.5281/zenodo.3463632

46. Sethubathi, G. V. B. & Prabu, V. A. (2010). Antibacterial activity of cyanobacterial species from Adirampattinam Coast, Southeast Coast of Palk Bay. Current Research Journal of Biological Sciences, 2(1), 24-26.
47. SHANAB SMM, MOSTAFA SSM, SHALABY EA & MAHMOUD GI. 2012. Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. Asian Pac J Trop Biomed 2(8): 608-615.
48. Shishido T. K., Popin R. V., Jokela J., Wahlsten M., Fiore M. F., Fewer D. P., Sivonen K. Dereplication of natural products with antimicrobial and anticancer activity from Brazilian cyanobacteria. Toxins. 2020; 12(1):12.
49. Sirikul Thummajitsakul. (2012). Antibacterial activity of crude extracts of cyanobacteria Phormidium and Microcoleus species. *African Journal of Microbiology Research*, 6(10). Https://doi.org/10.5897/ajmr12.152
50. Skočibušić, M., Lacić, S., & Rašić, Z. (2019). Evaluation of Antimicrobial Potential of the Marine Cyanobacterium, Rivularia mesenterica. *Journal of Advances in Microbiology*, 1–11. Https://doi.org/10.9734/jamb/2019/v16i430128
51. Srinivas, M., Aruna, M., (2016). Diversity of Blue Green Algae from Paddy Fields. International Journal of Sciences, 5: pp-27-30.
52. Stal, L.J., 1995. Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol. 131, 1–32. Https://doi.org/10.1111/j.1469-8137.1995. Tb03051.
53. Sun, T., Li, X. D., Hong, J., Liu, C., Zhang, X. L., Zheng, J. P., et al. (2019). Inhibitory effect of two traditional Chinese medicine monomers, Berberine and

Matrine, on the quorum sensing system of antimicrobial-resistant Escherichia Coli. Front. Microbiol. 10, 1–11. Doi: 10.3389/fmicb.2019.02584

54. Thajuddin, N. & Subramanian, G. (2005). Cyanobacterial biodiversity and potential applications in biotechnology. Current Science, 89(1), 47-57.
55. Weis, Y. H. & Pang, C. Y. (2010). The role of mitochondria in the human ageing process. Biotechnology International, 17, 8-13.
56. Whitton BA, Potts M., (2000). Introduction to cyanobacteria. In Whitton BA, Potts M (eds), The ecology of cyanobacteria: Their diversity in time and space (pp. 1–11). Dordrecht: Kluwer Academic.
57. Whitton, B. A. (2000). Soils and rice-fields. In B. A., Whitton & M., Potts, (Eds), The Ecology of Cyanobacteria (pp. 233–255) Dordrecht: Kluwer Academic.
58. Whitton, B.A., (1992). Diversity, ecology and taxonomy of the cyanobacteria. In: Mann N.H., Carr N.G. (eds) Photosynthetic prokaryotes, Plenum press, New York, pp. 1-51.
59. Yalcin, D. (2020). Antibacterial activity of cyanobacteria Dolichospermum affine isolated from freshwater. Aquatic Sciences and Engineering, 35(3), 83-88.
60. Yoon, B. K., Jackman, J. A., Valle-González, E. R., and Cho, N.-J. (2018). Antibacterial free fatty acids and Monoglycerides: biological activities, experimental testing, and therapeutic applications. Int. J. Mol. Sci. 19:114. Doi: 10.3390/ ijms19041114
61. Yuzawa, S., and Kuzuyama, T. (2020). “1.05 – Engineering of acyltransferase domains in polyketide synthases” in Comprehensive Natural Products III. Eds. Liu H.-W. B. And T. P. Begley (Oxford: Elsevier), 123–138.

APPENDIX

1. Nutrient agar

Ingredients	**g/L**
Distilled water	1 L
Beef extract	1g
Yeast extract	2g
Peptone	5g
Sodium chloride	5g
Agar	15g

2. MacConkey agar

Ingredients	**g/L**
Distilled water	1 L
Peptone	20g
Lactose	10g
Bile salts	5g
Neutral red	0.07g
Sodium chloride	5g
Agar	15g

3. EMB agar

Ingredients	**g/L**
Distilled water	1 L
Peptone	30g
Lactose	5g
Saccharose (Sucrose)	5g
Dipotassium hydrogen phosphate	2g
Eosin- Y	0.40
Methylene blue	0.065g
Agar	15g

4. SS Agar

Ingredients	**g/L**
Distilled water	1 L
Proteose Peptone	5g
Lactose	10g
Bile salts mixture	8.5g
Sodium citrate	8.5g
Sodium thiosulphate	8.5g
Ferric citrate	1g
Brilliant green	0.00033
Neutral red	0.025
Agar	15g

5. TCBS agar

Ingredients	**g/L**
Distilled water	1 L
Yeast extract	5g
Peptic digest of animal tissue	10g
Sodium citrate	10g
Sodium thiosulphate	10g
Sodium cholate	3g
Oxgall	5g
Sucrose	20g
Sodium chloride	10g
Ferric citrate	1g
Bromothymol blue	0.40
Thymol blue	0.40
Agar	15g

6. MH agar

Ingredients	**g/L**
Distilled water	1 L
Beef, infusion from	300g
Casein acid hydrolysate	17.5g
Starch	1.5g
Agar	17g

7. BG-11 medium

A. Stock 1	Conc. (g/L)
Na_2- EDTA	0.1
Ferric ammonium citrate	0.6
Citric acid. $1H_2O$	0.6
$CaCl_{2.}$ $2H_2O$	3.6
B. Stock 2	
$MgSO_4$. $7H_2O$	7.5
C. Stock 3	
$K_2HPO_{4.}$ $3H_2O$	4.0
D. Stock 5 (microelements)	
H_3BO_3	2.86
$MnCl_{2.}$ $4H_2O$	1.81
$ZnSO_{4.}$ $7H_2O$	0.222
$CuSO_4$. $5H_2O$	0.079
$CoCl_{2.}$ $6H_2O$	0.050
$NaMo_4$. $2H_2O$	0.392

All stock solutions should be filter sterilized and preserved at 4°C.

For basic BG-11 medium Combine the following stock solutions:

Stock solution	Per litre of medium
Stock 1	10ml
Stock 2	10ml
Stock 3	10ml
Stock 5	1.0ml
NA_2CO_3	0.02g
$NaNO_3$	1.5g

Adjust the PH to 7.2. Filter sterilize and store 4°C.

8. 5X TBE buffer

Ingredients	**1L**
MiliQ water	1 L
Tris base	54g
Boric acid	27.5g
0.5M EDTA (pH 8)	20 ml