Antioxidant and Antimicrobial Properties of Cyanobacteria Isolated from Kanjia Lake, Nandankanan.

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Abstract

This study investigates the antioxidant and antimicrobial properties of Planktolyngbya limnetica, a cyanobacterium isolated from Kanjia Lake, Nandankanan. The species exhibited significant antioxidant activity, demonstrated by its ability to scavenge free radicals and inhibit lipid peroxidation. Furthermore, P. limnetica showed potent antimicrobial effects against a range of bacterial and fungal pathogens, suggesting its potential as a natural source for therapeutic agents. These findings highlight the promising applications of Planktolyngbya limnetica in developing sustainable, bioactive compounds for medical and industrial purposes.

Keywords: Cyanobacteria, Antioxidant, Antimicrobial, Species, bioactive

Introduction

Cyanobacteria are oxygen evolving, gram-negative, photoautotrophic bacteria that can be found in virtually all imaginable habitats across the world. They can be found in habitats extending from the extremely cold deserts of the Arctic and Antarctic Zones to the very extreme hot springs, thus representing an interesting form of life in a wide range of aquatic and terrestrial environments [1]. This extraordinary expanse of cyanobacteria is actually a result of an important character that the cyanobacteria can boast of which we refer to as "adaptability." Their amazing success in adapting to such diverse and varying habitats can be attributed to a very long-standing evolutionary history that cyanobacteria enjoy. Some of the modern physiological and anatomical features of cyanobacteria that have helped in supporting their long history on Earth include an extremely high tolerance level of low oxygen and free sulfide, the ability to utilize H2S as a photoreductant in place of H2O, and the strong tolerance capacity for ultraviolet B and C radiations. Thus, these are some of the major adaptive features that have allowed the cyanobacteria to grow, flourish, and dominate in habitats where many other forms of life are absolutely unknown or may struggle to even survive. Interestingly, the origin of cyanobacteria and their basic anatomical features are typical bacterial type but their ecological, biological, and morphological features are quite specific and diverse too. The almost essential possession of some interesting features like the ability of buoyancy, performing oxygenic photosynthesis, and fixation of atmospheric nitrogen make the cyanobacteria more special and capable to adapt to varied conditions in both normal and stressed habitats [2, 3]. These are prokaryotic bacteria which are known to be the prior life form on earth around 3.5 billion years back. Conversion of Earth's anaerobic atmosphere slowly to oxygen-rich atmosphere and the cyanobacterial origin of plastids in plants are the two major evolutionary contributions made by cyanobacteria, thus finally supplying oxygen to the atmosphere through oxygenic photosynthesis for approximately 1.5 billion years. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry [4].

Review of Literature

Cyanobacteria are highly adaptable microorganisms found in various environments, ranging from freshwater to extreme salinity, and can survive under stresses like heat, cold, drought, and UV exposure. They play a vital role in aquatic ecosystems, converting CO2 and H2O into organic compounds via photosynthesis. Cyanobacteria have been used for therapeutic purposes since the 1950s, with recent research focusing on discovering novel bioactive compounds, including antioxidants and antibiotics. These organisms have significant metabolic flexibility, producing secondary metabolites in response to environmental stress. Cyanobacteria are particularly noted for their antioxidant and antimicrobial properties, making them a promising source for new therapeutic agents.

Antimicrobial activity

The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes –including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons; for instance, the antimicrobial activity of supercritical extracts obtained from the microalga Chaetoceros muelleri were related to itslipid composition [5]. However, the antimicrobial activity detected in several

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pressurized extracts from Dunaliellasalina may be explained not only by several fatty acids, but also by such compounds as α- and β-ionone, β-cyclocitral, neophytadiene and phytol. Efforts to identify the compounds directly responsible for those antimicrobial features – e.g. chlorellin, have been on the run, but are stillrelatively incipient owing the some new classes of compoundsfound. Algae are considered as diverse source of secondarymetabolites having a broad spectrum of biological activities such as antimicrobial, antiviral, antifungal, anti-allergic, anticancer, anti-fouling and antioxidant that have been used todevelop the pharmaceutical industries (**Figure 1**).

Antibacterial action

The past decade has witnessed a significant increase in the resistance of pathogenic bacteria to antibacterial agents with direct implications in human morbidity and mortality. Now attention has been paid to a more detailed understanding of the mechanisms underlying antimicrobial resistance – as well as to improved methods to detect resistance, new antimicrobial options for treatment of infections caused by resistant microorganisms, and methods to prevent emergence and spreading of resistance in the first place [6]. Most efforts were devoted to the study of antibiotic resistance in bacteria for several reasons: (i) bacterial infections are responsible for most community-acquired and nosocomial infections; (ii) the large and expanding number of antibacterial classes offers a more diverse range of resistance mechanisms; and (iii) the ability to move bacterial resistance determinants into standard, well-characterized bacterial strains facilitates more detailed studies of the underlying molecular mechanisms [7].

The first antibacterial compound from the microalga Chlorella, identified in 1944, was a mixture of fatty acids known as chlorellin, effective against both Gram-positive and Gram-negative bacteria (Pratt, 1944). Interest in isolating antibacterial compounds from microalgae has surged due to the emergence of multidrug-resistant strains like MRSA, which pose significant challenges in healthcare. This highlights the need for new antibiotics with unique biochemical mechanisms, as traditional antibiotics are often less effective against Gram-negative bacteria due to their complex cell walls [8].

Research has shown that microalgal extracts can exhibit potent antibacterial activity, particularly against Gram-positive bacteria. For instance, the diatom Phaeodactylum tricornutum produces eicosapentaenoic acid, which displays activity against both Gram-positive and Gram-negative bacteria, including MRSA, at micromolar levels. Other compounds, such as hexadecatrienoic acid, also demonstrate efficacy against Gram-positive pathogens like Staphylococcus aureus, with effects noted at low concentrations. Additionally, extracts from Haematococcus pluvialis show antimicrobial properties linked to short-chain fatty acids, targeting both Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus [9, 10].

The precise mechanisms by which these fatty acids exert their antibacterial effects are not fully understood but may involve damaging cell membranes, leading to nutrient leakage and impaired cellular respiration [11]. Some studies suggest that these compounds might affect various cellular targets, possibly inducing peroxidative processes. Research into fatty acids from Scenedesmus costatum has indicated that longer-chain fatty acids can induce bacterial cell lysis.

Notably, microalgal-derived oxylipins, particularly polyunsaturated aldehydes, exhibit significant antibacterial activity. For example, decadienal, derived from arachidonic acid, has shown strong effectiveness against MRSA and other pathogens, with low minimum inhibitory concentration (MIC) values. This compound also inhibits the growth of several marine and human bacteria, emphasizing the potential of microalgae as a source of novel antimicrobial agents. Overall, while research on microalgal antibiotics is still in its early stages, their diverse bioactive compounds hold promise for addressing antibiotic resistance [12] **(Table 1)**.

Reactive oxygen species

The necessity of compounds with antioxidant activity is increasing as it is realized that the formation of Reactive

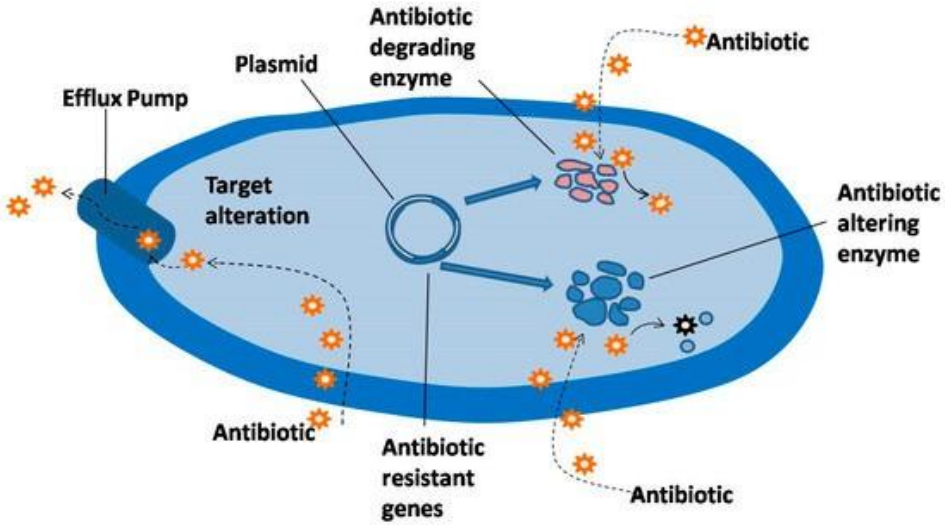

Figure 1. *Various ways of resisting the action of antibiotics.*

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Table 1. *Antibacterial features of selected compounds from microalgae.*

Microalga	Active compond	Target microorganism
Phaeodactylum t Ricornutum	Eicosapentaenoic acid	MRSA, *Listonella anguillarum, Lactococcus garvieae, Vibrio* spp.
Haematococcus Pluvialis	Short-chain fatty acids	*Escherichia coli, Staphylococcus aureus*
Skeletonema costatum	Unsaturated, saturated long chain fatty acids	*Vibrio* spp.
Euglena viridis	Organic extracts	*Pseudomonas, Aeromonas, E. coli Edwardsiella*, *Vibrio* spp.
S. costatum	Extra-metabolites	*Listeria monocytogenes*
Chlamydomonas reinhardtii, Chlorella vulgaris	Methanolic and Hexanolic extracts	*S. aureus*, *Staphylococcus Bacillus subtilis*, *E. coli*, *salmonella typhi*

Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) have been linked in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, immune disfunction, aging, neurodegenerative disorders and certain types of cancer. Oxygen free radicals disintegrate DNA, destroy cell membranes, and create havoc among cell's basic enzymatic metabolic processes. The formation of cancer cell in human body can be directly induced by free radicals. Furthermore, ionizing radiation, which causes free radicals, is well documented as a carcinogen [13] (**Figure 2**).

Antioxidant compounds from Cyanobacteria

Cyanobacteria are rich in reactive antioxidant molecules, including ascorbate and glutathione, as well as secondary metabolites like carotenoids (α- and β-carotene, fucoxanthin, astaxanthin) and mycosporine-like amino acids. They also produce flavonoids with anti-allergic and anti-viral properties, although synthetic antioxidants can have harmful side effects [14, 15]. Recent research has identified various antioxidants from different algal species, such as fucoxanthin in Hijikia fusiformis and phycocyanin in Spirulina platensis, highlighting the diverse potential of these organisms for producing bioactive compounds.

The antioxidant defense mechanism

Antioxidant defense mechanisms are essential for detoxifying Reactive Oxygen Species (ROS) and are organized into three lines of defense. Cells invest significant resources to mitigate the damaging effects of ROS, utilizing vitamins, micronutrients, and various enzymes. The first line of defense consists of primary antioxidants such as Superoxide Dismutase (SOD), catalase, and Glutathione Peroxidase (GPX), which directly neutralize harmful radicals [16].

SOD catalyzes the conversion of superoxide (O_2^-) into less reactive species. Catalase, a tetrameric enzyme found in most cells, breaks down hydrogen peroxide (H_2O_2) into water and oxygen. GPX, which contains selenium, reduces H_2O_2 and lipid hydroperoxides to water using reduced glutathione as a substrate. Glutathione is also recycled in cells via glutathione reductase, which reduces oxidized glutathione using NADPH. Additionally, Glutathione-S-Transferases (GSTs) help detoxify various compounds by conjugating them with reduced glutathione [17].

The second line of defense includes a variety of antioxidants such as Glutathione (GSH), vitamins C and E, uric acid, and flavonoids. GSH, the most abundant non-protein thiol synthesized in the liver, acts as a substrate for GPX and scavenges free radicals. Essential vitamins like β-carotene, vitamin C, and vitamin E cannot be synthesized by most mammals and must be obtained through diet [18].

The third line of defense comprises a complex group of enzymes responsible for repairing damage to DNA, proteins, and lipids caused by oxidative stress. These include lipases, proteases, DNA repair enzymes, and methionine sulfoxide reductases. Detection of oxidative stress often involves measuring compounds like conjugated dienes, hydroperoxides, and malondialdehyde, which result from free radical attacks [19].

Cyanobacteria, like other photosynthetic organisms, are subject to high oxygen concentrations and light, leading to free radical formation. The components of their photosynthetic machinery are particularly vulnerable to photodynamic damage due to the presence of polyunsaturated fatty acids in thylakoid membranes. However, cyanobacteria exhibit effective antioxidant systems that eliminate peroxides and free radicals generated during photosynthesis, enabling essential processes like nitrogen fixation [20].

Phenolic compounds, categorized into ten groups including flavonoids, play a vital role in antioxidant defense. Flavonoids, the most structurally diverse group, encompass over 5,000 known types. The antioxidant activity of these compounds is often linked to their degree of polymerization, with lower polymerization generally leading to higher antioxidant effectiveness (**Figure 3**).

Antioxidant enzymes

The intracellular levels of Reactive Oxygen Species (ROS) are influenced by their production and the antioxidant system's ability to remove them. Key antioxidant enzymes in mammalian cells include Superoxide Dismutase (SOD), catalase, and Glutathione Peroxidase (GPx). SOD converts superoxide radicals into hydrogen peroxide and oxygen, while catalase and peroxidases break down hydrogen peroxide into water. SOD and catalase function without co-factors, whereas

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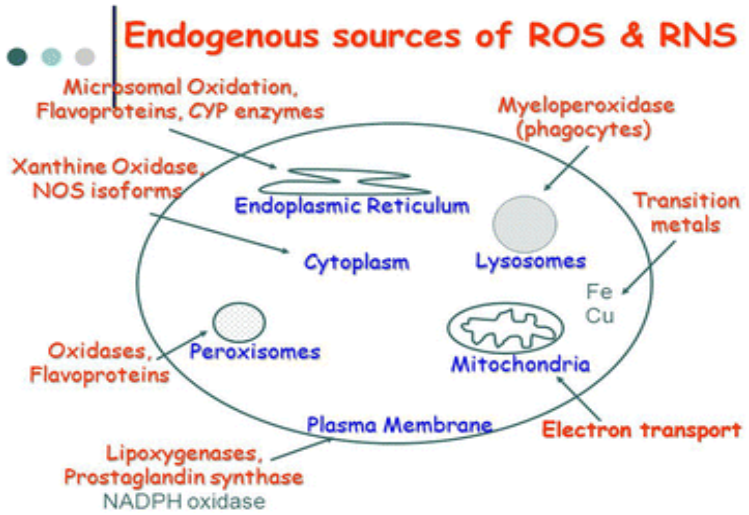

Figure 2: *Endogenous sources of reactive oxygen and reactive nitrogen species (ROS/RNS).*

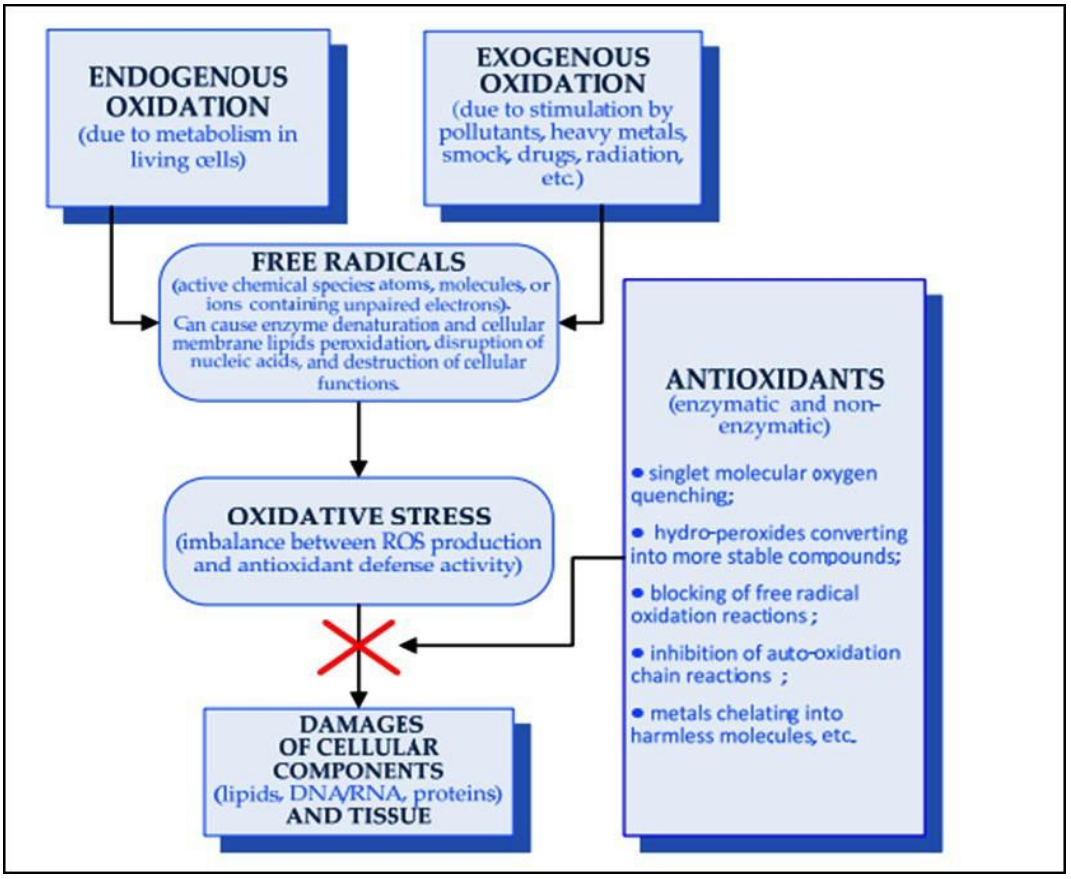

Figure 3: *Defense mechanism against damage by ROS.*

GPx requires several co-factors and has five isoenzymes. These enzymes are compartmentalized in various cell structures, allowing them to effectively reduce oxidative stress in specific cellular regions [21].

Materials and Method

Survey site

Kanjia Lake, located on the northern outskirts of Bhubaneswar, Odisha, is a significant natural lake covering 75 hectares, with a total wetland area of 105 hectares. Part of Nandankanan Zoological Park, it supports recreational boating and boasts rich biodiversity, including 37 bird species, 20 reptiles, 10 amphibians, 46 fish, and various macrophytes. Declared a wetland of national importance by the Ministry of Environment, Forest and Climate Change in 2006, the lake faces threats from quarrying, waste dumping, and unregulated real estate development.

Materials

We use different chemicals from different source. Chemicals used in the study were Nutriant broth, Nutriant agar,

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Tris buffer were obtained from Himedia (India). Sodium phosphate, Sodium carbonate, Sodium potassium tartarate ascorbic acid, gallicacid, tannic acid, butylated hydroxyl toluene, 2,2-Diphenyl-1-Picrylhydrazyl radical (DPPH), Ethylenediaminetetraacetic Acid (EDTA), Dimethyl Sulphoxide(DMSO), ammonium acetate, glacial acetic acid, trichloroacetic acid, sodium dihydrogen phosphate, disodium hydrogen phosphate and ferrozine. All the chemicals were purchased from Merck, SRL India and all solvents used were of analytical grade.

Cleaning of glasswares

Glasswares used for the experiments were first cleaned with liquid detergent/ powder in running tap water and are dried in a hot air oven. The dried glassware after cooling were immersed in chromic acid (potassium dichromate in concentrated sulphuric acid, 1% w/v) overnight followed by repeated washing in running tap water and finally rinsed with double distilled water. Washed glasswares were again dried in a hot air oven.

Sterilization

Sterilization of glasswares and the cultures medium were done by autoclaving for 20 minutes at 15lb pressure, 121 Cin an autoclave.

Culture condition

The cyanobacterial cultures were maintained in a culture room at a temperature 25± 1° C and 3000 lux light intensity with a photoperiod of 16h light and 8h dark with 50-60% relative humidity. Each treatment consisted of 4 replicates and repeated thrice. The liquid cultures in the flask and tubes were hand shaken daily 2-3 times to provide uniform light, aeration and nutrient to the suspension culture and to avoid sticking of cyanobacterial cell to the walls of the glass vessel which may result in uneven growth and subsequent experimental error.

Maintenance of Cultures

The organism used for experimental purpose were grown in liquid medium and were taken in their exponential stage, which is 6–12 days after inoculation. The stock cultures of isolated cyanobacteria were maintained in agar slant containing 1% w/v agar in the BG-11 medium.

Test organism

The test organisms (Planktolyngbya limnetica) were taken from the culture collection unit of microalgae (collected from Nandankanan Kanjia Lake of Odisha) which were maintained in unialgal condition in PG. Department of MITS School of Biotechnology, Bhubaneswar (**Figure 4**).

Media Preparation

Analytical grade chemicals and distilled water was used for the preparation of culture media. The formulation of BG-11 media was used throughout this studyto test the growth and culture pattern of the freshwater cyanobacteria. The composition of the media were given in the **(Table 2)** For antibacterial test nutrient agar medium was prepared by taking Ingredients for Nutrient Agar medium Peptone ($5gL^{-1}$), NaCl ($3gL^{-1}$) & Beef extract ($3gL^{-1}$).

Sub-culture

The cyanobacteria samples were separated into unialgal condition by repeated subculturing in enrichment media in both liquid and agar slants.

Biochemical tests

Growth Measurement

In every 2 days of incubation (0 -14 days) 5ml of cultured algal samples were taken for measurement of growth by spectrophotometer at 760nm.

Pigment Estimation

Chlorophyll

The chlorophyll and total cholorophyll was estimated at 663 nm spectrophotometrically.

Carotenoid

Thecarotenoidwasestimatedat470nm(spectrophotometrically) following the method.

Phycocyanin, Allophycocyanin, Phycoerythrin estimation: Phycobilliproteins were estimated of NFMC, Tiruchirappalli. Known volumes of cyanobacterial suspensions were centrifuged and the pellets were suspended in 3 ml of phosphate buffer. The contents were repeatedly frozen and thawed and

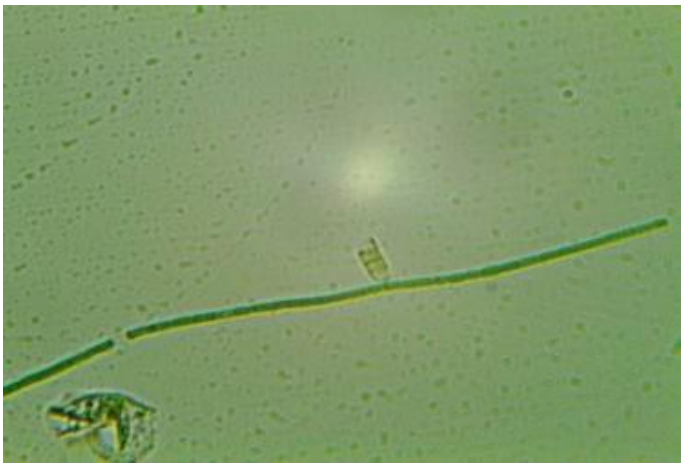

Figure 4: *Microscopic image of Test organism.*

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Table 2. *Pigment estimation.*

Species	No. of Days	Growth	Chl a	Total chl	Carotenoid	Phycobillin		
						PE	PC	APC
Phormidium mole	0 day	0.094	8.092	9.452	0.00026	0.000314	0.0024	0.000114
	2 day	0.241	9.401	10.981	0.00042	0.000406	0.00318	0.000206
	4 day	0.346	16.541	19.321	0.00075	0.000416	0.00322	0.000316
	6 day	0.54	21.42	25.02	0.00134	0.000465	0.00365	0.000403
	8 day	0.556	24.157	28.217	0.00179	0.000513	0.00313	0.000513
	10 day	0.689	38.199	44.619	0.00237	0.000535	0.00435	0.000635
	12 day	0.771	42.245	49.345	0.00416	0.000575	0.00475	0.000805
	14 day	0.966	76.041	88.821	0.0085	0.000738	0.00546	0.000838
Planktolyngbya limnetica	0 day	0.076	6.188	7.228	0.00027	0.000036	0.00067	0.000043
	2 day	0.099	7.497	8.757	0.00069	0.000158	0.00162	0.000137
	4 day	0.318	8.33	9.730	0.00117	0.000184	0.00176	0.000364
	6day	0.352	11.662	13.622	0.00141	0.000241	0.00215	0.000467
	8 day	0.395	12.138	14.178	0.00181	0.000278	0.00248	0.000528
	10day	0.426	19.873	23.213	0.00218	0.000314	0.00289	0.000654
	12 day	0.487	24.514	28.634	0.0031	0.000369	0.0314	0.000649
	14 day	0.69	27.618	32.248	0.00568	0.0000457	0.0405	0.000757

centrifuged in order to facilitate complete extraction. The supernatants were pooled and the absorbance was measured at 565, 615 and 652 nm against phosphate buffer blank. Calculations were done using the following equations,

Phycocyanin (PC) mg/ml= A615 - (0.474 x A 652) /5.34

Allophycocyanon (APC) mg/ml = A652 - (0.208 x A 615) /5.09

Phycoerythrin (PE) mg/ml = A565- 2.41 (PC) - 0.849 (APC) /9.62

Protein estimation

The Protein was estimated at 750 nm spectrophotometrically following the method of Lowry et al(1951) using Bovine Serum Albumin (BSA) as standard protein.

Graded volumes (0.02 - 0.1 mg/ml) of protein solutions were pipette out into a series of test tubes and made upto 1 ml using distilled water. 0.25 ml of the test solution was pipetted out into a series of test tubes and made up to 1 ml using distilled water. 5 ml alkaline copper reagent was pipetted out in to all the tubes. It is mixed well and allowed to stand at room temperature for 10 minutes, 0.5 ml Folin's reagent was added to all the tubes, mixed well and incubated at room temperature for 30minutes. The absorbance was read at 660nm.

Preparation of crude extracts

Fresh algal biomass was harvested through filtration from the late exponential phase to stationary phase culture and shade dried. The dried biomass was blended in to coarse powder. For the active metabolites, 1gm of powdered cyanobacterial biomass was extracted fractionally using methanol, acetone, benzene for 24-48 hours each successively. The crude extracts of known amount were stored for further use. All the extracts were preserved at +40C.

Phytochemical analysis of crude extracts

The qualitative phytochemical screening of cyanobacterial extracts was carried out following standard methods such as: test for Phenols (Ferric chloride test), Tannins (Braymer's test), Flavonoids (Alkaline reagent test), Saponins (Foam test), Terpenoids & Sterols (Salkowki's test), Glycosides (Legal's test) and Alkaloides by Wagner's Test and through Spectrophotometric analysis using a UV-VIS Spectrophotometer (Shimadzu, model UV- 2450) capable of producing monochromatic light in the range between 200- 800 nm for measuring the absorbance.

Chemical composition of extracts

The extracts were prepared in DMSO which is an organosulfur compound with the formula (CH3)2SO. This colourless liquid is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water.

Phytochemical screening

Cyanobacteria extracts were prepared as illustrated above in 50 mL methanol, acetone, and benzene,the extracts were concentrated till reached to about 10 mL. The extracts were analyzed for the presence of alkaloids, glycosides, phenolic compounds, flavonoids, saponins, Tannins, Steroids, Anthrocyanin, Quinones, and Resins.

Phenolic compounds: In extract (1 mL) few drops of Hydrochloric acid were added. Yellowish brown color indicated the presence of Phenolic compounds.

Flavonoid (Ferric chloride test)

Few drops of Ferric chloride were added into the extracts to brown precipitate.

Saponins

Each extract (3 mL) was taken in a tube. The suspension was vigorously shaken. The formation of stable foam was taken as an indication for the presence of saponins.

Alkaloids (Wagner's test)

Few drops of Wagner's reagent were added at the side of the test tube. The formation of reddish-brown precipitate showed the presence of alkaloids.

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Glycoside test

1 mL of concentrated sulphuric acid was added to sample reddish brown colour indicate a positive test for glycosides.

Tannins (Ferric chloride test)

Few drops of ferric chloride solution were added to the test solution. Brackish precipitate showed the presence of Tannins.

Steroids

To 1 ml of test solution, few drops of acetic acid were added and then few drops of conc. sulphuric acid was added to the above mixture. Reddish brown colour showed the presence of steroids.

Anthrocyanin

A small amount of extract was treated with 2 ml of NaOH and observed. Blue green colour was indicate the presence.

Quinones

To 1 ml of the test sample Alcoholic KOH solution was added separately. Colour ranging red to blue showed the presence of quinones.

Resins

To 1 ml of test solution 2-3 ml of copper sulphate solution was added and the contents were mixed for 2 minute and then solution was allowed to separate .Green colour precipitate showed the presence of resin.

Quantitative analysis of phytochemicals present in cyanobacteria

Total Phenolic Content (TPC)

Folin-Ciocalteu method was used to determine the total phenoliccontent in the four cyanobacterial extracts.An amount of 100µl of Folin-Ciocalteu was added toeach extract (500µl) and incubated for 15 minutes at room temperature in the dark. A saturated sodium carbonate (2500µl) was added and incubated for a further 30 minutes at room temperature before reading the absorbance at 760nm using a spectrophotometer(GeneSyn 20, Thermo, USA), and gallic acid was used in the construction of the standard curve. Estimation of the phenolic compounds was carried out in triplicates.The results aremean values and expressed as mg of gallic acid equivalent (GAE)/g of dry extract.

Total Flavonoid Contents (TFC)

The aluminum chloride colorimetric method as described by Chang et al (2002) with some modifications was used for this analysis. Each extract (1 ml) in methanol was separately mixed with 1 ml of methanol, 0.5 ml of 1.2% aluminum chloride, 0.5 ml of 0.12 M potassium acetate and 2.8 ml of distilled water. Mixtures remained at room temperature for 30 min;the absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer(GeneSyn 20, Thermo, USA). Total flavonoid content of the extract was expressed as mil-ligram equivalents to quercetin per gram (mg QE/g) dry weight of the extract (Ordonez etal. 2006)

Statistical Analysis

All analytical determinations and measurements were performed in triplicates. Values of different parameters are expressed as the mean ± standard deviation

Antimicrobial activity

Test organisms

The strains of Escherichia coli (MTCC-443), Staphylococcus aureus (MTCC-96), Vibrio cholera (MTCC-3906), were obtained from IMTECH, Chandigarh and were maintained on suitable agar medium at 4°C until testing.

Characteristics of Test pathogens

Bacillus subtilis (MTCC-441)

Bacillus subtilis is a Gram-positive bacterium, rod-shaped and catalase-positive. It was originally named Vibrio subtilis by Christian Gottfried Ehrenberg and renamed Bacillus subtilis by Ferdinand Cohn in 1872 (subtilis being the Latin for 'fine'). B. subtilis cells are typically rod-shaped, and are about 4-10 micrometers (µm) long and 0.25–1.0 µm in diameter, with a cell volume of about 4.6 fL at stationary phase. As with other members of the genus Bacillus, it can form an endospore, to survive extreme environmental conditions of temperature and desiccation. B. subtilis is a facultative anaerobe and had been considered as an obligate aerobe until 1998. B. subtilis is heavily flagellated, which gives it the ability to move quickly in liquids. B. subtilis has proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of cellular differentiation. In terms of popularity as a laboratory model organism, B. subtilis is often considered as the Gram-positive equivalent of Escherichia coli, an extensively studied Gram-negative bacterium.

Staphylococcus aureus (MTCC-96)

Staphylococcus aureus is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although S. aureus usually acts as a commensal of the human microbiota it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of S. aureus such as methicillin-resistant S. aureus (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for S. aureus has been approved.

Escherichia coli (MTCC-443)

E .coli is a bacterium; the name was given by Bavarine paediatrician, Theoder Escherich, in 19th century. The

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bacterium was first seen in the intestinal flora of infants and he described as a normal inhabitant individual named as Bacterium coli commune. It is an anaerobic microorganism with prototypic communal species and generally causes diseases ranging from diarrhoea to ulcerative colitis. E. coli is the first organism which showed a good studying model for scientific research like gene regulation and played a vital role in molecular biology.

Determination of the Inhibitory Effect of the Algal Extracts

The tests were performed by agar well-diffusion method using Mueller-Hinton agar plates,50 µl of each extract was loaded into the wells. Indicator organisms were spread on Mueller-Hinton agar plates. The solidified media plates were swabbed with 0.1 ml of each test pathogens for which the turbidity the broth was set equivalent to 0.5 McFarland just before swabbing. A well punch machine was used to create wells of 6 mm at equal distances. In these wells, 50 µl of eachextract from each dilution was placed (each plate received 5000-0.05 µg dry weight equivalent). In one well, 50 µl of DMSO served as negative control. Ampicillin was used as a positive control on separate plates for each tested bacteria.The inoculated plates were incubated for 24 h at 37°C for bacteria. After incubation for 24 hours, a clear zone around a disc was evidence of antibacterial activity. Diameter of the zones of inhibition was measured in millimeters.

Assessment of antioxidant activity

DPPH Radical Scavenging Activity

One milliliter of the fraction solutions (100, 200,300,400 and 500 µg/ml in methanol) was added to 1ml of a DPPH solution (0.1mM in methanol). After a 30 min of reaction at room temperature, the absorbance of the solution was measured at 517 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blanksolution (no sample). The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging activity (%) = (A0 – A1) /A0 ×100

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

RESULTS

Pigment Estimation

Two cyanobacterial species were grown in BG 11 media and the rate of growth was measured and their photosynthetic pigments and protein was estimated in two days intervals up to 14 days. Among the two species P. molle exhibited higher growth rate followed by P. limnetica. P.molle showed higher chlorophyll a,and total chlorophyll (76.041, 88.821.)mg/ml content followed by and P. limnetica (27.618, 32.248,) mg/ml, respectively.

Concentration of carotenoid was higher in P.molle (0.0085) mg/ml followed by P. limnetica.(0.00568) lower carotenoid content.

P.molle exhibit higher phycocyanin(0.00546)mg/ml, phycoerythrin(0.000738,)mg/ml, Allophycocyanin(0.000838,) mg/ml content followed by P.limnetica [22] (**Table 2**).

Antibacterial activities of the isolated Species

Antibacterial activities of two species of cyanobacteria tested against bacteria *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacilus subtilis.* In this study benzene extraction yields higher antimicrobial activity than acetone and methanol, whereas in others acetone extraction is better than methanol and benzene. Benzene extracts of *Planktolyngbya limnetica* showed highest zone of inhibition against *E. Coli*(19.8mm) *and Klebsiella pneumoniae* (19.7mm)Similarly the acetone extracts of *Phormodium molle* depicted zone of inhibition 16.4mm and its methanolic extract showed 15.3mm inhibition zone against *Stapylococcus aureus* (**Figure 5-8**). Whereas Benzene extracts of *Planktolyngbya limnetica* exhibited 16 mminhibition zones against *Stapylococcus aureus.* Benzene extract was less effective against test pathogenic bacteria. Thus among the two species benzene extracts of *Planktolyngbya limnetica* showed effective inhibition zone against both Gram- as well as Gram+ pathogens (**Table 3**).

The methanol (M), acetone (A) and benzene (B) extract of selected two freshwater cyanobacteria [(a) P. molle (b) P.limnetica] tested against four bacterial pathogens shown below.

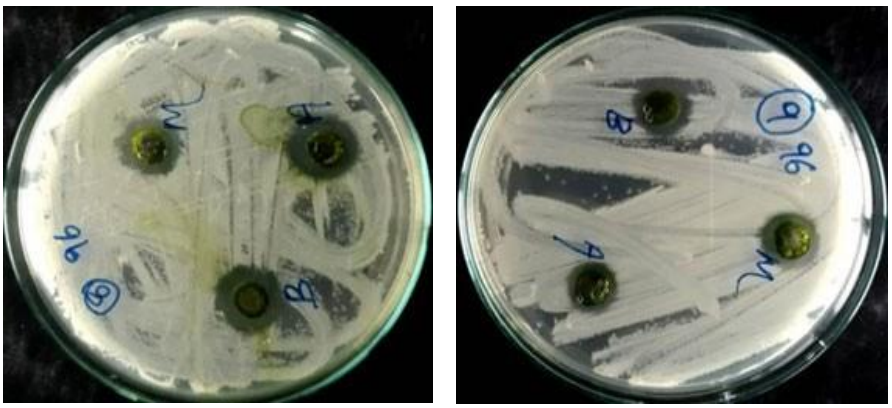

Figure 5: *Antibacterial activity against S. aureus.*

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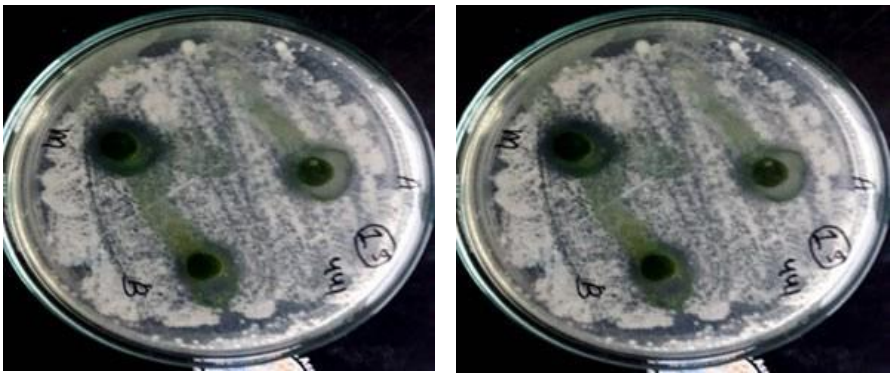

Figure 6: *Antibacterial activity against B. Subtilis.*

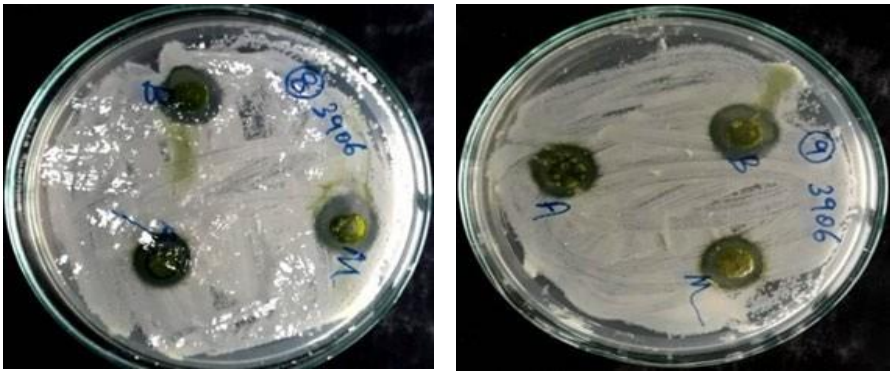

Figure 7: *Antibacterial activity against Klebsiella pneumonia.*

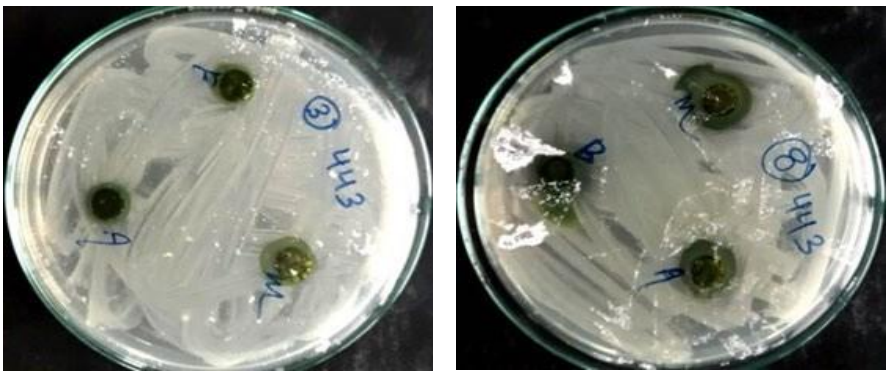

Figure 8: *Antibacterial activity against E. coli.*

Table 3: *Antimicrobial activity of cyanobacterial species.*

Cyanobacterial species	Solvents	Gram +ve		Gram –ve	
		Staphylococcus aureus	***Bacillus subtilis***	***Klebsiella pneumonia***e	***Eschercichia Coli***
Antibiotics (Control)		28.5	28.25	29.83	25.5
Phormidium molle	**methanol**	15.3	14	12	14
	acetone	16.4	11	9	11
	benzene	14	13.5	9	11
Planktolyngbya limnetica	**methanol**	14.8	13	14.8	12.3
	acetone	11	13	12.9	13.1
	benzene	16	14.3	19.7	19.8

Discussion

The antioxidant activities of crude cyanobacterial extracts were assessed using three complementary biochemical methods to address the limitations of single tests in measuring the complex in-vivo interactions of antioxidants. The DPPH assay, known for its simplicity, speed, and reliability, effectively evaluated the total antioxidant capacity of the extracts, which rely on electron or hydrogen donation. Among the species tested, Phormidium molle exhibited the highest antioxidant activity, followed by Planktolyngbya limnetica, whose activity was comparable to the commercial antioxidant BHT.

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In antimicrobial testing, methanol and benzene extracts of cyanobacteria showed the strongest activity across both species. This antimicrobial potential may not only serve as a defense mechanism for the cyanobacteria but also represent a valuable source of bioactive compounds for pharmaceutical applications. Screening of cyanobacteria has identified several promising antimicrobial compounds. In this study, the benzene extract of Planktolyngbya limnetica was the most effective for extracting antimicrobial agents, while acetone proved most effective for Phormidium molle. No significant differences in antimicrobial activity were found between acetone and methanol extracts. These variations could be due to differences in secondary metabolite production, extraction methods, and assay protocols. Overall, cyanobacteria from Nandankanan Kanjia Lake, Odisha, India, are promising sources of natural antioxidants and antibiotics, warranting further research to identify their bioactive compounds.

Conclusion

This study investigated the antioxidant, phytochemical, and antimicrobial properties of cyanobacteria from Nandankanan Kanjia Lake, Odisha, India. Freshwater organisms, known for their bioactive compounds, were examined for their potential to yield new molecules. Results showed varying antioxidant activity in the cyanobacterial extracts, with Phormidium molle demonstrating the highest activity, followed by Planktolyngbya limnetica, whose activity was comparable to commercial antioxidants. The correlation between high radical-scavenging activity and elevated phenolic content highlights the role of phenolic compounds in antioxidant mechanisms. Both P. limnetica and P. molle exhibited strong antimicrobial activity against Staphylococcus aureus and E. coli, respectively, suggesting that these cyanobacteria are valuable sources of bioactive compounds.

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