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Insight into the Potential Cyanobacterial Metabolites and their Screening Strategies

S. Pooja and Niveshika

Department of Botany, St. Joseph's College (Autonomous), Bengaluru-27, India.

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Cyanobacteria are the first oxygenic photosynthesis performing prokaryotes. They are considered to have fast growth, amenability to genetic modifications towards photo-autotrophy. Have the ability to grow under heterotrophic conditions with minimum available sunlight to obtain energy by utilizing organic carbon as its substrate. Cyanobacteria are diversely spread in marine, freshwater, terrestrial habitats which differ from each other concerning their structural and functional metabolism. It produces bioactive compounds which are toxic to animals as well as humans which are produced in freshwater habitats whereas marine species of cyanobacteria produce secondary metabolites which are involved in the production of new drugs and also show potential in various fields such as Biotechnological applications, pharmacology, agriculture sustainability, and environmental remediation. Cyanobacteria also produce non-toxic compounds which help in protecting plants by producing phytohormones, siderophores, and UV protective or absorbing compounds. Marine cyanobacterial bioactive compounds are involved in several bioactivities such as antiviral, antialgal, antiprotozoal, antifungal activities, etc. Freshwater species are involved in forming harmful cyanobacterial blooms which are highly toxic to animals as well as humans (Ex: cyanotoxins, hepatotoxins, etc). Different strategies are used to detect the cyanobacterial compounds under in-vivo and in-vitro cultures. To analyze the quality and safety of water, screening methods are necessary to detect possible toxic compounds present in the environmental habitats. Screening methods include microscopy assay, physiological methods, chemical methods, biochemical-based methods, and molecular-based methods. All these methods of screening help in characterizing, identifying the cyanobacterial toxins and also have a few limitations in their reliability, sensitivity, and limit in the detection of the compounds.

Keywords: Biotechnological application; Cyanobacteria; Secondary metabolites; Screening strategies.

Cyanobacteria is a gram-negative photoautotrophic bacterium that is said to be the first prokaryote to get involved in oxygenic photosynthesis[1,2]. They are considered an ancient group of photosynthetic prokaryotes[3]. Also, their ability towards photo-autotrophy, fast growth, and amenability to genetic modifications are quite immense[4–8]. Cyanobacteria are the primitive prokaryotes to perform the oxygenic photosynthesis using both the photosystems P1 and P2 (P1= P700 nm and P2= P680 nm) to produce energy, water, carbon dioxide, inorganic compounds for their growth. They also have the capability of growing under heterotrophic conditions with minimum sunlight to obtain energy by utilizing organic carbon as a substrate[9–12]. The cyanobacterial

*Corresponding author E-mail: niveshika87@gmail.com

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classification was proposed in the year 1985 and divided into 4 orders namely: Chroococcales, Nostocales, Oscillatoriales, Stigonematales, and their phyla also identified such as Chroococcales, Gloeobacterales, Pleurocapsales[7]. Later it was classified into 5 orders. They are Chroococcales, Pleurocapsales, Nostocales, Oscillatoriales, Stigonematales[13] as shown in (Table 1). The relative quantity of biomolecules was separated from the filamentous form of cyanobacteria: Nostocales order (Genera includes *Lyngbya, Oscillatoria, and Symphloca*[5].

Cyanobacteria are populated diversely in marine, freshwater habitats, or terrestrial environments in terms of morphology (morphologically, cyanobacteria can be unicellular or filamentous with having spherical, rod, spiral shapes and they often grow in large colonies Ex: *Oscillatoria, Nostoc, Spirulina*[7]), physiology and metabolism and it can also survive in extreme conditions such as hot spring water, geotherms as seen in *Synechococcus* species, high temperature and so on[13,14].

Cyanobacteria produce harmful blooms under eutrophic conditions due to the presence of high levels of phosphorus and nitrogen[13,15]. Cyanotoxins are harmful to animals as well as humans and are produced in freshwater habitats whereas the marine habitat species of cyanobacteria produce bioactive compounds which are involved actively in the production of new drugs such as antiviral, antibiotic, anticancer, etc.[3,16]. Screening of un-purified extracts has been proven to be an active method in identifying organisms that produce potentially useful compounds[17].

Secondary Metabolites from Cyanobacteria

Over some time, the cyanobacteria have evolved to produce various secondary metabolites to get through the changes in the surrounding environment such as high temperature, high pH, high dissolved phosphorus, and nitrogen[18]. Secondary metabolites are often called Natural products with low molecular weight organic molecules that have multiple biological activities[19]. Cyanobacteria are known to produce around 1100 unique secondary metabolites[20]. Among these, almost 300 nitrogen-containing secondary metabolites have been reported from the marine prokaryotic cyanobacteria[5]. Exceptionally, marine cyanobacteria have a very rich origin of bioactive compounds compared to freshwater and terrestrial cyanobacteria due to the varied bioproducts obtained from these marine species. These secondary metabolites show potential in various fields such as biotechnological applications, pharmacology, agriculture sustainability, and environmental remediation. The cyanobacterial secondary metabolites play an important function in the improvement of drugs mainly in the field of cancer and infection[21]. Improved knowledge and understanding of cyanobacterial metabolites and their utilization in drug development has raised a new perspective of utilizing cyanobacteria in the field of medicine[9,22]. Cyanobacteria are found to be involved in activities like an antibiotic, antiviral, anticancer, cytotoxicity, anti-inflammatory, enzyme inhibitor, free radical scavenger[2,23]. Figure 1 shows different cyanobacterial metabolites.

Secondary metabolites are the essential origin of new composition which leads to medicating in many crucial sickness areas[22]. When cyanobacterial species grow rapidly to form harmful blooms, they can produce large amounts of unique and bioactive secondary metabolites[24]. Secondary metabolites from cyanobacteria perform numerous roles such as defensive mechanisms against other organisms including antibiotics, fungicides, etc., as a metal transporting agent, as facilitators of symbiosis, as photo-protectants, as antioxidants, as allelochemicals in signaling[14,25].

Toxic Secondary Metabolites from Cyanobacteria

Cyanobacterial toxins are well studied and vastly associated with the health effects on the human and healthy environment. The first Scientific literature on the toxicity of secondary metabolites in cyanobacteria was reported by *George Francis* due to increased deaths of livestock in South Australia after drinking water which contained high consumption of cyanobacteria in lake Alexandra[26]. In Cyanobacteria, secondary metabolites are divided into toxic metabolites and non-toxic secondary metabolites. Here, non-toxic cyanobacterial compounds usually help in plant growth, cell division, and the release of nutrients. Toxic secondary metabolites are based on which organ they affect (animals and humans). Unicellular freshwater species such as *Microcystis aeruginosa* have a very high source of peptide metabolites and have adverse effects on human

health[27]. Toxic metabolites show a bad impact on both animals and humans.Some lakes were infected by cyanobacterial blooms causing diseases by releasing their toxic substances that lead to different sorts of problems to the cattle, fishes, humans, etc[24]. Bloom forming species of cyanobacteria are known to produce the secondary metabolites which belong to different classes, among them are the neurotoxins and hepatotoxins[27]. The majority of the toxic secondary metabolites (Ex: cyanotoxins) were produced from the freshwater habitats of cyanobacteria such as harmful blooms which lead the humans (to fall sick) and the aquatic organisms (lethal deaths)[28]. The production of the potent neurotoxins (nervous system) and hepatotoxins (liver cells) show potentially dangerous consequences [29]. Benthic forms of cyanobacteria form clusters or mats as a part of the biofilms which helps in photo-autotrophy[30]. There are a few toxic secondary metabolites from cyanobacteria: Cyanotoxins such as hepatotoxins, neurotoxins, dermatotoxins, etc[18].

Cyanotoxins

Cyanotoxins are natural toxins produced from freshwater cyanobacteria due to a decrease in nutrient sources[31]. Cyanotoxins weigh around 299Da produced by cyanobacterial species *viz. Aphanizomenon isatschenkoi*, *Anabaena circinalis*, *Cylindrospermopsin raciborskii, Planktothrix* species[29]. This acts as a channel blocker as it blocks the VGSC in neurons[32] in the environment, there is a threat to animal and human health[33]. Cyanotoxins are harmful to higher animals, humans (rarely)[34]. Cyanotoxins are rarely ingested by humans in high amounts but if a person is exposed for a long period it might lead to chronic effects which promote the development of further carcinogenic problems[35]. Compared to other cyanotoxins, hepatotoxins are naturally faced by humans and animals in bloom formation of cyanobacteria[29]. Research on their toxicological and ecotoxicological properties revealed that there are possible ways to employ naturally toxic bioactive metabolites called Allelochemical drugs[37]. Allelopathy involves using the bioactive metabolites by one species to slow down the growth of sympatric species which shows potential to compete with the resource such as algaecides, herbicides, and insecticides[26]. The cyanotoxins are natural algaecides that slow down the growth rate in other cyanobacteria[18]. Cyanotoxins are divided into alkaloid neurotoxins and cyclic-peptide hepatotoxins. Deaths from these toxins are most likely due to intrahepatic hemorrhagic and hypovolemic shock[36]. Hepatotoxins and neurotoxins are intracellular cyanobacterial metabolites. They are produced by only particular strains of the cyanobacterial species[38].

Hepatotoxins

The most common cyanotoxins in freshwater are hepatotoxins produced by a few cyanobacterial species belonging to *Microcystis, Anabaena, Nostoc, Planktothrix, Nodularia*, etc[39]. The variation in the structure of hepatotoxin has been found in the filamentous brackish water cyanobacterial species such as Nodularia spumigena[36]. Sometimes, the temperature influences the growth and production of the hepatotoxin whereas warmer temperature may directly or indirectly promote the growth and levels of the cellular toxicity in hepatotoxins[40]. Hepatotoxins are taken up by hepatocytes triggering illness. Hepatotoxins are responsible for the poisoning of wildlife, humans worldwide[29]. Most commonly the cyanobacteria encounter hepato-toxicosis involving the hepatotoxins[41]. The release of the hepatotoxin from the cyanobacterial species also has an allelopathic effect on other phytoplanktons. Hepatotoxins are highly stable under sunlight and resistant to UV radiations [42]. Hepatotoxins affect massive hemorrhages and disruption in the liver as well as kidneys and exposure to this toxin occurs mostly through the diet or by direct contact with the contaminated water. It is known to have high resistance to digestion in the gastrointestinal tracts of eukaryotes because the presence of peptide bonds linked to D-amino acids are not sustainable to normal enzymes. Thereby it shows a high risk to consumers of animal products that have been contaminated [43]. Hepatotoxin is comprised of microcystin (cyclic peptide) produced by *Microcystis aeruginosa*, and also by some other genera such as *Oscillatoria* sp, *Anabaena* sp, *Nostoc* sp, etc. and Nodularin (pentapeptide) by *Nodularia spumigena* and Cylindrospermopsin produced by *Cylindrospermopsis raciborskii* [41]. Both the microcystin and nodularin have a significant impact on water quality[44] also have

similar toxicity mechanisms but the morbidity of cylindrospermopsin may rely on metabolic modification in liver[29].

Microcystin (MC)

Microcystin is a cyclic heptapeptide and highly hepatotoxic non-ribosomal peptide produced by a few cyanobacterial species[37]. Their growth in freshwater leads to animal deaths and most of these microcystins are found growing in brackish water or freshwater habitat[20]. The biosynthesis of MCs is increased under high light and red-light conditions[37]. Hepatotoxic blooms are seen all over the world and microcystins are found common in these blooms[36]. Hepatotoxic microcystins are produced by bloom-forming species through a complex of non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS)[17]. These have been studied most commonly and found to slow down the growth of other photoautotrophs[40]. Microcystin-LR is the virulent member of MC variants and it has the potential to treat cancer due to cytotoxicity[45]. Microcystin-LR weighs around 994 Da and its structure is Cyclo (-D-Ala-L-Leu-D-MeAsp-L-Arg-Adda-D-Glu-Mdha) here, Adda is 3-amino-9-methoxy-2,6,8, trimethyl-10-phenyl deca-4,6-dienoic acid; D-MeAsp is D-erythro-Beta-iso aspartic acid; Mdha is N-Methyl-dehydroalanine 22[,36,46]. Uptake of MC-LR into the liver can be suppressed by bile salts competitively such as taurocholate or by a competitive substance named antamanide, sulfo-bromo-phthalein, rifampicin, etc.[37] Microcystin inhibits the subclasses of threonine or serine amino acids, phosphatases type PP (1 and 2A) with a broad range of protein substrates (targeted)[2]. Primary targets for MCs are eukaryotic protein phosphatase[47].

Nodularin (NOD)

Nodularia produces the nodularin by cyanobacterial species *Nodularia spumigena*, this genus is well known due to its toxicity nature which is specific to form the cyanobacterial blooms (highly toxicant)[49]. Nodularin weighs around 824 Da and is termed as NODLN[36]. It is a diazotrophic filamentous cyanobacteria[50]. Nodularin surroundings extend from slight brackish water, saline-alkaline inland lakes, and few are found rarely in the freshwater habitats and soil[51]. Nodularin is closely related to microcystin[19]. Nodularin is synthesized by a multi-enzyme compound containing NRPS and PKS as domains[50] Nodularin shares a highly similar biosynthetic pathway of Microcystin[15]. Toxicological characteristics of nodularin are to inhibit the threonine or serine protein phosphatases such as PP1 and PP2A[37]. Exposure to nodularin causes oxidative stress in wildlife such as mussels, fishes, marine fucus vesiculosus[52].

Cylindrospermopsin (CYN)

Cylindrospermopsin is an alkaloid hepatotoxin isolated from freshwater cyanobacterial species named *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum* and its natural analogues are cytotoxins which provokes hepatotoxicity in humans[53]. Cylindrospermopsin weighs around 415.43Da[54]. The precursor of CYN is formed by the unique activity of L-arginine-glycine amidinotransferase Cry A[15]. CYN is classified as hepatotoxin because of its nature to damage the hepatocyte and liver such as Lipidosis, hemorrhage, necrosis. CYN is also produced by benthic forms of cyanobacterial species (*Lyngbya wollei*)[30]. CYN is the most frequently studied hepatotoxin after Microcystin so far and their distribution is increasing day by day, they are known to be an effective cytotoxin that affects different organs and their functions in animals, plants causing fatal deaths in cattle and outbreaks in human health situations by causing the illness[49]. In vitro, their decomposed products are toxic to humans and their presence of harmful characters in CYN is huge and primarily it includes the cytotoxicity[55]. It inhibits the glutathione, protein synthesis, and cytochrome P450, also characterized by a tricyclic carbon skeleton attached to the hydroxymethyl uracil, a sulfate group, Guanidine[15,54]. There are 3 different variants of CYN that have been identified such as 7-deoxy cylindrospermopsin, Cylindrospermopsin, 7-cylindrospermopsin based on the presence of substituents on 7th carbon[53]. Mode of action and uptake mechanism is known in Cylindrospermopsin[15]. In rodents, it has affected liver, kidney, heart damage including their hepatocellular vacuolation, cytotoxicity, etc.[49]. The main harmful effect after exposure to CYN is the inhibition of protein synthesis due to reaction with eukaryotic translation protein synthesis[55]. Their biological activity involves the interruption

of various metabolic pathways and also causes oxidative stress by increasing the concentration of free oxygen radicals[54].

Neurotoxins

Neurotoxins are alkaloids[43]. Cyanotoxins affect the nervous system either in the central or peripheral region due to chemical and biological agents known as neurotoxins. The chemical structure of neurotoxins is closely related to CYN[56]. Many of the neurotoxic substances from marine cyanobacteria are known to target the voltage-gated Sodium channels (VGSC)[15]. Neurotoxins of the cyanobacteria are composed of 2 groups with two low molecular weight alkaloids such as anatoxins (anatoxin-a, homoanatoxin-a, anatoxin-a(s)) and saxitoxins. Neurotoxins destroy the natural growth of the neural impulses to muscles causing paralysis and respiratory failure leading to animal death[29]. The mode of action is well defined based on their targeting organs[56]. Marine cyanobacteria are considered as a communicator of potent neurotoxins which acts as either activator or blocker[34]. The new classes of cyanobacterial metabolites are defined by their exceptional integration of NRPS & PKS-subunits with long unusual 15-carbon linear polyketide chain, tri-heterocyclic ring consisting of 2 á-methyl thiazolines and a thiazole[33]. The PKS extends the residue of isoleucine with an uncertain number of extra amino acids to form the cyclic structure. Ingestion of neurotoxins causes death in animals due to respiratory fatigue and failure[53]. Symptoms of this toxin cause staggering, muscle fasciculation, gasping, convulsions, and opisthotonos seen in birds. Anatoxin-a and homoanatoxin-a are closely connected in terms of their structure with cholinergic nicotinic agonists which adhere to the neuronal nicotinic acetylcholine receptors[38]. Anatoxin-a (commonly called as very fast death factor-VFDF)[57] is isolated from the freshwater cyanobacteria and it is secondary Bi-cyclic amine with 2-acetyl-9-azabicylo (4,2,1) non-2-ene with a molecular weight of about 165 Da. The production of neurotoxic compound anatoxin-a has been determined from Oscillatoria or Planktothrix occasionally and homoanatoxin-a is produced from stratifying and structurally similar cyanobacteria species called *Tychonema bourelly*[34]. These two toxins are potent neurotoxins produced by freshwater cyanobacterial species of different genera such as *Anabaena, Aphanizomenon, Oscillatoria, Phormidium, Cylindrospermum*[36,58].

Neurotoxins such as anatoxin-a alter the neuromuscular transmission[18,58]. Anatoxin-a is isolated from cyanobacterial species such as *Anabaena flos-aquae, Aphanizomenon flos-aquae, Planktothrix* which acts as receptor binder binding irreversibly to acetylcholine [28]. Anatoxin-a is considered to be the potent drug of postsynaptic cholinergic nicotinic leading to depolarizing neuromuscular blockade. And it provokes the activity of acetylcholine which causes paralysis and muscle cramping[29].

Anatoxin-a(s) is a phosphate ester with cyclic N-hydroxy-guanine and their mechanism is to synthesize the organophosphate insecticides such as parathion, malathion, etc. Anatoxin-a (s) is isolated from single cyanobacterial species *Anabaena* such as *A. flos aquae, A. lemmermanni*. Anatoxin-a(s) weighs about 252Da[56]. It inhibits the acetylcholinesterase and interferes in muscle contraction[38]. This toxin causes muscle fatigue and failure[29]. This is a neurotoxic alkaloid that is capable of inhibiting the acetylcholine ester [58].

Saxitoxin is also known as paralytic shellfish toxins (PST) which causes the poisoning in shellfish by paralyzing it; these are closely related to alkaloids in terms of morphology [53]. PST is fast-acting neurotoxins that slow down the rate of nerve conduction by blocking the sodium (Na) channels without affecting the potassium (K) concentration. It is a neurotoxic guanidinium derivative with two functional groups of amines [28].

Dermatoxins

Cyanotoxins causing skin irritation are known as dermatoxins [18]. There are two types of toxins: lyngbyatoxin and aplysiatoxin [46]. These two toxins are produced from the *Lyngbya majuscula* [26]. Lyngbyatoxins and aplysiatoxin possess a polyketide backbone containing amino acid constituents[16]. Lyngbyatoxin and debromoaplysiatoxin are extremely inflammatory and they have structurally different secondary metabolites [37]. There is a special activator called protein kinase C (PKC)[15]. Lyngbyatoxin is known to cause swimmer's itch. When a person is exposed to this toxin, Lyngbya majuscula produces a few toxins which result in a dermatitis-like condition in swimmers causing inflammation, blisters, and tumors [17]. These compounds also act as potent

tumor promoters[15]. *Lyngbya majuscula* results in contact dermatitis and is found toxic in fishes and grazer species [26].

Endotoxins

Endotoxins are also known as Irritant toxins or Lipopolysaccharides (LPS). LPS is well known for its effect on the alimentary tract of bacteria such as *E. coli, Salmonella* species, *Vibrio cholera*, *Pseudomonas aeruginosa*. Lipopolysaccharides are isolated from the genera such as *Microcystis, Anabaena, Spirulina, Oscillatoria*. Liposaccharides contain lipid-A, polysaccharide (core region), and outer polysaccharide chain[37]. Lipopolysaccharide is an obligate region of the outer layer of cells in gram-negative bacteria. It differs from enteric bacteria by lacking phosphates and having a high mixture of unsaturated long fatty acid chains and hydroxy fatty acids. In mammals it is best-known to cause fever, it is involved in septic shock syndrome (SSS) where this is aggravated, toxicant, and induced in the liver and kidney [17]. Table 2 showed the toxic secondary metabolites and their causes.

Non-toxic Secondary Metabolites From Cyanobacteria

Few cyanobacterial species also produce non-toxic metabolites which help in different ways in protecting them such as phytohormones, siderophores, and UV protective/absorbing compounds[18].

Phytohormones

Phytohormones help the plant in terms of their growth, cell division, cell differentiation, and nutrient release. They are produced by *Acutodesmus dimorphus, Synechococcus* sp, *Phormidium corium* etc[18]. Phytohormones such as auxins, indole acetic acid, cytokinin, gibberellins, play significant roles in the germination of the shoot and root lengths [59]. Phytohormones regulate different developmental stages and responses of plants under certain environmental conditions[60]. The release and production of phytohormones by cyanobacterial species have very important ecological consequences [57]. The advantage of this is, it has a broad spectrum of activity which is needed for the development of normal plants in both *in-vivo* and *in-vitro* cultures[13]. The production of phytohormones such as auxins, cytokinin, improves the condition of a plant and is active in physiological responses such as wounding, herbivores' pathogen attacks in salinity, and drought stress. Nostocales of cyanobacteria play a vital role in the paddy fields because of their ability to fix the atmospheric nitrogen and phytohormones supplied for crop growth[61].

Siderophores

Siderophores are known as sideramines and are formed by the synthesis of iron chelators[62]. Siderophores are nothing but to obtain iron in limited conditions. Many bacteria secrete soluble amounts of iron chelators which compete with the environmental iron molecules [40]. Siderophores help in the uptake, soluble, transportation of iron and these are complex molecules that solubilize the ferric ions at the range of pH 7.4 [25]. They have an extremely high affinity towards iron molecules. Iron transport factors are on the boundary line of primary, secondary metabolite compounds because it is required for growth and stimulating the growth under Fe-deficient conditions. These are found in soil that is produced microbially and enterobacterial antibiotics are isolated from human fecal extracts. Micro-organisms show both reduced and advanced affinity towards ferric ions in solubilizing and transporting. Fluorescent siderophores can induce disease suppression because *Pseudomonas produces*[63]. Negative mutants of siderophores either promote plant growth under certain conditions or fail to protect against the disease[25]. It is also involved in antibiotic activity due to the ability to take up Fe-siderophores complexes such as nocardamine and deferri-triacetyl fusigen[63].

UV-Protective Compounds

For energy production, the cyanobacteria depend on the light, and at a comparable instance, they are exposed to harmful UV radiation[15]. Micro-organisms show different ways in overcoming this damage with UV light, UV avoidance, UV absorption/sunscreen compounds, synthesis of radiation-absorbing pigments, DNA repairing process[15,64]. Cyanobacteria produce two sunscreen compounds when they are exposed to harmful UV radiations, they are; scytonemin, mycosporine-like Amino acids (MAAs) [15]. With the help of these two compounds, the cyanobacteria can overcome the harmful UV radiations toxicity and are exploited in cosmetic industries[18], which was found to defend the skin from UV damage and helped to balance the antioxidant defense system.

Sun-screening compounds such as scytonemin and MAAs facilitated cyanobacteria to grow in extreme conditions on bare rocks and in the open ocean [13].

Mycosporine like Amino Acids-(MAAs)

MAAs are water-soluble, colorless compounds with cyclohexane chromophores connected with nitrogen substituents of the amino acids [3]. MAAs are isolated from the cyanobacterial genera such as *Anabaena, Nostoc, Lyngbya, Synechococcus, Synechocystis*[18]. The basic function of this compound is to assist the cells from alteration by absorbing the UV radiations, it consumes the energy without reactive oxygen species (ROS) scavenging, also protecting the skin from UV damage, defense against oxidative thermal stress [3]. Mycosporine-like amino acids are synthesized purely when they are exposed to UV-B[19]. Cyanobacteria can match the UV protection by exploding the manufacture of MAAs when they are unprotected from harmful UV radiations[64]. MAAs are involved in antioxidant activity and also absorb light in UV-A and UV-B ranges with a maximum absorbance of 310-360nm[13,64]. They are present primarily in the cytosol of cells and found on outer cell membranes such as in the *Nostoc* community and facilitate the photoprotective nature with the ability to spread energy without producing ROS. Four enzymes actively participate in the synthesis

Table 1. Cyanobacteria are classified in 5 orders

Sl. No	Orders	Species	Habitat	Form
1	Chroococcales	*Microcystis* sp	Freshwater	Unicellular
		Synechococcus sp	Marine water	
		Synechocystis sp	Freshwater	
2	Pleurocapsales	*Hyella caespitosa*	Marine water	Unicellular
3	Nostocales	*Anabaena* sp	Freshwater	Filamentous
		Nostoc sp	Terrestrial	
4	Oscillatoriales	*Oscillatoria* sp	Freshwater	Filamentous
		Lyngbya majuscula	Tropical marine water	
5	Stigonematales	*Fischerella muscicola*	Freshwater	Filamentous

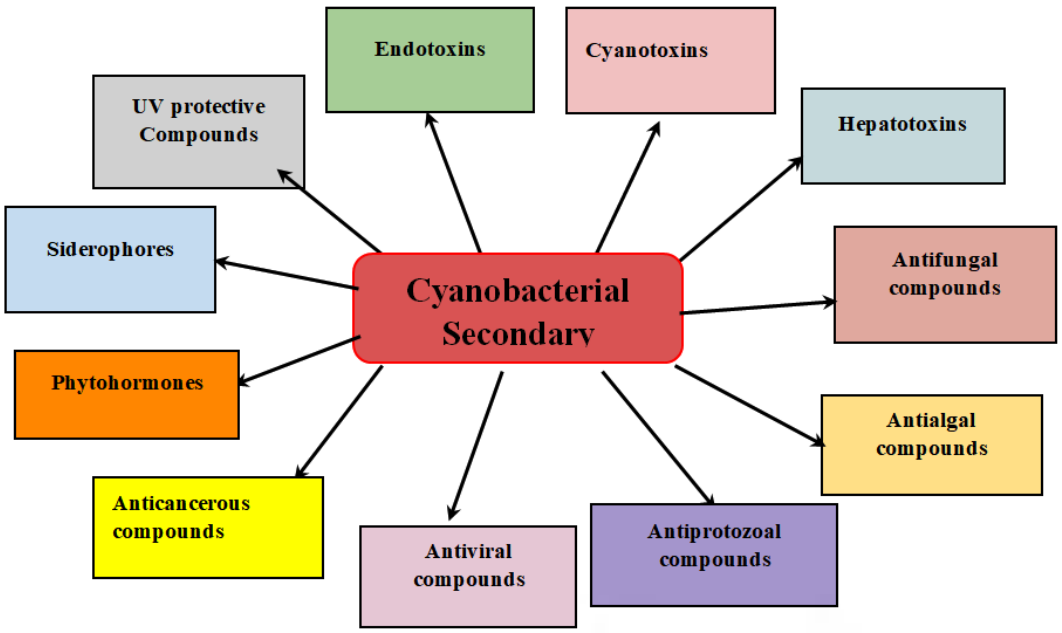

Fig. 1. Diagrammatic representation of secondary metabolites reported from Cyanobacteria

of MAAs-shinorine in *Anabaena variabilis* ATCC29413, dehydroquinase synthase homologue DHQS, O-methyl-transferase O-MT, ATP grasp ligase, an NRPS-like enzyme whereas ATP grasp ligase and NRPS- like enzymes are closely related to amino acid attachment to cyclohexanone region helps in the formation of imine[19].

Scytonemin

Scytonemin shows similar features as MAAs and they can block the UV radiations up to 90% [18]. Scytonemin is isolated from *Lyngbya* sp, *Anabaena* sp,[18]. It is present in the sheaths of a few cyanobacteria which live in extreme conditions such as on bare rocks and in open oceans [64]. Scytonemin (544Da) is a dimeric indole phenolic alkaloid, lipid-soluble, the yellow-brown pigment that acts as a passive sunscreen compound in the protection of cyanobacteria against UV light in marine and freshwater environmental conditions [19,64]. The maximum absorbance is in the 380 nm range. By random genetic activations, the gene cluster is liable for producing the scytonemin from *Nostoc punctiforme* [65]. It is restricted only to cyanobacteria and biosynthesized in response to

Table 2. Toxic secondary metabolites and their causes

S. no	Toxic Metabolite		Weight	Causes	References
1	Cyanotoxins		-	Harmful to higher animals including humans (rarely). There are possible ways to develop bioactive metabolites called allelochemical drugs. Also involved in the formation of toxic cyanobacterial blooms.	18,29,34,37
A	Hepatotoxins	Microcystin (MC-LR)	994Da	Humans may get exposed to MC-LR through their diet which results in liver damage.	45
		Nodularin (NOD)	824Da	It causes oxidative stress in mussels, fishes, marine fucus vesiculosus.	52
		Cylindrospermopsin (CYN)	415.43Da	Causes fatal deaths in cattle and outbreaks in human health by causing illness.	49
B	Neurotoxins	Anatoxin-a	165Da	Potent agonist of postsynaptic cholinergic nicotinic leading to depolarizing neuromuscular blockade.	29
		Homoanatoxin-a	-	Cholinergic nicotinic agonist binds to neuronal nicotinic acetylcholine receptors and functions same as Anatoxin-a.	57
		Anatoxin-a(s)	252Da	Synthesizes organophosphate insecticides such as parathion and malathion.	38
		Saxitoxin	299Da	It causes poisoning in shellfish by paralyzing them.	53
C	Dermatotoxins		-	It causes skin irritation. commonly known as Swimmer's itch.	17
D	Endotoxins		-	It causes fever in humans, animals and is concerned with septic shock syndrome (SSS).	18,26

UV-A radiations [19]. The biosynthesis pathway starts with tryptophan and tyrosine[15]. After scytonemin is synthesized, it is eliminated from the outer protective covering of cyanobacteria. Factors to regulate these compounds include UV radiations, Salinity, oxidative stress, nutrient deficiency[64]. It is chiefly engaged in photoprotection by UV radiations and also has anti-inflammatory activity with no cytotoxicity against non-dividing cells. Scytonemin inhibits polo-like kinase 1 (PLK1) which is present in phosphorylation of activation of proteins such as cdc25C and it allows to repress cell division. So, it is considered a promising compound used in pharmaceutical fields such as Anti-cancer drugs, Sunscreen agents, anti-inflammatory drugs etc[3].

Bioactivities of Cyanobacteria

The secondary metabolites of marine cyanobacteria show a wide range of bioactivities which is related to natural environments such as anticancer, anti-infective, anti-inflammatory, antifungal, antialgal, antibacterial, antimicrobial, etc where their functions are not well known but show different roles in pharmaceutical/medical areas such as treating the diseases and biological disorders. Bioactive compounds of marine cyanobacteria have high potentiality in pharma industries.

Anticancer Activity

There is a crucial need to develop brand-new anticancer drugs because tumour cells are processing resistivity against accessible drugs such as Taxanes which is leading to failure in the aiding of cancer treatment (chemotherapeutic). Niveshika et al. (2017) reported a novel cyancompound 9-Ethyliminomethyl-12-(morpholin-4-ylmethoxy)-5,8,13,16-tetraaza -hexacene-2,3-dicarboxylic acid (EMTAHDCA) from freshwater cyanobacteria *Nostoc* sp. MGL001 has anticancer properties. It was found that cyanocompound EMTAHDCA induced significant cytotoxic response against Dalton's lymphoma ascites (DLA) cells with an inhibitory concentration (IC50) value of 372.4 ng/mL in a dose and time dependent manner after 24 h of incubation[66]. Several marine cyanobacterial compounds interact with certain significant molecular targets which are involved in anticancer activity resulting in controlling the death of tumor cells, this targets to start the compounds to slow down the proliferation of cancer cells by inducing apoptotic cell death[67]. Marine cyanobacteria from the unexplored environments, there is the source of compounds that leads to the discovery of new drugs [67]. There are about 62 different isolates from different biomes that tested for anticancer activity and all these isolates belong to 23 genera amongst five orders of cyanobacteria which was identified in Brazil[2]. More than half of marine cyanobacterial species are potentially utilized for the natural process of secondary metabolite compounds which are impressive in damaging cancer cells by causing apoptosis [5]. Several compounds have developed

Table 3. Non-Toxic Metabolites and their causes

S. no	Non-Toxic Metabolite		Causes	References
1	Phytohormones		It helps in terms of growth, cell division, cell differentiation, nutrient release.	60,61
2	Siderophores		It induces the disease suppression and negative mutants of siderophores and is involved either in promoting growth factors or failing to protect against disease.	25
3	UV protective Compounds	Mycosporine like Amino acids (MAA)	Protects the cells from alteration by riveting UV radiation to consume the energy without reactive oxygen species (ROS) scavenging, protects skin from UV damage, defense against oxidative thermal stress.	3,64
		Scytonemin	It blocks UV radiation up to 90% and biosynthesis in response to UV-A radiation.	18,19

as templates for development of new Anticancer drugs[67]. These drugs play an important role in treating many deadly and biological disorders[19]. such as Curacin A and Dolastatin 10 have gone through all pre-clinical and objective trials as potency anticancer drugs.

Curacin A is produced by *Lyngbya majuscula* and shows the high potentiality of anticancer activity through the suppression of tubulin polymerization[57]. It is a unique thiazole-containing compound and found to have high potentiality against breast cancer[22].

Dolastatin 10 is a bioactive compound that is isolated from Symploca species. It is a pentapeptide compound with 4 (unique) amino acids; dolavaline, dola isoleucine, dolaphenine. This bioactive compound of anticancer activity acts as a potent antiproliferative agent[22]. An analog of dolastatin 10 is soblidotin TZT-1027, it is different from dolastatin in absence of thiazoline ring from the dolaphenine residue[5]. It is biosynthesized by NRPS-PKS enzymes to disrupt the microtubule formation[57]. Cryptophycins are one of the most potent anticancer agents which were produced by marine cyanobacterial species named Nostoc sp GSV224[8]. Cryptophycins were first identified for their antifungal activity against cryptococci. Later, Moore and his co-workers isolated the same

Table 4. Cyanobacterial bioactivities and their causes

S. no	Cyanobacterial Bioactivity	Causes	References
1	Anticancer activity	Anticancer activity results in controlling the death of tumor cells by inducing apoptotic cell death. Ex: Dolastatin 10, Cryptophycin F, Apratoxin A shows anticancer activity.	5
2	Antibacterial activity	Antibacterial compounds are effective towards Gram-positive and Gram-negative bacteria and the discovery of new drugs has become essential to control the bacterial infection during its resistance. Ex: Hapalindole, Noscomine shows antibacterial activity.	68,70
3	Antiviral activity	Several viral diseases are spreading dramatically which shows impacts on human health and society. Ex: Cyanovirin-N, Microvirin, Scytovirin shows antiviral activity.	75,76
4	Antifungal activity	Pathogenic fungi are developing resistance to numerous antifungal drugs. But few bioactive compounds have shown antifungal activity such as Hassallidin A, B & D, Hectochlorin, Calophycin, etc.	70,81
5	Antialgal activity	Antialgal compounds possess the growth inhibitory activity of algae by targeting their photosynthesis, respiration, enzyme activity, oxidative stress induction. Ex: Cyanobacterin, Nostocyclamide, etc shows antialgal activity.	87,88
6	Anti-inflammatory activity	Bioactive compounds have great involvement to produce effective drugs due to their competency with significant biological activity. Ex: C-phycocyanin shows anti-inflammatory activity by inhibiting arachidonic acid metabolism.	91
7	Anti-protozoal activity	Drug discovery against anti-protozoal diseases is very slow because bioactive compounds are developing resistance towards specific drugs. But few compounds such as Heirridin B, Carmabin A, venturamide A & B show anti-protozoal activity.	68,113,114

compound from the Nostoc sp and explained their toxicity against tumor cell lines[68]. This compound found activities against both drug-sensitive and drug-resistant tumor cell lines[5,58]. Cryptophycins show 1000 times more effective anticancer activity other than any bioactive compounds[18,23]

Apratoxin A is an effective cytotoxic compound isolated from Lyngbya majuscula species in the marine habitat of cyanobacteria. It is considered a cytotoxic compound due to its action mechanism in weakening the fibroblast growth factor signaling pathway[5]. Apratoxin A is best-known for causing G1 phase cell cycle arrest resulting in apoptosis[22]. Many anticancer drugs act as apoptotic modulators to destroy the outcast cells. These apoptotic cells develop complex structural alterations which allow the identification of tumor cells. During the early stages, cells become smaller in size, having dense cytoplasm with thin organelles in it[66]. Cell extracts from *Cyanobium* sp; CENA154, *Nostoc* spp; CENA64 & CENA 69, *Oxynema* sp; CENA135 have anticancer properties against murine colon cancer line CT-26 and lung cancer 3LL found in Brazilian cyanobacterial species[2].

Antibacterial Activity

The secondary metabolites from marine cyanobacteria represent a large source of Bioactive

Table 5. Screening methods used to detect the presence of cyanobacterial metabolites

Sl. No	Screening Methods		Uses	References
1	Microscopy assay		This method is used to monitor the cyanobacterial community present in water bodies. Two different microscopes are used as epifluorescence and Inverted light microscopy	120
2	Physicochemical methods		In this method, cyanobacterial water bodies are used to detect the growth conditions including weather, nutrient availability, and presence or absence of photopigments.	125
3	Molecular based methods	PCR	Used to detect cyanobacterial toxins in marine forms and mainly achieved by amplifying the 16SrRNA gene used for identification of prokaryotes.	133
		qPCR	It is a quantitative method compared to the PCR method and helps in detecting and quantifying cyanobacterial specific genes 16SrRNA.	138
		Microarray	It is utilized to form screening of gene reflection on a genomic scale using a microarray chip (provides quantitative information on the amount of nucleic acid present in an environmental sample).	144
4	Biochemical based methods		Used to detect biochemical properties of cyanobacterial toxins. ELISA, ligand binding assay, enzyme inhibition assay, and colorimetric assay are used as biochemical methods.	41,143
5	Chemical methods		Used to detect cyanobacterial toxins in water by liquid separation methods such as HPLC, NMR, Mass spectrometric methods (LC-MS/MS, MALDI-TOF, LC-TOF). Among these NMR is considered to be less efficient for quantitative analysis.	151,161

compounds and these are known for their therapeutic effects. Certain marine cyanobacterial compounds are recognized as major producers possessing antibacterial activity. An increasing rise in bacterial strains against antibiotics is encountered in past years. So, to solve this issue there is a huge demand for antibacterial compounds in pharmaceutical fields[68]. Specifically, the secondary metabolites from marine cyanobacteria have developed a rich source of new therapeutics[3]. Antibacterial compounds are most effective towards gram-positive and gram-negative bacteria and the discovery of new drugs has become very essential to control bacterial infection during antibacterial resistance. Major antibacterial compounds of cyanobacteria belong to the orders Nostocales and Oscillatoriales [69].

Hapalindole is an indole alkaloid isolated from *Fischerella* sp. It has shown antibacterial activity against gram-positive, gram-negative bacteria such as E. coli ATCC25992 and *Staphylococcus aureus* ATCC25923. It is considered as sodium channel modulators[1]. All the antibacterial alkaloids are indole-containing compounds[1]. The 12-epi-hapalindole E isonitrile compound's mode of action is to hinder the RNA polymerase of bacteria[70].

Noscomine is a di-terpenoid isolated from the *Nostoc commune* EAWAG 122b and this compound showed antibacterial activity against *Bacillus cereus, Staphylococcus epidermidis* and *E.coli*. Carbamide cyclophanes is a paracyclophane that is detached from the *Nostoc* sp CAVN 10 shows reasonable antibacterial activity against *Staphylococcus* resulting in minimum inhibitory concentration (MIC) value of nano molar limit because of its methicillin-resistance [68]. Of late, the alkyne containing polyketides-anaephenes were found to be active against *Staphylococcus aureus*. In peptides, the N-methylation of phenylamine is considered as a crucial factor against antibacterial activity. Niveshika et al (2016) reported novel cyanocompound 9-Ethyliminomethyl-12-(morpholin-4-ylmethoxy)-5,8,13,16-tetraaza -hexacene-2,3-dicarboxylic acid (EMTAHDCA) isolated from *Nostoc* sp. MGL001 has antibacterial properties[71]. The cyanocompound exhibited growth inhibiting effects against the gram negative bacterial strains and produced a maximum zone of inhibition at 150 µg/mL concentration[71]. Even insects have resistance against polluted bacteria by producing antibacterial proteins such as cecropins, attacins, defensins, lysozyme, diptericin, sacrotoxis which causes lysis or bacteriostatic, sometimes by attacking the parasite[25].

Antiviral Activity

Increasing viral impedance and their actions on antiviral drugs have transformed into a major issue in the medical area. Several viral diseases are spreading dramatically which are showing impacts on human health and society. Few deadly viruses are namely severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), ebola, swine flu, and now coronavirus disease in 2019 (COVID 19) [72].

Lectins are antiviral proteins that were isolated from cyanobacteria. These are monomer proteins with low molecular weight resulting in the repressive specificity for glycoproteins[73]. There are three members of lectins that are isolated from cyanobacteria. They are Cyanovirin-N (CV-N), Microvirin (MV-N), Scytovirin (SVN) [74–76]. Cyanovirin-N is 101 amino acids long isolated from Nostoc ellipsosporum. CV-N is potent against HIV 1 and 2, in nano-concentrations, Simian immune-deficiency virus (SIV), and other lentiviruses [22]. Also involved in inhibition of herpes virus, measles virus in in-vitro culture[75]. Also recovered to stamp down the division in Hepatitis C virus, Influenza virus[77]. Microvirin (MV-N) weighs around 14.3 kDa. MV-N is isolated from cyanobacterial Microcystis aeruginosa PCC7806 and shares about 33% of their individuality with effective Anti-Human Immune-Deficiency Virus (HIV)[78]. Their mode of mechanism helps in the inhibition of virus-cell interaction [76]. Scytovirin (SCV) [68] shows antiviral activity by intrusive nature with various tracks in the viral fusion activity. SCV is a 95% amino acid with five disulfide bonds isolated from the binary compound infusion of *Scytonema* varium[22]. SCV binds to the covering of glycoprotein HIV (such as gp120, gp160, and gp41) and sets off the low-level nanomolar concentrations[68]. Calcium spirulan 74kDa is isolated from *Arthrospira plantesis* which shows the activity against viruses admit HIV-1, Herpes simplex virus type 1 (HSV-1), Human Cytomegalovirus (HCMV), Influenza virus[18]. The structure of Calcium spirulan consists of sulfated polysaccharides[79]. Sulfoglycolipids such as sulfolipids have antiviral activity and it

is isolated from cyanobacterial genera *Lyngbya, Phormidium, Scytonema*. This shows the inhibition of HIV 2 DNA polymerization function, reverse transcriptase of HIV1. The presence of fatty acid chains of sulfo glycolipids is mandatory for activity.

Antifungal Activity

Pathogenic fungi are developing resistance to numerous antifungal drugs[80]. So, there is an urgent need to find out new antifungal compounds which show good resistant [70]. Cyano-peptides such as Hassallidin A & B, D, hectochlorin, lyngbyabellin A & B, majusculamide C, laxaphycins, scytophycin, calophycin, etc are identified as antifungal compounds. In cyanobacteria, the majority of antifungal compounds are produced from orders such as Nostocales, Oscillatoriales, Stigonematales[81]. Hassallidin A & B are cyanobacterial glycosylate lipopeptides are isolated from Hassillia sp. It shows antifungal activity against *Candida* species with 4.8 ìg/mL as MIC value[67] whereas Hassallidin D is stranded from Anabaena species shows antifungal activity against *Candida albicans, Candida krusei* with the MIC value of d"2.8 ¼g/mL[82]. The cyclic tri decapeptides and tolybyssidins A & B are two antifungal compounds that show antifungal activity against the yeast Candida albicans and are isolated from collective refined cyanobacterium *Tolypothrix byssoidea* EAWAG 195[83]. The Laxaphycins are well-known antifungal compounds isolated from two cyanobacterial species namely *Anabaena laxa* [84] and *Lyngbya majuscula*[17]. Hectochlorin is isolated from *Lyngbya majuscula* with unique characters called 2 DHIV units and the gem-di-chloro-group exhibits potent antifungal activity against *Candida albicans* and anti-proliferative activity for actin stabilization. Hectochlorine structurally resembles Lyngbyabellins A & B[57]. Hectochlorin A is the acyl CoA synthetase homologue that activates free hexanoic acid and provides hectochlorin synthesis[15]. Lunatoic acid is an antifungal agent isolated from *Cohliobocus lunatus* that helps in inducing chlamydospore formation [25]. Cryptophycin is first reported as an antifungal compound but later Moore and his co-workers found the same compound from the *Nostoc* strain exhibiting anticancer activity against tumor cell lines. So, they named this compound anticancer activity[58].

Antialgal Activity

Antialgal compounds result in damaging the cellular morphology, SOD (Superoxide dismutase) activity, reduce chlorophyll-content, it also induces ROS etc[85]. Antialgal activity is highly restricted to certain cyanobacterial genera such as *Fischerella, Nostoc, Anabaena, Calothrix, Scytonema* containing nitrogen-fixing property with heterocystous filamentous cyanobacteria[86]. Antialgal compounds possess growth inhibitory activity of algae by targeting their photosynthesis, respiration, enzyme activity, oxidative stress induction[87]. Fischerella A is an antialgal compound isolated from *Fischerella muscicola* which has unique enediyne; two heterocyclic moieties show the antialgal activities along with antifungal activity[4]. Cyanobacterin is isolated from *Scytonema hofmannii*. It is identified and characterized as chlorinated ã-lactone89. Cyanobacterin is a cyanobacterial toxin that is considered an allelopathic compound that exhibits growth inhibition in cyanobacteria, eukaryotic algae, in various higher plants [26]. Cyanobacterin precisely inhibits photosystem II[89]. Cyanobacterin compounds LU-1 and LU-2 are involved in the inhibition of electron transport in photosystem 2 and they don't show similarity in terms of morphology. Cyanobacterin LU-1 is isolated from *Nostoc linckia* and it is found to inhibit cyanobacteria, algae except in non-photosynthetic microbes. Whereas LU-2 inhibits only the cyanobacteria[26]. The 2'-deoxyadenosine is a bioactive compound isolated from *Streptomyces jiujiangensis* JXJ 0074T. This bioactive compound damages the vegetative cells by crumpling, collapsing, expanding, perforating, breaking the filamentous cyanobacteria[85]. Nostocyclamide is a cyclic peptide that appears as an uncoupler of electron transport in photosynthesis and exhibits the inhibition of cyanobacteria. It is considered an allelopathic compound[26].

Anti-inflammatory Activity

Secondary metabolites from the marine cyanobacterial species show great participation in producing effective drugs due to their unique structure frameworks and competency with significant biological activity such as anti-inflammatory activity [23]. Chemically assorted compounds were found to induce the anti-inflammatory activity[66].

The C-phycocyanin is a water-soluble pigment isolated from the marine cyanobacterial species *Spirulina platensis* and shows hepatoprotective effect due to inhibition of cytochrome P450 by mediated reactions involved in reactive metabolites formation, ability to act as radical scavenging, or sometimes both91. The C-phycocyanin exhibits anti-inflammatory activity due to its noesis to suppress the arachidonic acid metabolism and scavenging free oxygen radicals. This compound shows anti-inflammatory activity along with antioxidant activity[91]. The spirulina exhibits anti-arthritic effects due to the antioxidant and anti-inflammatory activities of its component phycocyanin[92]. Different species of marine cyanobacterial genera *Lyngbya* are known to produce secondary metabolites[93]. Microcolin A & B is a bioactive compound isolated from the blue-green algae Lyngbya [94]. Their mode of mechanism is unidentified but both microcolin A and B are structurally distinguishable in terms of immuno-suppressive drugs. Microcolin A is a lipo-peptide present commonly in the benthic form of cyanobacteria and it can reduce the survival and inhibit the settlement of larvae Porites astreoides by unsettling the natural biomes[95]. Malyngamide is a lipopeptide isolated from *Lyngbya* sp[93]. The majority of Malyngamide S, X are isolated from *Bursatella leachii*[96], Malyngamide O, P is isolated from cyanobacterial species Stylocheilus *longicauda*[97]. These compounds show a broad spectrum of bioactivities such as anti-inflammatory, cytotoxicity, antimicrobial etc[98]. In murine RAW 246.7, the macrophage cell line is treated with LPS and exhibits anti-inflammatory activity by inhibiting induced nitric oxide production100. When the production of nerve growth factor (NGF) and the organic process factors are increased at the central nervous system (CNS), it causes in suppressing the inflammation by shifting immunity response to the anti-inflammation and restrictive mode in the specific brain environment[100]. This production in brain cells is induced by pro-inflammatory and anti-inflammatory cytokines like Interleukin-1 (IL-1), (IL-4), (IL-5), tumor necrosis factors (TNF-á), transforming growth factor-beta (TGF-â), interferon beta (IFN-â) by NFkâ signaling [101,102].

Antiprotozoal Activity

Several natural products have been identified in cyanobacteria against the anti-protozoal activity such as malarial parasite plasmodium, protozoan parasites such as Trypanosoma also called sleeping sickness or Chagas disease, and Leishmania (leishmaniasis)[103]. Many compounds are active in this activity by also showing cytotoxicity by limiting their drug usage. Medicine discovery against anti-protozoal diseases is very dragging because these bioactive compounds from cyanobacterial species are developing resistance towards the specific drugs[68]. There is imperative demand for more efficient drugs to be discovered because there are no new medicines that are available for tropical disease treatment [104]. The antiprotozoal compounds are lipophilic phenolic ambigols which is isolated from Fischerella ambigua, heirridin B is isolated from *Phormidium ectocarpi* and *Cyanobium* sp[105,106], dolophoenanthridine is an alkaloid calothrixin [107]isolated from marine cyanobacteria Calothrix[107], the carmabin A, dragomabin, dragonamide A is linear lipopeptides[108], the lagunamide A-C is a cyclic depsipeptide and Malyngolide dime is a cyclopeptide is isolated from *Lyngbya majuscula* [109,110], Venturamide A & B is a cyclic peptide isolated from *Oscillatoria* sp[111], gallinamide A is a linear peptide and this is isolated from *Schihzothrix* [112,113]. All these compounds have antiprotozoal activities which are further used in pharmaceuticals to discover new drugs. Table 3 showed cyanobacterial bioactivities and causes.

Screening Strategies of Bioactive Compounds from Cyanobacteria

Traditionally, the new drug disclosure is a wet laboratory process that is completely experimental and this process helps to identify the main compound in bioactivity. The new drug discovery and its remedial solutions is an extended process, very costly[114]. The extracts and refined compounds are time-tested against specific drug targets. Pharmacologists attempt to speed up this screening process by developing new strategies under in-vivo and in-vitro cultures. Viewing of cyanobacteria for pharmacologically bioactive compounds has received considerable attention during past decades[115]. The screening of isolated compounds from different natural sources is a common way to find out new biologically active compounds. To analyze the quality and safety of water the screening methods are necessary to

detect possible toxins present in the environment samples[116]. Enormous drawing of potency drugs with their targets are being commonly known through a variety of broad speed with the new application including DNA Sequencing, micro-array or 2D gel electrophoresis, mass spectroscopy assays and so on[117]. Clustscan, NRPS-PKS, NP-searcher are genome screening programs that are used to predict the locations of gene clusters and their constitution of putative products [118]. Different screening methods help to identify or detect the presence of toxins in cyanobacteria, namely microscopy assay, physicochemical methods, molecular-based methods, biochemical-based methods, and chemical methods. Every method has particular boundaries in terms of sensibility, reliableness, and limit of sense. All these methods in screening help to characterize, identify cyanobacterial toxins.

Microscopy Assay

This technique is employed for monitoring the cyanobacterial community present in the water bodies; it can be either freshwater or marine water habitat. Microscopy assay is done by cell counting which helps to monitor the cyanobacterial blooms. There are two microscopes used in this analysis as epifluorescence and inverted light microscopy[119]. In epifluorescence microscopy, the cells are counted in autotrophs by using a 515-560 nm absorption range whereas in heterotrophs the cells are counted by using a blue light source at 420 to 490nm[119,120]. The inverted light microscopy is used to examine the phytoplanktonic communities in freshwater and marine water environments[121]. With the help of these two microscopies, the abundance of specific cell colonies in cells/ ML helps to determine the target organisms. Due to dense colonies presence may give false cell estimations and non-homogenous cell distribution. For example, the aggregation of some marine cyanobacterial colonies such as Microcystis doesn't allow its individuality at a particular level based on its geomorphology[122,123]. Flow cytometry is used to detect phytoplankton because of its higher sensitivity than any other microscopic approach[124]. The main disadvantage of this microscopy technique is that there is no possible way to differentiate between toxic and non-toxic cyanobacteria.

Physicochemical Methods

In this method of screening, the cyanobacterial water bodies are used to detect the growth conditions including weather, nutrient availability, presence or absence of photopigments[125]. Estimation of the presence of nitrogen and phosphorus in water, temperature, and light intensity may favor the cyanobacterial bloom formation in water bodies. Change in weather mainly impacts the bloom growth promotion by modifying seasonal variations in warming of air and water which adds nutrients resulting in increased bloom formation. Cyanobacteria contain the accessory pigments such as Chlorophyll a, phycocyanin, and phycoerythrin[126]. Chlorophyll-a is present in various organisms, so it is considered a good indicator of total autotrophic phytoplankton[127]. Phycocyanin and Phycoerythrin are cyanobacterial specific pigments. The phycocyanin is the accessory pigment present in freshwater cyanobacteria[128,129] and phycoerythrin is the accessory pigment dominating in marine habitat[131]. These pigments help to estimate the total biomass of cyanobacteria. The spectrophotometric method is a fast and straightforward pigment quantification based on the relative abundance intensity at a specific wavelength[131].

Molecular-based Methods

The specificity, reliability, and speed of this molecular-based method can overcome the disadvantage of Microscopy assay by detecting and quantifying the cyanobacterial toxin encoding genes. Due to its high sensitivity, it enables the early warning of cyanobacterial toxin in water bodies long before the cyanobacterial bloom formation[132]. So, this method is considered a more efficient monitoring method. It includes polymerase chain reaction (PCR), Real-time quantitative polymerase chain reaction (qPCR), and microarray.

The sequence and diversity of NRPS-PKS cluster genes in-vivo acculturation in cyanobacteria demonstrates the degeneration PCR screening, cloning, sequencing of fragments constitutes as performing to determine potency gene targets in diversified cyanobacterial lineages[62]. The structure of cyanobacterial metabolites is characterized by using isotopically labeled precursors giving the biogenesis and a specific gene template and it is known by using degenerate PCR amplification. Gerwick and co-workers identified the biosynthetic gene clusters for Apratoxin-a; they used a single cell as PCR to template the whole genome of the

producer. Then the genome was sequenced and the cluster was known using various screening[47]. PCR technique is used to detect the cyanobacterial toxins in marine forms where the host of the primers have developed for aiming the MC gene cluster and also the toxicologic assessment of micro-organisms including the cyanotoxins, nodularin, neurotoxins[133]. This technique is mainly achieved by amplifying the 16SrRNA gene used for the identification of prokaryotes. This formulation has been utilized to identify different genes associated with CYN manufacture and MC producing from MC strains[134,135]. The multiple PCR is used to target the 16SrRNA to detect the toxic contamination of MC in regular dietary supplements[136].

The qPCR is a quantitative method compared to PCR in terms of sensitivity with the detection limit of *Microcystis* spp and *Cylindrospermopsin raciborskii*[137,138] and helps in detecting and quantifying the cyanobacterial specific genes 16SrRNA such as *Microcystis* specific rRNA genes[139] There are two different methods used in qPCR technique such as SYBR green and TaqMan[63]. SYBR green consists of a fluorescent molecule that can intercalate double-stranded DNA. There is a disadvantage of unspecific binding which might occur during the amplification[63]. The TaqMan method is considered as a short sequence added to the sample. In this method, the amplification is highly specific[140].

Microarray is a technique that is utilized to make a screening of gene expression on the genomic scale. Using a microarray chip, it analyses efficiently and quickly by providing quantifiable information on the amount of nucleic acid in the environmental sample[141]. This technique performs gene expression analysis by targeting the RNA retro transcribed into complementary DNA (cDNA) to identify genetic variation such as Single nucleotides polymorphism (SNP) and its mutation. DNA chip method is formulated to discover the microcystin (MCs) gene (mcy) and nodularin (NOD) synthetases (nda) producing cyanobacterial genera such as *Anabaena, Microcystis, Planktothrix, Nostoc, Nodularia*[142].

Biochemical-based Methods

There are various methods to detect the biochemical properties of cyanobacterial toxins. Among those methods, enzyme-linked immunosorbent assay (ELISA), Ligand binding assay, enzyme inhibition assay. ELISA uses antibodies either monoclonal or polyclonal for quantifying and detecting the cyanobacterial toxins in water samples[143,144]. ELISA is active along with analytical techniques namely HPLC and LC-MS for more high-fidelity MCs analysis. Because this method is delicate, low-level of expertise needed with limited ridge life may undervalue the denseness of toxins. Antibodies have been generated with different cross quality against MC-LR and this is with success governing the MC content in environmental samples[145,146]. An indirect competitive ELISA is derived from polyclonal antibodies because there is a good cross-reactivity against a range of purified MC variants[146]. A direct competitive ELISA is used to detect MCs in water samples also considered as good cross-reactivity with MC variants[147].

Measurements are used by two techniques: The radio-isotopic technique based on 32 phosphates radiolabeled substrate[148] and the colorimetric assay based on p-nitrophenyl phosphate substrate[149]. The radio-isotopic technique is dependent on the radiolabeled proteins[38]. The phosphatase inhibition is also used for speedy observance of the MC producing bloom toxicity[41].

Chemical Methods

This method is used to detect the cyanobacterial toxins in water are majorly fluid-based separation such as high-performance liquid chromatography (HPLC), Nuclear magnetic resonance (NMR), and various Mass spectrometric techniques namely triple quadrupole mass spectrometry (LC-MS/MS), Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, liquid chromatography time of flight (LC-TOF) are available to different cyanobacterial toxins.

Cyanotoxins are chromatographically separated under higher pressure in LC columns by a liquid that was crowded with tiny particles[150]. Reverse HPLC is used but saxitoxins, â-N-methylamino-L-alanine (BMAA) are polar substances that are separated by hydrophilic interaction LC (HILC)[151,152]. The need for standardization for many MC variance makes the detection difficult and their results are generally expressed as MC-R equivalents[125,153]. Liquid chromatography-mass spectrometry (LC-MS) is an analytical technique that helps to detect,

identify and confirm the presence of cyanotoxins in environmental samples and it is also considered a highly selective and sensitive technique. This combines the physical detachment capacity of LC mass analysis capabilities of MS and detection of the MC, NOD, CYN is easy. The analysis is performed by ELISA or antithetic LC-MS methods to detect and determine certain isomers[154]. Detection of BMAA is also difficult due to its smaller molecular structure. There are five different methods or techniques used to detect the BMAA compound in marine cyanobacteria such as HPLC with fluorescence, UV, MS discovery etc[155].

LC-MS/MS is an analytical technique that is highly sensitive and selective towards unequivocal detection and quantification of unknown toxins in environmental samples[156]. CYN has a different molecular structure when compared to others but they are analyzed together by LC-MS/MS by applying particular MS-MS transitions. No other method is found to detect the CYN[157]. MALDI-TOF is a speedy, exclusive, and delicate analytical method with advanced declarations allowing the exact mass measurement and sensing of compounds founded on their molecular formula. In this ion's mass to charge ratio is determined in terms of their time measurements[158]. MCs are peptides, and are readily hypersensitive to sensing with MALDI- TOF MS[159]. This technique provides support to HPLC by detecting cyanotoxins in minute-quantities such as a cyanobacterial colony not available as purified standards. Unlike LC-MS, LC-TOF-MS has a reward to produce the accurate measurement providing good selectiveness in convoluted samples[160]. NMR is an analytical technique that studies the magnetic property of certain atomic nuclei and provides detailed information about the structure of the material. This technique is not so useful for quantifiable analysis of cyanotoxins[161]. Table 4 showed screening methods used to detect the presence of cyanobacterial metabolites

CONCLUSION

Cyanobacteria show the high potentiality to create aggregative populations in the surroundings as they are present as common members of plant communities in marine, freshwater, and brackish water throughout the world. Cyanobacteria are known to produce secondary metabolites which are either useful such as research fields, pharmaceuticals, drug discovery, etc., or harmful by producing toxins that cause cyanobacterial blooms, diseases to animals and wildlife, and even fatal deaths. Numerous secondary metabolites have been identified and sporadic from marine cyanobacterial species which are time-tested for different forms of bioactivities such as anti-cancer, antialgal, antifungal activities, etc. They also offer many advantages as their cultures are readily established and their manufacture can be optimized to give property yields in industrial-level scales. In particular, the application of molecular biology and DNA amplification technology is used to detect the toxins that provide advanced significance in water quality management. Many screening methods are developed to monitor the water assessment of public health risks. The impact of toxins on the water environment is important in the future because of its relation to human health.

Within the scope of this review, several facets of environmental monitoring to detect cyanobacterial toxins by using different screening methods were discussed and analyzed. Different types of complementary approaches were presented such as microscopy assays, physicochemical methods, molecular-based methods, biochemical-based methods, and chemical methods, and their respective advantages and disadvantages were discussed. Among them, the molecular-based method allows first screening of cyanobacterial toxins and gives primary response about the presence of an approached sample in the environment. After concluding the potentially toxic cyanobacteria further methods are carried out as final confirmation of its toxic status.

The review mainly illustrates the cyanobacterial toxins and their causes to the environment, cyanobacterial secondary metabolites which are involved in bioactivities, screening methods to detect the presence of cyanobacterial toxins which prevents future health risk and improves water management.

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Conflict of interest

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