C-PHYCOCYANIN: A POTENT BIOACTIVE COMPOUND

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Abstract

Cyanobacteria (blue-green algae) are photosynthetic prokaryotes which are also used as food by humans for a long time. They are rich in a variety of bioactive compounds, proteins, vitamins, antioxidants, antimicrobials and anti-carcinogenic biomolecules. The bioactive potential of C-phycocyanin isolated from cyanobacteria has been well established as antiviral, anti-tumour, antibacterial, anti-diabetic, anti-HIV and a food additive. This review basically presents a thorough approach about the various key applications of cyanobacteria derived bioactive compound C-phycocyanin together with highlights of future thrust areas in its research related to its production and potential medical applications.

KEYWORDS: *Cyanobacteria, microalgae, bioactive compounds, toxins, C-phycocyanin.*

1. Introduction

Cyanobacteria are oxygenic photosynthetic prokaryotes and are broadly distributed in the natural ecosystems like freshwater and seawater. Natural water contains numerous organisms like blue-green algae, phytoplankton, zooplankton, and fish. Natural and synthetic chemical compounds called pesticides hold an essential place in agriculture and economics. Pests like various algae, weeds, fish, nematodes, fungi, bacteria and insects can have a devastating effect on crop yield either by direct destruction of the crop or by competing for nutrients in the soil. Pesticides are used globally and extensively for the control of such pests. Satisfactory crop yield is impossible without the use of pesticides despite the public awareness that now exists about the harmful effects of pesticide use. Besides agriculture, pesticides are widely used in industrial, domestic and marine environments. Pollution of natural waters particularly fresh and sea water implies that it contains a lot of inorganic and organic substances introduced by human activities which change its quality and are harmful to many living organisms, including man (Anand *et al*., 1980). Cyanobacteria belong to the kingdom Monera and division Cyanophyta and the most primitive

forms of life on earth. The cellular structure of cyanobacteria is simple prokaryote and performs photosynthesis. These likewise resemble animals in having complex sugars like glycogen on their cell membrane. These include edible and toxic species like *Nostoc*, *Spirulina* and *Aphanizomenon*. They have the petition of being a raw unprocessed food, rich in carotenoid, chlorophyll, phycocyanin, amino acid, minerals and many other bioactive components. The nutrient content depends on the location and environment in which the algae are grown. The environment includes altitude, temperature and sun exposure that can significantly affect the lipid and pigment content in algae. Algae grown in canals and rivers differ from that of the sea. Prokaryotic photosynthetic microorganisms are rich in biologically active secondary metabolites. Phycocyanin is a biologically active nutrient compound which is isolated and purified from a variety of cyanobacterial species (De Jesus Raposo *et al*., 2013). Phycocyanin can be obtained from different species, such as *Spirulina* sp., *Synechocystis* sp., *Aphanizomenon* sp., *Phormidium* sp., *Lyngbya* sp., and *Synechococcus* sp. (Shen *et al*., 2008), has been separated and studied.

Phycocyanin belongs to the phycobiliprotein (PBP) family (Stadnichuk *et al*., 2015); it is characterized by a deep and intense blue colour. According to the coloured molecules, phycobiliproteins can be divided into three categories: phycoerythrin (PE, PE is red), phycocyanin (PC, PC is blue) and allophycocyanin (AP, AP is bluish green) (Grossman *et al*., 1993). Phycocyanin is a type of photosynthetic assistant protein which can efficiently capture light energy. Phycobiliprotein is one of the components of phycobilisome, which is a supramolecular protein complex that auxiliarily collects light energy. Phycobilisome plays an important role in photosynthesis energy absorption and transmission (Kirst *et al*., 2014). Phycobiliprotein acts as an antenna molecule in algae photosynthesis, which can absorb light energy and can be capable of efficiently delivering light energy to a reaction centre containing chlorophyll by a non-radioactive process (Watanabe *et al*., 2014).

2. Bioactive compounds found in cyanobacteria

Cyanobacteria contain up to 50-70% protein, 30% lipids, over 40% glycerol, up to 8-14% carotene and high concentration of vitamins B1, B2, B3, B6, B12, E, K, D, etc., compared with other plants or animals. The potential of microalgae biomass for big pharma practical uses is definitely great (Kuddus *et al*., 2013). Cyanobacteria contain abundant bioactive compounds that can be harnessed for commercial use. Initially considered as nuisances agents or laboratory

curiosities in water bodies, but at the present cyanobacteria form an important component of integrated nutrient management in agriculture and are exploited in commercial biotechnological schemes (Priyadarshani *et al*., 2012) as a source of pigments, phycocolloids, vitamins, immuno-diagnostic agents and therapeutics and for biofuel production (Shen *et al*., 2008).

2.1 Cyanovirin-N

Cyanovirin-N (CV-N) (fig.1) is a unique, 101 amino acid long and 11 KDa protein. It was discovered as a constituent of a cultured cyanobacterium, *Nostoc ellipsosporum.* It was recognized in a screening effort as a highly potent inhibitor of diverse laboratory-adapted strains and clinical isolates of HIV-1, HIV-2, and SIV. Afterwards, the structure of CV-N was solved, first by NMR spectroscopy and later by X-ray crystallography at a resolution of 1.5 Å. The two structures are similar. The CN-V monomer consists of two similar domains with 32% sequence identity to each other. In the crystal structure, the domains are connected by a flexible linker region, forming a dimer by intermolecular domain swapping. It has a potent virucidal activity that possesses the ability to inactivate all strains of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) as well as other viruses such as those of influenza and Ebola. The mechanism(s) underlying the HIV-1-inhibitory activity of CV-N remain unclear. CV-N binds with high affinity to gp120, the external subunit of the HIV envelope glycoprotein (Env); evidence suggests the anti-HIV-1 effects of CV-N are mediated through this interaction (Boyd *et al*., 1997).

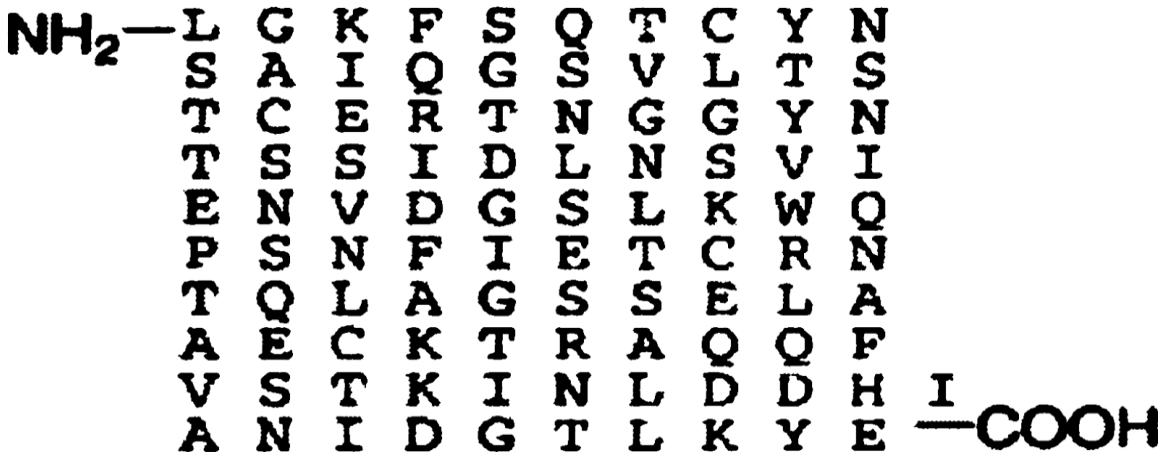
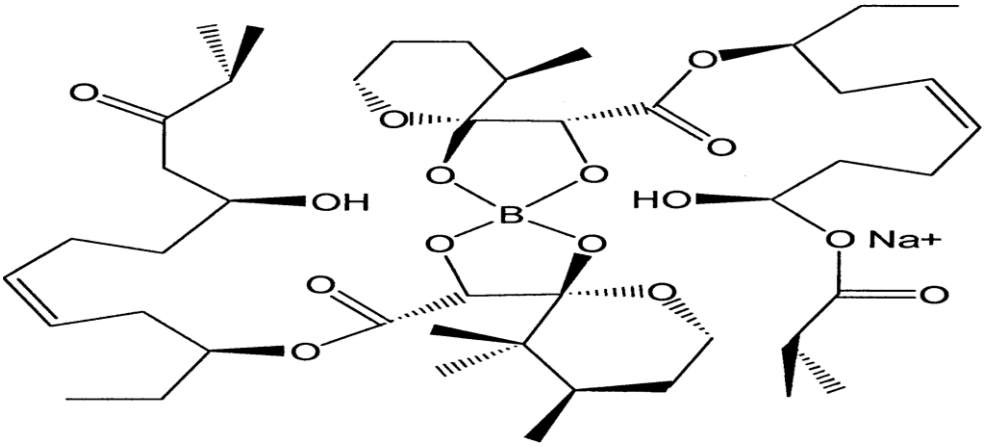


Fig.1. Cyanovirin N amino acid sequence (Burja *et al.*, 2001).

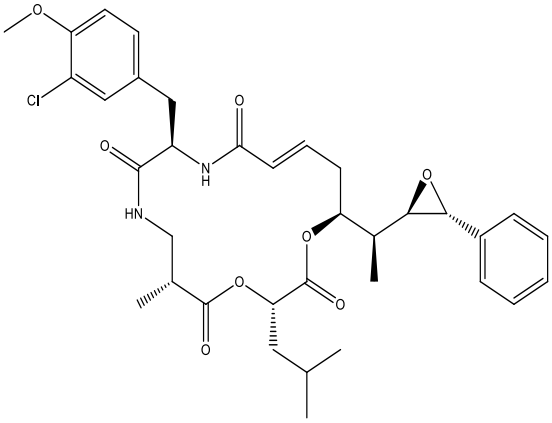
2.2 Borophycin



Borophycin (fig.2) is a boron-containing metabolite isolated from marine strains of cyanobacteria *Nostoc linckia* and *Nostoc spongiaeforme var. tenue.* The gross structure of this boron-containing compound was determined by spectral methods and it's relative stereochemistry established by X-ray crystallography. Borophycin is made up of two identical halves with an overall structure reminiscent of the ionophoric antibiotics boromycin (2) and aplasmomycin (3). The biosynthesis of 1 differs from the biosynthesis of 2 and 3. All three compounds are acetate-derived polyketides that utilize a C3 precursor for the starter unit and methionine for the methyl branches on the polyketide chain. Whereas phosphoglycerate or phosphoenolp

yruvate has been suggested to be the C3 starter unit in the biosynthesis of 2 and 3, the C3 starter unit for the biosynthesis of 1 is derived from acetate and methionine, but not propionate (Boyd *et al*., 1997).

2.3 Cryptophycin



Cryptophycin (fig.3) first isolated from *Nostoc sp.* ATCC 53789 is a potent fungicide and lipopeptides. Cryptophycinsare a class of dioxadiazacyclohexadecenetetrone cytotoxins with a potent ability to induce tubulin depolymerization. Similar to the maytansinoids, cryptophycinsbind microtubules at the vinca-binding site eventually leading to a mitotic arrest. Initial preclinical data with synthetic versions of cryptophycinssuch as

LY355703 revealed promising antitumor effects in mammary and prostate xenograft models, which facilitated the transition of LY355703 into human clinical trials. However, as was the demise of several other tubulin inhibitors in clinical trials, the doses required for LY355703 to achieve therapeutic efficacy elicited significant toxicities, thus precluding its use as a stand-alone therapeutic agent (Jiang *et al*., 2017). Before cryptophycin could be converted into a warhead for an ADC, it first had to be modified with a suitable handle for linker attachment. Bouchard et al. synthesized a variant of cryptophycin, cryptophycinanalog 1, which included an amine handle while retaining potency similar to that of the parental cryptophycin (Murugan *et al*., 2012, Gonzalez *et al*., 1999).

2.4 Lipopeptides

Approximately 68% of the natural products derived from cyanobacteria contain nitrogen. Analysis of 424 marine cyanobacterial natural products show that 40.2% are lipopeptides (cyclic or linear), 5.6% are of pure amino acid, 4.2% are fatty acids, 4.2% macrolides and 9.4% are amides (Chen *et al*., 2015). For example, several molecular forms of 'hassilidins' have been isolated from *Hassallia sp*., 'puwainaphycins' have been characterised from *Cylindrospermum alatosporum* and 'anabaenolysins' from *Anabaena sp*. They are often distinctive in that the fatty acid component (C_{12} to C_{18}) contains a hydroxyl group in position 2 and an amine group in position 3; usually the fatty acid chain is saturated, but at least one C_{18} fatty acid has six double bonds (two groups of three in conjugation), while others contain methyl branches and methoxyl groups. Dragomide E from *Lyngbya majuscule* (a marine cyanobacterial species) has five amino acids in a linear peptide linked to an acetylenic C_8 fatty acid. Although the biological properties of these lipopeptides have barely been explored, some are known to have anti-fungal actions or cytolytic activities against mammalian cell lines. Hapalosin (fig. 4), a cyclic desipeptide isolated from the cyanobacteria, *Hapalosiphon welwitschii*, has a reversing activity against MDR (multidrug resistance) derived from P-glycoprotein (Kashihara *et al*., 2000).

2.5 Protease Inhibitors

Five classes of protease inhibitors have been reported from the toxic genera of cyanobacteria: they are micropeptins, aerugenosins, microginins, anabaenopeptins and microverdins. More recently, Rohrlack *et al.* have suspected microviridin J, a newly discovered protease inhibitor

produced by *Microcystis* strain UWOCC MRC, of causing the lethal moulting disruption observed by Kaebernick *et al*., 2001. Protease inhibitors have been isolated from a variety of freshwater cyanobacterial blooms and mats, i.e., *Microcystis*, *Lyngbya*, *Nostoc* sp. and *Oscillatoria* . Further, a wide range of proteases has been shown to be inhibited by crude extract or purified compounds from cyanobacteria including *Oscillatoria.* Alike other cyanobacteria, compounds from *Oscillatoria* have also shown to inhibit major serine protease family member enzymes, i.e., trypsin, chymotrypsin, elastase and plasmin (Shin *et al*., 1995).

2.6 Phycobiliprotein

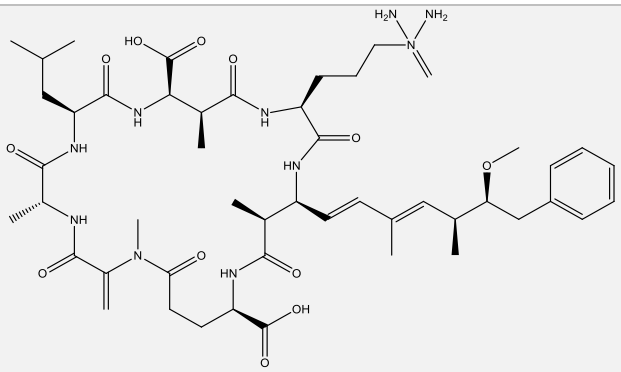
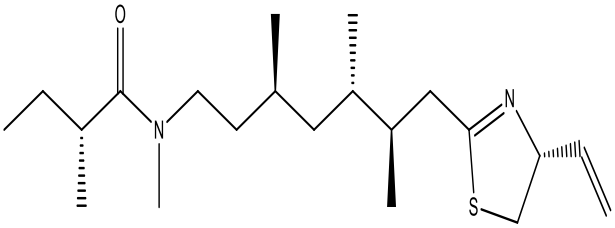
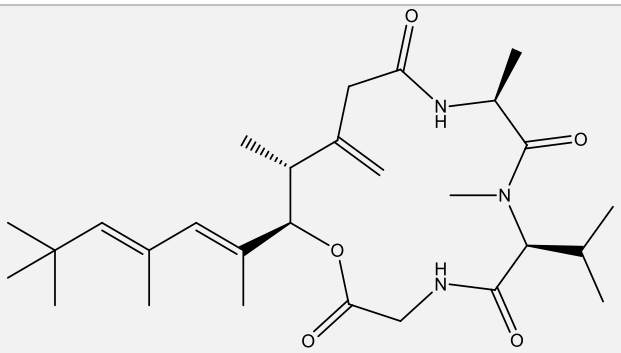
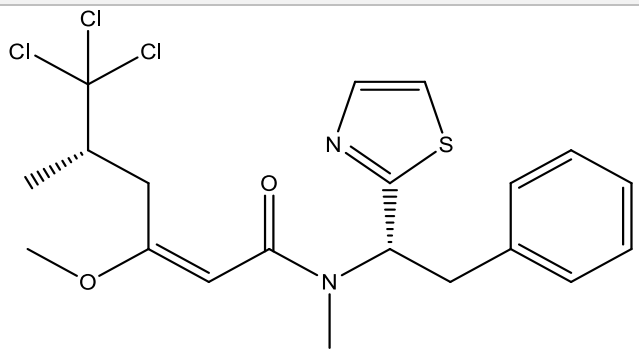
Phycobiliproteins are a group of coloured proteins present commonly not only in cyanobacteria (blue-green algae) but also in red algae, cryptomonads, etc. They are broadly commercially used in foods, cosmetics, biotechnology, pharmacology and medicine. Commercially, Phycobiliproteins are high-value natural products with actual and/or potential biotechnological applications in nutraceuticals and pharmaceuticals, food and cosmetic industries as well as in biomedical research and clinical diagnostics. The use of phycobiliproteins as non-toxic and non-carcinogenic natural food colourants is gaining importance worldwide in the view of the potential toxicity and carcinogenicity of the synthetic food colourants, moreover, their therapeutic value has also been demonstrated (Moraes *et al*., 2011, Pandey *et al*., 2013). Generally, they are categorized into three main types namely phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) which differ in their spectral properties (Sun. *et al*., 2003, Chu *et al*., 2012). Phycobiliproteins also are the 6 main light-harvesting chromoproteins in a certain type of marine algae (Silveira *et al*., 2007). These compounds are classified according to different criteria (such as structure, spectra of absorption, colour, etc.). Moreover, based on absorbance wavelength, the phycobiliproteins existing in cyanobacteria and red algae are commonly categorized into four groups known as, phycoerythrin (PE; λmax= 490-570 nm), phycoerythrocyanin (PEC; λmax= 560-600 nm), (3) phycocyanin (PC; λmax= 610-625 nm) and allophycocyanin (AP; λmax= 650-660 nm) while in the cryptomonads, exist two classes of phycobiliproteins: phycoerythrin (PE; λmax= 540 - 570 nm) and phycocyanin (PC; λmax= 610-650 nm). Also based on their colours, phycobiliproteins are classified into two large groups namely: phycoerythrin (red) and phycocyanin (blue); furthermore, phycocyanins are subdivided

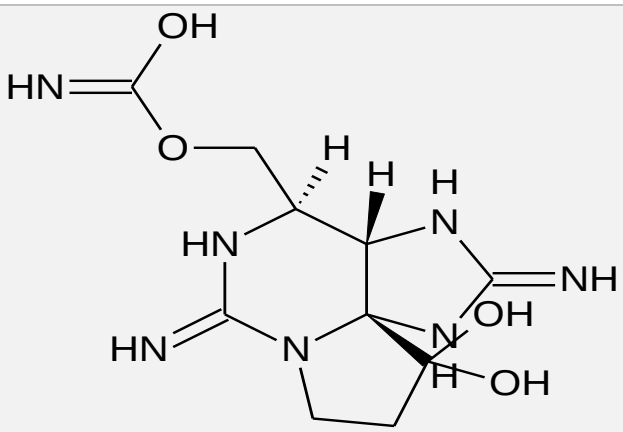
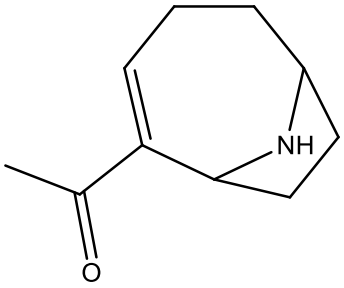
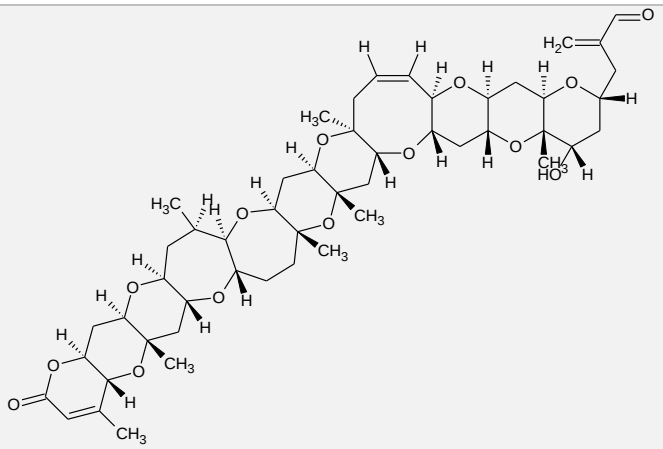
into C-phycocyanin (C-PC), R-phycocyanin (R-PC) and allophycocyanin (APC) (Kuddus *et al*., 2013).

2.7 Phycocyanin.

Phycocyanin is a biologically active nutrient compound which is isolated and purified from a variety of seaweeds (De Jesus Raposo *et al*., 2013). Phycocyanin obtained from different species, such as *Aphanizomenon* sp., *Spirulina* sp., *Phormidium* sp., *Lyngbya* sp., *Synechocystis* sp. and *Synechococcus* sp., has been separated and studied. Phycocyanin belongs to the phycobiliprotein (PBP) family (Madamwar *et al*., 2015), which is characterized by a deep and intense blue colour. According to the coloured molecules, phycobiliproteins can be divided into three categories: phycoerythrin (PE, PE is red), phycocyanin (PC, PC is blue), and allophycocyanin (AP, AP is bluish green) (Chen *et al*., 2013). Phycocyanin is a kind of photosynthetic assistant protein which can efficiently capture light energy. Phycobiliprotein is one of the components of phycobilisome, which is a supramolecular protein complex that auxiliarily collects light energy. Phycocyanin has a deep and intense blue colour and consists of α and β subunits. In general, the α and β subunits of the phycocyanin form a stable heterodimeric monomer (αβ) and then polymerize it into a multimer (αβ) n (n=1~6) (Watanabe *et al*., 2014). Most phycocyanins are present as a trimer (αβ) 3. The α and β subunits of C-phycocyanin have similar 3D structures; however, their sequences are different (Adir *et al*., 2003). The α and β subunits contain about 160 to 180 amino acid residues, respectively. The molecular weight of α and β subunits ranges 10~19kD and14~21kD. It has been reported that phycocyanin has anti-oxidative function, anti-inflammatory activity, anti-cancer function, anti-bacterial, immune enhancement function, liver and kidney protection pharmacological effects (Storf *et al*., 2001).

3. TOXINS FROM CYANOBACTERIA

Toxins	Isolated from	Application	Structure
Hepatotoxins	Anabaena, Hapalasiphon, Microcystis, Nostoc and Oscillatoria	Destructive to liver cells.	 Microcystin
Kalkitoxin	Lyngbyamajuscula	Neurotoxin	
Antillatoxin	*L. majuscula*	Activation of voltage-gated sodium channels	
Barbamide	Curacao strain of *L. majuscula*		

Saxitoxin	*Alexandriumcatenella, A.minutum, A.ostenfeldi, A. tamarense, Gymnodiniumcatenatum*	Neurotoxic alkaloids	
Anatoxins	*Anabaeneflos-aquae*	Secondary amines and are postsynaptic depolarizing neuromuscular blocking agents	
Brevitoxins	*Ptychodiscus brevis*	Neurotoxins	

4. Bioactive potential of C-phycocyanin

4.1 Antibacterial activity

Various organic and aqueous extracts of *S.platensis* were screened for antibacterial activities by agar diffusion method against human pathogens (Mala *et al*., 2009). Water extract showed

maximum activity against *K.pneumoniae*. This result could be due to the water-soluble pigment C-phycocyanin from *Westiellopsis* sps. (fresh water Cyanobacterium) tested for their antibacterial activity against *B.subtilis*, *Pseudomonas* species and *Xanthomonas* sps. and all were inhibited by C-phycocyanin. Four Bacterial species were used which were isolated from clinical samples and are pathogenic to human. Out of four bacterial isolates, *Pseudomonas aeruginosa* does not respond to all the concentration of C-phycocyanin. The maximum zone of inhibition obtained with *S.aureus* and the zone of inhibition to *K.pneumoniae* was lower than the result of Mala et al., 2009. They used standard strain, but here the isolates were from a clinical sample which may have previous exposure to drugs that may attribute to the lower zone of inhibition (Shahzad *et al*., 2010).

4.2 Anti-inflammatory activity

Effects of C-phycocyanin and 5-ASA on Determination of myelo peroxidase (MPO) activity and on damage scores in colitis induced by acetic acid in rats Colitis induced by acetic acid in rats resulted in significant increases in colonic MPO content with respect to normal control rats. Though, the former effect was reversed in the group of rats with colitis which was previously treated with C-phycocyanin. The greater inhibitory effect on MPO activity was achieved by C-phycocyanin which indicates a reduction of neutrophil infiltration in colonic tissue, also substantially decreased MPO activity in rats with colitis. With these findings, histological evaluation of colonic tissues revealed essentially normal mucosa in the non-treated control group, in contrast with mucosal haemorrhage, severe inflammatory cell infiltration, submucosal oedema and focal ulceration in acetic acid-treated rats. Rats pre-treated with phycocyanin, there was only slight submucosal oedema, minimal subepithelial haemorrhage and mild inflammatory cell infiltration. Thus, the colonic damage score was reduced in the group pretreated with the greatest dose of phycocyanin (Gonzalez *et al*., 1999).

4.3 Antioxidant Activity

The DPPH inhibition by C-phycocyanin extract, reveal that applied concentration of C-phycocyanin showed inhibition of more than 15% at the highest concentration. Ferric Reducing Antioxidant Potential (FRAP) Assay Ferric reducing the potential result of ferric reducing capacities of selected concentrations of C-phycocyanin. The trend for the ferric ion reducing

activities of C-phycocyanin tested was similar to the DPPH scavenging activities. C-phycocyanin possesses dose-dependent ferric reducing capacities. A dose-dependent increase was noticed in the case of C-phycocyanin (Mahfooz *et al*., 2017).

4.4 Antidiabetic potential:

The effect of C-phycocyanin was seen on KKAy mice. The blood glucose level in the mice of the control group remained high, while those in both the PC group and the pioglitazone group were significantly lower. PC was more effective in lowering the blood glucose level than pioglitazone. Compares 24 h random blood glucose levels measured on day 20. Again, the random glucose levels of both the PC and pioglitazone treatment groups were significantly lower than that of the control group. Treatment with PC and pioglitazone increased significantly the content of glycogen in liver and muscle as compared to untreated KKAy mice (Ou *et al*., 2013).

4.5 Anticancer potential

When C-PC treated tumour cells HT-29 and A549, it was reported that cell cycle was blocked in the cell cycle G0 / G1 phase, DNA synthesis was blocked, and thus, tumour cell proliferation was inhibited. Similarly, when C-PC treated human breast tumour cell MDA-MB-231 and human squamous carcinoma cell 686LN-M4C1, these tumour cells were found to have different degrees of cell cycle arrest in G0 / G1 phase. Phycocyanin could increase the expression of p21, meanwhile, Phycocyanin could down-regulate the expression of Cyclin E and CDK2 in the MDA-MB-231 cell. Moreover, phycocyanin could prevent K562 cells into S phase and the cells were arrested in G1 phase. Additionally, it was found that phycocyanin blocked G2 / M cell cycle progression and induced apoptosis of PANC-1 cells. Chunyan Wang also confirmed that phycocyanin caused cell cycle G2 / M arrest and induced apoptosis in human hepatoma cell line HepG2. It was interesting to note that several groups reported the mechanism of PC-mediated cell cycle arrest (Jiang *et al*., 2017).

It was found that ATRA and C-phycocyanin combination treatment of HeLa cells could significantly reduce the dose and side effects of ATRA. The combination therapy can significantly down-regulate anti-apoptotic protein Bcl-2, up-regulate the expression of pro-apoptotic Caspase-3 protein, inhibit cell cycle-related CDK-4 and Cyclin D1 protein expression,

inhibit complement regulatory protein CD59 expression and induce the HeLa cell apoptosis (Yang *et al*., 2014).

5. Conclusion

Cyanobacteria produce a wide range of toxins and other biomedical interesting bioactive compounds. One of its potent bioactive compounds is C-phycocyanin. Extensive research studies revealed that C-phycocyanin has a rich potential to act as a drug. C-phycocyanin might be a promising bioactive compound and can be used as a remedy for various diseases comprising cancer, diabetes, inflammation. It also acts as anti-oxidant, anti-bacterial, antiviral, anti-fungal agents which further suggest its involvement in combating a large aura medical infestation including ageing. This review summarizes some recent developments in applications of C-phycocyanin. Although significant initial studies have already be done and but deep-rooted extensive research still be needed in coming future to extract hidden information about C-phycocyanin and efforts should have to be made in order to attain economical excess production of C-phycocyanin by rDNA technology through increasing its nutritional and pharmacological value by protein engineering and other techniques.

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