Isolation and Characterization of Value Added Compounds From Fresh Water Strains of *Nostoc Linckia*

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A Minor Project Report submitted

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Summary

Isolation of microalgae has a large scope of utility towards the benefits of mankind. Cyanobacteria, the blue green alhae are one of the most diverse groups of Gram- negative photosynthetic prokaryotes widely distributed throughout the world. Cyanibacteria produce a wide range of compounds including secondary metabolites with various biological actions like anticancer, antimicrobial, antifungal or anti- inflammatory and other pharmacological activities (Kaushik et. al, 2009; Kailash, Bhardwaj and Bahuguna, 2010). Research into bioactive metabolites has led to discoveries of Cyanobacteria which are toxic to other Cyanobacteria or green algae inclusive of its rich source for various commercial, pharmaceutical or toxicological products (Rainer, 2006)Increasing energy demand and depleting fossil fuel sources have intensified the focus on biofuel production.

Microalgae have emerged as a desirable source for biofuel production because of high biomass and lipid production from waste water source. Microlagae being natural sources of various nutrients and growth promoting factors are used, nowadays, as an alternative biofertolizer. Due to their diversity and availability, microalgae have a wide scope of research and discovery in all sectors related to life science. It has, therefore, become an important objective to identify the water bodies surrounding the city and isolate the microalgal and algal species.

Phycobiliproteins such as phycocyanin and phycoerythrin have been purified extensively from many *Nostoc* sp. wherein phycoeythrin yield and purity have shown to be more than that of phycocyanin. So far, no concrete report has been published with regard to purification of phycobiliproteins from *Nostoc linckia*.

The study showed the efficacy of cost effective chemicals and methods to isolate and purify the phycobiliproteins, particularly phycoerythrin at the laboratory scale. The protocol developed in this study has four major steps viz. preparation of crude extract, one step 65% ammonium sulphate precipitation, dialysis and anion exchange chromatography using DEAE Cellulose and acetate buffer.

This extraction and purification protocol formulated is efficient enough to obtain high purity phycoerythrin of final purity 4. 35 while the final purity of phycocyanin was 3. 753. Since, purity ratio more than 4.0 is generally accepted (Patel et. al, 2004), the procedure has scope to be modified so as to obtain a higher purity of phycocyanin using acetate buffer alone.

The protective effect of extracts on oxidative DNA strand breakage was evaluated with pBR322 plasmid DNA. Oxidative modification of DNA has been suggested to contribute to aging and various diseases including cancer and chronic inflammation (Kumar et. al,). The plasmid DNA was mainly of the super coiled form (band at the bottom) as in lane- 1- DNA (pBR322 plasmid alone) and open circular form (band at the top) as in lane- 2- DNA and Fenton's reagent, where nicking was caused by hydroxyl radicals (Gigure 7). Inhibitory effect of the phycoerythrin and phycocyanin on plasmid DNA nicked caused by hydroxyl radicals was seen. In presence of Fenton's reagent, the super coiled form decreased and converted into open circular form. Addition of phycocyanin and phycroerythrin significantly inhibited the formation of open circular form compared to positive control. This may be attributed to the scavenging activity of phycocyanin and phycoerythrin on the hydroxyl radicals generated from the Fenton reagent. The phycobiliproteins were also non-toxic towards the plasmid DNA as the circular for of the supercoild DNA remained intact even after treatment with the proteins. The phycobiliproteins exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization indicates that the proteins may also well stabilize lysosomal membranes. Stabilization of lysosomal membrane is crucial point in limiting the inflammatory response via inhibiting the release of lysosomal constituents of activated neutrophil. Some of the NSAIDs are known to have membrane stabilization properties which may be contributing to their anti- inflammatory property. Phycocyanin and phycoerythrin were more efficient in protecting the RBC membrane as compared to the standard drug, Diclofenac. Phycocyanin expressed better activity than phycoerythrin. Proteinases are involved in arthritic reactions. The main source of proteinase is the lysosomal granules of neutrophils. Earlier it was reported that leukocytes proteinase play important roile in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995).

The Diclofenac drug exhibited better proteinase K inhibition activity as compared to the phycobiliproteins. Phycocyanin was a more potent inhibitor as compared to phycperythrin.

The phycoproteins, however, exhibited strong protective and inhibitory activities without being toic to DNA.

The proteins have potential to be explored extensively in this line of research