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Research Article

Phytochemical screening and estimation of value added compounds from *Nostoc linckia*

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Abstract: The aqueous and ethanol extracts of lyophilized cultures of *Nostoc linckia* isolated from Kukkarahalli lake, Mysore underwent phytochemical analysis wherein flavonoids, saponins, terpenoids, tannin and steroids were detected in the aqueous and ethanol extracts while alkaloids were not observed in either extract. Phycobiliproteins were detected in the aqueous extract only while carotenoids and chlorophyll were detected in ethanol extract only. The total protein content, total free amino acids, total carbohydrate content, total flavanoid content, total phenolic content and total ascorbic acid content in aqueous extract was found to be 44.42 µg BSAE/ mg extract, 42.22 µg GE/ mg extract, 18 µg GlcE/mg extract, 6.01 µg RE/ mg extract and 9. 77 µg GAE/ mg extract respectively, while the ethanol extract values were 2.46 µg BSAE/ mg etract, 6.17 µg GE/ mg extract, 4.5 µg GlcE/mg extract, 1.14 µg RE/ mg extract and 0.992 µg GAE/ mg extract respectively. Total ascorbic acid content was 20µg/ mg extract in both aqueous and ethanol extracts. The phycoerythrin, phycocyanin and allophycocyanin content were 0.108 mg/ml, 0.032 mg/ml and 0.006 mg/ml respectively while chlorophyll a and caroteneoid content were 1.203 mg/ml and 0.723 mg/ml respectively. The aqueous extraction was more efficient process than ethanol extraction of value added compounds from *N. linckia*.
Keywords: lyophilized cultures, *Nostoc linckia*, Phycobiliproteins, flavanoid

INTRODUCTION

According to the recent FAO database, the total number of undernourished people in the world, almost 870 million, is still unacceptably high, and eradication of hunger remains a major gobal challenge. Hence, the search for alternative food ingredients remains of utmost importance. For centuries indigenous populations have used microalgae for varius application. Edible blue- green algae like Nostoc, Arthrospira (Spirulina) and Aphanizomenon speies have been used for thousands of years [18]. Algal biomass have appeared to be a new and unconventional protein aource in the arly 1950s in order to supply the insufficient protein supply to the world's population that was increasing [1,2]. Large scale factories producing tons of algal biomass were established to collect value added compounds like proteins, β-glycans, carotenoids, phycobiliproteins etc [3,4]. In addition to being considered as an unconventional source of proptein, they are also sourced of essential amino acids as they can synthesize all amini acids essential for human and animal [5]. Carbohydrates in the form of starch, glucose and other polysaccharides can be found in microalgae. Due to their overdigestibility there is no limitation to using dried whole microalgae in foods and feeds [1]. Microalgae also represent a valuable source of nearly all essential vitamins like A, B1, B6, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid [1]. Microalgae are also rich in pigments such as chlorophyll, carotenoids and phycobiliproteins. These pigments have commercial applicants as food colorants, diet supplement, and fluorescent label for immunolabeling experiments including nutraceutical potential by exhibiting anti-inflammatory activity. Thus, their composition gives microalgae interesting qualities, which can be applied in human and animal nutrition. Limited extensive work and concrete report has been made about the commercial potential of *Nostoc linckia*. It has been utilized mainly as bioremediation algae. Two classes of morphological mutant clones of *Nostoc linckia* was isolated after its treatment with nitrosoguanidin [21]. Both classes of mutants expressed reduced nitrogenase activity. The potential of spent biomass of hydrogen producing cyanobacterial strain *Nostoc linckia* from a hydrogen fermentor was studied for decolorization of a tri- phenylmethane dye, crystal violet [6]. The water-methanoloc extracts from freeze dried masses was found to exhibit moderate acetylcholinesterase inhibitory activity of 30- 65% [7]. The antioxidant and antimicrobial activities of the ethanolic extract have already been investigated wherein β- carotene was the

main carotenoid detected [8]. However, no elaborate reports have been made regarding the utilization of *Nostoc linckia* as source of human or animal nutrition or dietary supplement. We have thus explored such application by determining and estimating the value added compounds present in *Nostoc linckia*.

MATERIALS AND METHODS

Culturing and collection of *Nostoc linckia*:

Nostoc linckia isolated from Kukarrahalli Lake, Mysore, was mass cultured and maintained in BG-12 medium, in 12/12 hr light/dark conditions, under continuous agitation a 20 ^{0}C. The biomass was harvested and lyophilized giving a dry powder. The dried powder was used for further extraction and analysis.

Phytochemical Screening

Preliminary phytochemical screening for alkaloids, flavonoids, sapponins, tannins, steroids, terpenoids, carotenoids, chlorophyll and phycocyanins was conducted for the aqueous and ethanol extracts using standard procedures [9].

Estimation of phycoconstituents

Phycoconstituents such as proteins free amino acids, carbohydrates, ascorbic acid, phenolics and flavanoids were estimated for aqueous and ethanol extracts using standard protocols [10, 11]. The results are expressed as µg/ mg extract and compared. The phycobiliprotein content was estimated for the aqueous extract while the chlorophyll a and carotenoid content was estimated for the ethanol extract, and expressed as mg/ml respectively [12, 13].

Statistical Analysis

Data were expressed has mean of the triplicate values ± standard deviation .The data was statically analyzed using Origin 5.0 wherein p values< 0.05 were considered as significant.

RESULTS

Phytochemical Screening

The preliminary phytochemnical screening was carried out in order to determine the presence of various phytococtituents in aqueous and ethanolnextracts of Nostoc linckia (Table- 1). The study has shown the presence of flavonoids, saponins, terpenoids, tannin and steroids in the aqueous and ethanol extracts. While phycobiliproteins such as phycocyanins were detected in the aqueous extract only, carotenoids and chlorophyll were detected in ethanol extract only. Alkaloids were not observed in either extract.

Table-1: Preliminary screening of cyanobacterial species for the presence of certain phytoconstituents

[+: present-: absent]

Test	*Nostoc linckia*	
	Water extract	Ethanol extract
Alkaloids	_ve	_ve
Saponnins	+ve	+ve
Tannins	+ve	+ve
Steroid	+ve	+ve
Flavonoid	+ve	+ve
Terpenoids	+ve	+ve
Carotenoids	_ve	+ve
Phycocyanins	+ve	_ve
Chlorophyll	_ve	+ve

Estimation of phycoconstituents

The total protein content, total free amino acids, total carbohydrate content, total flavanoid content, total phenolic content and total ascorbic acid content in aqueous extract was found to be 44.42 µg BSAE/ mg extract ($p < 0.01$), 42.22 µg GE/ mg extract ($p <0.001$), 18 µg GlcE/mg extract ($p< 0.001$), 6.01 µg RE/ mg extract ($p< 0.001$) and 9. 77 µg GAE/ mg extract ($p < 0.001$) respectively, while in the ethanol extract the values were 2.46 µg BSAE/ mg etract, 6.17 µg GE/ mg extract, 4.5 µg GlcE/mg extract, 1.14 µg RE/ mg extract and 0.992 µg GAE/ mg extract respectively. Except for total ascorbic acid content which was equal in both aqueous and ethanol extracts (20µg/ mg extract), the phycoconstituents of the aqueous extract were higher than of the ethanol extract. the aqueous extraction method was more efficient than the ethanol extraction method.

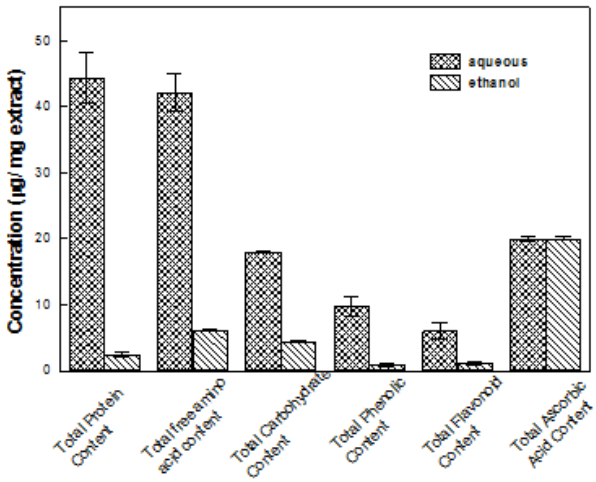

Fig-1: Figure showing the in aqueous and ethanol extracts. Values are represented as mean of the triplicate values ± standard deviation

The phycobiliproteins were estimated wherein phycoerythrin was the highest at 0.108 mg/ml followed by phycocyanin at 0.032 mg/ml and allophycocyanin at 0.006 mg/ml. the chlorophyll a content was higher at 1.203 mg/ml as compared to carotenoids at 0.723 mg/ml.

Table-2: Quantization of chlorophyll a, phycocyanin, phycoerthrin, allophycocyanin(mg/ ml)

Phycocyanin (mg/ml)	0.032 ± 0.007
Allophycocyanin (mg/ ml)	0.006
Phycoerythrin (mg/ ml)	0.108 ± 0.010
Chlorophyll a (mg/ ml)	1.203 ± 0.013
Carotenoids (mg/ ml)	0.723 ± 0.002

DISCUSSION

The study has shown that the aqueous extract contained higher prevalence and quantity of phycoconstituents than ethanolic extract except for vitamin c, chlorophyll and carotenoids. Wide arrays of secondary metabolites which allow the group to dominate under systems of high herbivory and extreme nutrient and light conditions have been isolated from a number of cyanobacterial genera from different geographical locations. Two main reasons to attract the attention of researchers towards these vast structurally diverse cyanobacterial secondary metabolites are acute toxicity of toxins produced by several freshwater cyanobacterial blooms and their harmful effect on animals and human health, and potential therapeutic use of these secondary metabolites. Phenolic compounds have been extensively studied for their antioxidant properties as they act as free radical terminators [14]. The antioxidant and antimicrobial potentials of ethanolic extract of *N. linckia* [8] has been studied and attributed to the β- carotene content. The main functions of chlorophyll and carotenoids are light harvesting andphotoprotection, necessary for the growth of the cyanobacteria by photosynthesis. The major carotenoids in cyanobacteria are β- carotene; its hydroxyl derivatives, zeaxanthin and nostoxanthin; its keto derivatives, echinone and canthaxanthin; and the carotenoid glycosides, myxol 2'- glycosides and oscillo 2, 2'- diglycosides [15]. Phycobiliproteins, phenols, terpenoids, saponins, tannins and polysaccaharides act as bioactive compounds. Phycocyanin is a water soluble pigment, which is known to exhibit antioxidant, anti-inflammatory, hepato protective effects [16, 17]. The pigments contained in the cells of cyanobacteria are currently used by the food, cosmetic and pharmaceutical industries. The therapeutics of phycobiliproteins like antiplatelet, immunomodulatory, anti hepatoxi, anti-inflammatory, neuroprotective and anti- carcinogenic effects are well explored [18, 19, 20]. The increase in the current demand of consumers for natural products due to the fact that some dyes and synthetic antioxidants may trigger allergic and carcinogenic process[2] has made obtaining pigments and natural antioxidants important.

The aqueous extraction was determined to be a more efficient process for most of the value added nutrients as compared to ethanol extraction. The ethanol extraction however was more efficient in extracting the antioxidant pigments like chlorophyll and carotenoids.

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