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RESEARCH ARTICLE

BIOCHEMICAL ANALYSIS OF CYANOBACTERIAL SPECIES ISOLATED FROM PADDY FIELDS OF WARANGAL DISTRICT, TELANGANA STATE, INDIA

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ABSTRACT

The aim of the present work was to study the biochemical constituents of four species of Cyanobacteria isolated from paddy fields of Warangal district. The biochemical constituents were analyzed in terms of chl-a, carotenoids, total proteins, total carbohydrates and phycobiliproteins content of four Cyanobacterial species, *Anabaena circinalis, Nostoc punctiforme, Oscillatoria princeps* and *Phormidium mucosum* were analysed. The analysis showed that maximum chlorophyll-a content in *A.circinalis* (2.52±0.06) and minimum in *P. mucosum* (1.23±0.43), whereas the maximum carotenoids content in *O.princeps* (1.57±0.01) and minimum in *A.circinalis* (0.98±0.14), the maximum amount of total protein in *N.punctiforme* (2.89±0.09) and minimum in *P. mucosum* (1.98±0.07), also maximum amount of total carbohydrate in *A.circinalis* (3.21±0.25) and minimum in *P. mucosum* (2.07±0.09), whereas the maximum Phycocyanin content in *O.princeps* (5.08±0.12) and minimum in *P. mucosum* (3.45±0.02), the highest allophycocyanin content in *P. mucosum* (3.94±0.51) and lowest in *O.princeps* (1.05±0.01), and the maximum Phycoerythrin content in *N.punctiforme* (3.56±0.71) and minimum in *O.princeps* (1.02±0.57) respectively. The results of this experiment revealed that in food, pharmaceutical, cosmetics industries and biotechnological applications.

Key words: Anabaena circinalis, Nostoc punctiforme, Oscillatoria princeps and Phormidium mucosum Cyanobacteria, Biochemical analysis

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INTRODUCTION

Cyanobacteria are the oxygenic, photosynthetic diverse gram-negative prokaryotes are found in almost aquatic and terrestrial habitats. Cyanobacteria occupy almost every niche of the earth, including fresh and salt waters, paddy fields, hot springs, arid deserts, and polar regions (Thajuddin and Subramanian, 2005**,**Wilkie *et al.*, 2011).Their diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkylophilic, planktonic to barophilic, fresh water to marine including hypersaline (salt pans). Cyanobacteria are an ancient group of prokaryotic with the ability to perform functions like nitrogen fixation and photosynthesis. The capacity of several Cyanobacteria to fix atmospheric nitrogen is a significant biological process of economic importance (Santra, 1993). Rice is the most widely grown food grain crop; it serves as the staple food for about half of the population in world. Cyanobacteria in recent years have application in various fields like biotechnology, pharmacology, agriculture etc (Fatma *et al*., 1994).

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The presence of bioactive compounds from Cyanobacteria has possesses biological activities, such as antibacterial, antifungal, antiviral, anti-neoplastic, antifouling, antioxidant, anti-inflammatory, anticoagulant, antienzymatic, cytotoxic and anticancer activities (Ayyad *et al*., 2003; Rodriguez *et al*., 2008; Rath and Priyadarshani, 2013; Shaieb *et al*., 2014; Carvalho *et al*., 2013; Reginald and Darling, 2015; Schaeffer and Krylov, 2000)**.** Cyanobacteria are recognized to be prolific producers of bioactive compounds drawing interests as a source of various nutraceuticals, biomass and pigments (Cifferi *et al.*, 1985; Pulz *et al.*, 2004; Tan, 2007; Cardozo *et al*., 2007). This work aimed to obtain cyanobacterial isolates from paddy fields, which have the ability to produce the metabolites such as proteins, exopolysaccharides, pigments and carbohydrates which are valuable substances with potential applications in the food, pharmaceutical and cosmetics industries.

MATERIALS AND METHODS

Study Area

Collection of sample and culture condition: The experiments of this work were carried out at Department of Botany, Kakatiya University, Warangal. In this study Water and Soil samples containing Cyanobacterial species were collected from various sites of paddy fields of Warangal district, Telangana state and were kept in the plastic vials to transfer to the lab.

The cyanobacterial samples were cultured directly in inorganic BG-11 media. The cyanobacterial axenic cultures were developed by repeated sub culturing method using BG-11 (Rippka *et al.*, 1979) media with and without combined nitrogen. Purified Cyanobacteria cultures were transferred into 250 ml conical flasks containing 100 ml of inorganic BG-11 medium with pH 7.2 and incubated under controlled conditions of continuous fluorescent white light 3000 Lux at 26 ± 2ºC in16:8 (light: dark) photoperiod regime. After 28 days, the cultures were harvested for the analysis of biochemical studies.

Identification of Cyanobacterial species: Identification of cyanobacterial species were done microscopically based on morphological as well as taxonomical observation, the length and the width of the vegetative cells also the width of the sheath, type of spores, presence or absence of hormogonia, presence or absence of spores and its position, number of heterocysts and its repetition, presence of akinetes and its type, the nature of cell wall, presence or absence of gas vacuoles, as well as pigment color was taken in consideration according to standard monographs (Desikachary, 1959; Santra, 1993). The photomicrographs were taken using with a fluorescent microscope. The obtained species were identified as *Anabaena circinalis, Nostoc punctiforme, Oscillatoria princeps* and *Phormidium mucosum* shown in (Figure-1).

Estimation of Total Proteins

The estimation of total protein was done by the method of Lowry *et al.*, (1951). The cultures were centrifuged at 7000 xg for 10 minutes. From the pellets 20 mg was treated with 10% TCA and centrifuged at 10,000 rpm for10 minutes. The resulting pellet was resuspended in 0.1NNaOH and boiled for 30 minutes, cooled and then centrifuged to eliminate light scattering materials. The supernatant was made up to known volume. To 0.1 ml of supernatant, 0.9 ml of distilled water and 5 ml of alkaline copper reagent were added and allowed to stand for 10minutes, finally 0.5 ml of Folin-ciocalteau reagent added. The absorbance was measured after 30 minutes in spectrophotometric readings at 750 nm against the Bovin Serum Albumin as blank.

Estimation of Total Carbohydrates: The total carbohydrate was determined according to Dubois *et al.,* (1956). 10 ml algal suspension was centrifuged at 7000 rpm for 10 minutes. The supernatant was discarded and 20 mg of pellets was taken in a test tube. Then it was hydrolyzed with 2 ml of concentrated H_2SO4 were added and mixed thoroughly and tubes placed in water bath maintained at 25 ^{0}C for 30 minutes. The color developed was measured at 490 nm using UV-visible spectrophotometer against glucose as blank.

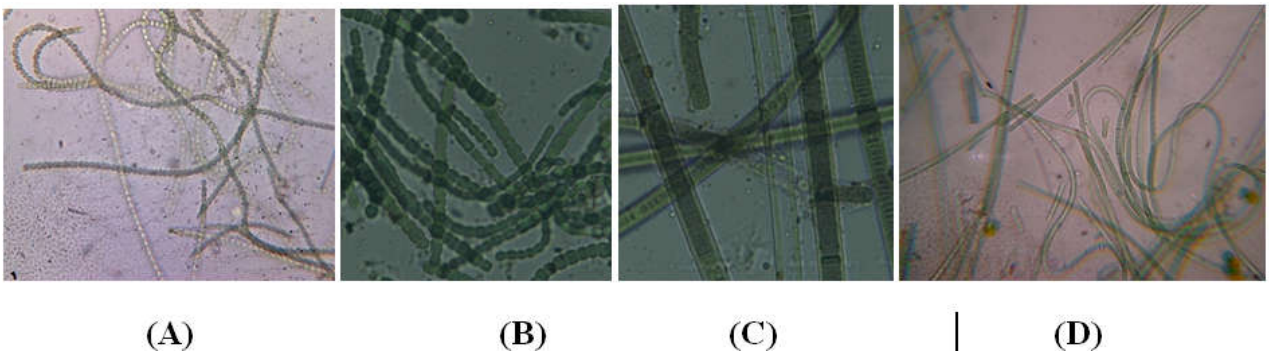

Figure 1. Cyanobacterial species, (A): *Anabaena circinalis*, (B): *Nostoc punctiforme*, (C): *Oscillatoria princeps,* (D): *Phormidium mucosum*

Biochemical analysis

Estimation of Chlorophyll-a: Estimation of chlorophyll-a was determined by adapting the method described by Mckinney (1941). 10 ml of homogenized Cyanobacterial suspension was centrifugation at 7000 rpm for 10 min and then discarded the supernatant and transferred the cyanobacterial pellet to a test tube and added 10 ml of 90% methanol. Shake the contents and placed the tubes covered with aluminium foil in a water bath at 60˚C for 30 min. The absorbance from supernatant was measured at 665 nm using UV-visible spectrophotometer against methanol as blank.

Estimation of Carotenoids: Estimation of total carotenoids was determined by the method described by Jensen (1978). 10 ml homogenized Cyanobacterial suspension was taken and centrifuged at 4000 rpm for 10 min. Discarded the supernatant and added 3 ml 85% acetone and subjected to repeat freezing and thawing until the pellet becomes colorless. Measured the volume of the extract and make up the final volume upto 10 ml with 85% acetone and read the O.D. at 450 nm using 85% acetone as blank and calculated the total amount of carotenoids in µg/ml using UV-visible spectrophotometer.

Estimation of Phycobiliproteins

Estimation of phycobiliproteins was determined by the method described by Bennett and Bogorad (1973). 10 ml Cyanobacterial biomass was homogenized and centrifuged at 7000 rpm for 10 min. The pellets were suspended in 5 ml of 0.05 M phosphate buffer (pH=6.8). The contents were repeatedly freezed in 4ºC and thawed at room temperature. The supernatants containing pigments were pooled and the absorbance was measured at 562 nm, 615 nm and 652 nm against phosphate buffer as blank by using UV-visible spectrophotometer for phycocyanin, allophycocyanin and phycoerythrin respectively.

Statistical Analysis: The obtained data were statistically analyzed and the results were expressed as mean ± standard error (SE) of three independent replicates (n=3) by using Graph Pad Prism version 5.03 (Graph Pad Software, Inc.,).

RESULTS

Four Cyanobacterial species were isolated from paddy fields which were *Anabaena circinalis, Nostoc punctiforme, Oscillatoria princeps* and *Phormidium mucosum* under the 4

genera including the two heterocystous and two non-heterocystous forms. The biochemical analysis of Chlorophyll-a, Carotenoids, Protein, Carbohydrates, and phycobiliproteins content of four Cyanobacterial species were presented in (Table-1). The Chlorophyll-a had showed a very significant content. The maximum Chlorophyll-a present in *A. circinalis* (2.52±0.06), followed by *N. punctiforme* (2.01±0.34), *O. princeps* (1.82±0.21), and minimum in *P. mucosum* (1.23±0.43). The Carotenoid was found to be highest content in *O. princeps* (1.57±0.01) followed by *P. mucosum* (1.25±0.87), *Nostoc punctiforme* (1.20±0.25) and the lowest content was found in *Anabaena circinalis* (0.98±0.14). The maximum protein content was found in *N. punctiforme* (2.89±0.09) and minimum in *P. mucosum* (1.98±0.07).

Table 1. Biochemical constituents of four Cyanobacterial strains collected from paddy fields of Warangal district

Cyanobacterial Species	Chlorophyll a (μgml^{-1})	Carotenoid (μgml^{-1})	Protein (μgml^{-1})	Carbohydrate (μgml^{-1})	Phycocyanin (μgml^{-1})	Allo Phycocyanin (μgml^{-1})	Phycoerythrin (μgml^{-1})
Anabaena circinalis	2.52±0.06	0.98±0.14	2.56±0.32	3.21±0.25	4.96±0.06	2.53±0.72	2.12±0.03
Nostoc punctiforme	2.01±0.34	1.20±0.25	2.89±0.09	3.10±0.23	3.85±0.01	3.22±0.86	3.56±0.71
Oscillatoria princeps	1.82±0.21	1.57±0.01	2.25±0.23	2.37±0.02	5.08±0.12	1.05±0.01	1.02±0.57
Phormidium ucosum	1.23±0.43	1.25±0.87	1.98±0.07	2.07±0.9	3.45±0.02	3.94±0.51	2.54±0.12

Values are the means of three replicates ± standard error

The protein content present in *A. circinalis, O. princeps* were (2.56±0.32 and 2.25±0.23) respectively. The carbohydrate content was highest in *A. circinalis* (3.21±0.25) followed by *N. punctiforme* (3.10±0.23), *O. princeps* (2.37±0.02) and *P. mucosum* showed lowest content (2.07±0.09). The phycocyanin (PC) content had shown very significant higher content in *O. princeps* (5.08±0.12) followed by *A. circinalis, N. punctiforme* (4.96±0.06 & 3.85±0.01), similarly the minimum in *P. mucosum* (3.45±0.02) respectively. Likewise the allophycocyanin (APC), the highest content was recorded in *P. mucosum* (3.94±0.51) followed by *N. punctiforme* (3.22±0.86) and *A. circinalis* (2.53±0.72). However the lowest content of allophycocyanin was observed in *O. princeps* (1.05±0.01). The Phycoerythrin (PE) content was maximum in *N. punctiforme* (3.56±0.71), followed by (2.54±0.12 & 2.12±0.03) in *P. mucosum* and *A. circinalis.* Whereas the lowest content of PE was found in *O. princeps* (1.02±0.57) under the study.

DISCUSSION

The results of the biochemical analysis of Cyanobacteria isolated from paddy fields showed the high amount of biochemical contents. In the present study the highest Chlorophyll-a was present in the *A. circinalis.* Result indicated that chlorophyll-a content increased with increasing incubation period. Similarly El Sheekh *et al*., (2015) recorded highest Chlorophyll-a in *N. calcicola, A. variabilis* and *N. linkia* and Amalina and Jayashree (2017) reported maximum Chlorophyll-a in the *L.holdenil*. The highest Carotenoid content was recorded in *O. princeps* followed by *P. mucosum*, *N. punctiforme* respectively. The results are in agreement with Bakiyaraj *et al*., (2014), Narayanan *et al*., (2006) who stated that the maximum carotenoids content in *O. pseudogeminata* and *Nostoc* respectively. In the present study the level of protein and carbohydrate were observed in *A. circinalis, N. punctiforme, O.princeps* and *P. mucosum.* From the study, maximum protein content was observed in *N. punctiforme.* Similarly Thamizh and Sivakumar, (2012) suggests that the results on total soluble proteins and total carbohydrates increased. The protein content was more than the carbohydrates in *A. aconstricta*. But in *O. curviceps*, the level of total carbohydrates was high when compared with total protein. Gribovskaya *et al.,* (2009) reported that the protein and carbohydrates were found in the *Oscillatoria* sp. Mishra *et al*., (2004) reported that the protein and carbohydrates were present in the *Anabaena* sp. and *Calothrix* sp. in the soil cyanobacteria. The present study shows the level of phycocyanin was more than allophycocyanain and phycoerythrinin in the present investigation. The highest Phycocyanin pigment was observed in *O. princeps.* Badrish *et al.*, (2006) reported similar type of result of phycocyanin in *Oscillatoria* sp. The highest allophycocyanin content was recorded in *P. mucosum.* Similarly, Narayan *et al*., (2006) reported that the highest content of allophycocyanin presented in *Anabaena,* whereas the maximum content of Phycoerythrin present in *N. punctiforme* while Narayan *et al*., (2006) reported in *Nostoc* and *Calothrix*, Phycoerythrin was highest observed and *N.calcicola* showed highest Phycoerythrin content (El Sheikh *et al*., 2015) respectively. Moreover the optimum contents of Phycocyanin, phycoerythrin, and allophycocyanin reported by (Tyagi *et al*., 1980). In the present study, carbohydrate and protein are found to be highest in *A. circinalis* and *N.punctiforme* and also lowest in *P.mucosum*, respectively.

Conclusion

The present study reveals that the four Cyanobacterial strains isolated from paddy fields of Warangal district, are demonstrated to be a rich source of chlorophyll-a, carotenoids, proteins, carbohydrate and phycobiliproteins. Due to rich biochemical contents these Cyanobacterial strains may have the potential use for in the agricultural, food industry and biotechnological applications.

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REFERENCES

Amalina Paul and Jayashree Rout, 2017. Biochemical evaluation of some Cyanobacterial strains isolated from the lime sludge wastes of a Paper Mill in Southern Assam (India). *Phykos*., 47(1):8-15.

Ayyad, S., Abdel-Halim, O., Shier W.T. and Hoye, TR. 2003. Cytotoxic hydroazulene diterpenes from the brown alga *Cystoseira myrica*. Z. *Natuforsch C Biosci*., 58: 33-38.

Badrish, S., Beena, K., Ujjval, T. and Datta, M. 2006. Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata*-Isolated from the rocky shores of Bet-Dwarka,Gujarat. *India Process Biochem.,* 41: 2017-2023.

Bakiyaraj, R., Baskaran, L. and Senthilkumar, T. 2014. Isolation and identification of cynobacteria (*Oscillatoria*

pseudogeminata G.schmid) from marine water and its potential on remediation of pesticide. *Int. J. Curr. Microbiol. App.Sci.,* 3 (3):256-267.

Bennett, A. and Bogorad, L. 1973. Complimentary chromatic adaptionin a filamenttous blue-green alga. *J. Cell Biol*, 58: 419-435.

Cardozo, K.H.M., Guaratini, T., Barros, M.P., Falcao,V.R., Tonon, A.P., Lopes, N.P., Campos, S., Torres, M.A., Souza, A.O., Colepicolo, P. and Pinto, E. 2007. Metabolites from algae with economical impact. *Com. Biochem. Physiol.,* 146: 60-78.

Carvalho, L.R.A. and Costa-Neves, G.A.A. 2013. Conserva, Biologically activecompounds fromcyanobacteria extracts: *invivo* and *invitro* aspects, *Braz.J.Pharma*., 23(3): 471-480.

Ciferri, O, and Tiboni, O. 1985. The biochemistry and industrial potentials of *Spirulina*. *Annu. Rev. Microbiol.,* 39: 503-526.

Desikachary, T.V.1959. *Cyanophyta.* Indian Council of Agricultural Research. New Delhi. pp.1-686.

Dubois, M., Gilles, R.A., Hamilton, F.K., Robers, P.A. and Smith, F. 1956. Calorimetric method for determination of sugar and related substances. *Anal. Chem*., 28: 350-356.

El Sheekh, M.M., Zayed, M.A., Faiza, Elmossel. K.A. and Reham Hasan, S.A. 2015. Isolation, identification and biochemical compounds of Cyanobacteria isolatesfrom saline soils in kafr el-sheikh governorate**.** *J.Agric.Chem.and Biotechn.,* Mansoura Univ.Vol. 6 (9): 331- 344.

Fatma, T., Sarada, R. and Venkataraman, L.V. 1994. Evaluation of selected strains of *Spirulina* for their constituents. *Phykos,* 33: 89 -97.

Gribovskaya., Kalacheva, G.S., Bayanova, Yu I. and Kolmakova, A.A. 2009. PhysiologyBiochemical Properties of the Cyanobacterium *Oscillatoria deflexa. Appl. Biochem and Microbiol.,* 45(3): 285-290.

Jensen, A. 1978. Chlorophylls and carotenoids. In: Hellebust JA, Craigie JS(eds) Handbook of phycological methods, physiological and biochemicalmethods. *Cambridge University Press,* Cambridge, pp. 59-70.

Lowery, O.H., Rosebrough, N.J., Fair, A.L. and Randall, R.J. 1951. Protein measurement with the folin-phenol reagent. *J. Biochem,* 193: 269- 275

Mackinney, G. 1941. Absorption of light by chlorophyll solutions. *J Biol Chem.,* 140:315-322.

Mishra, U., Pabbi, S., Dhar, D.W. and Singh, P.K. 2004. Floristic abundance and comparative studies on some specific nitrogen fixing blue green algae isolated from soil of J&K state. *Ad. Plant.Sci.,* 17(3): 635-640.

Narayan, K.P., Shalini., Saurabh, Tiwari., Sunil Pabbi. and Dolly Wattal Dhar, 2006. Biodiversity analysis of selected Cyanobacteria. *Curr. Sci*., 91 (7): 947-951.

Pulz, O. and Gross, W. 2004. Valuable products from biotechnology of microalgae. *Appl. Microbiol. Biotech*., 65:635-648.

Rath, B. and Priyadarshani, I. 2013. Antibacterial and antifungal activity of marine cyanobacteria from Odisha coast*. Int. J .Curr .Trends. Res.*, 2: 248-251.

Reginald, Appavoo, M., Darling Femi G., 2015. Eurihaline Microalgae-A Novel substance againstpost operative pathogen*. In.t J. Dev. Res.*, 5(1): 3030-3033.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria*. J. Gen. Microbiol,* 111: 1- 61.

Rodriguez-Meizoso, I., JaimeL, Santoyo, S., Cifuentes, A., Garcia-Blairsy, R.G., Seniorains, F.J. and Ibainiez, E. 2008. Pressurized fluid extractionofbioactive compounds from *Phormidium* species. *J .Agric. Food. Chem.*, 56: 3517-3523.

Santra, S.C. 1993. Biology of rice fields blue-green algae. Daya publishing House. Delhi. 110035.pp-1-202.

Schaeffer, D.J. and Krylov, V.S. 2000. Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicol .Environ. Saf*., 45: 208-227.

Shaieb, F A., Issa, A.A. and Meragaa, A. 2014. Antimicrobial activity of crudeextracts of cyanobacteria *Nostoc commune* and *Spirulina platensis*. *Arch BiomedSci.,* 2: 34-41.

Tan, L.T. 2007. Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochem,* 68: 954-979.

Thajuddin, N. and Subramanian, G. 2005. Cyanobacterial biodiversity andpotential applications in biotechnology. *Curr.Sc.i*, 89: 1- 10.

Thamizh, Selvi, K. and Sivakumar, K. 2012. Ultrastructure and biochemical analysis of *Anabaena* and *Oscillatoria* sp. (Cyanobacteria). *Int. J. Rec. Sci. Res.,* 3 (5): 329- 335.

Tyagi, V.V.S., Mayne, B.C. and Peters, G.A.1980. Purification and initial characterization of phycobiliproteins from the endophytic cyanobacterium of *Azolla. Archives of Microbiol.,* 128 (1):41-44.

Wilkie, AC., Edmunson, SJ. and Duncan, JG. 2011. Indigenous algae for local bioresource production: phycoprospecting. *Energy for Sustainable Development*, 365-371.

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