ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (261-270), Month: July - September 2018, Available at: www.researchpublish.com

Studies on cyanobacteria: *Spirulina* isolated from Satara district, Maharashtra

[1]Priyadarshani Kamble, [2]Vanita Karande

[1]Asst. Prof., Department of Botany
[1]S. M. Dr. Bapuji Salunkhe College, Miraj, India
[2]Asso. Prof., P.G.Department of Botany
[2]Y. C. Institute of Science, Satara
[1]Email: kpriyasharu@gmail.com

Abstract: **The aim of this work is to study occurrence of genus *Spirulina* and biochemical composition of *Spirulina subsalsa* isolated from various localities of Satara district, Maharashtra. Four species of *Spirulina* were recorded from the study area viz., *Spirulina subsalsa* Oersted ex Gomont, *Spirulina meneghiniana* Zanardini ex Gomont, *Spirulina major* Kutzing ex Gomont and *Spirulina gigantea* Schmidle. Out of these *Spirulina subsalsa* was cultured in conical flasks under lab conditions. Algal biomass was harvested at its exponential phase. Fresh as well as dried biomass was subjected to biochemical analysis. The metabolites considered for biochemical analysis were pigments viz., chlorophyll a, carotenoids and Phycobiliproteins; carbohydrate, protein, lipids, Nitrate reductase (NR) activity, Total flavonoid content (TFC),Total Phenolic content (TPC),Total antioxidant capacity (TAC) and Vitamin C. It showed significant amount of chlorophyll- a i.e. 6.510(mg/ml) with 3.800 (mg/ml) carotenoid content. Total phycobiliproteins recorded was 5.60 (mg/ml) with 0.800 (mg/ml) Phycoerythin, 1.800 (mg/ml) Phycocyanin and 3.00(mg/ml) allophycocyanin respectively. It showed 8.00 % (dry wt.) Carbohydrates, 49.00 % (dry wt.) proteins and 25.00% (dry wt.) lipids which makes it superfood. In addition Total flavonoid content (TFC) recorded was 3.970 (mg./g equivalent of Rutin);Total Phenolic content (TPC) 0.305(mg./g GAE),Total antioxidant capacity (TAC) 0.525(mg./g AAE), and Vitamin C 0.452(mg./g). *Spirulina subsalsa* have been found with promising biochemical characterization which may be exploited further in future.**

Keywords: **Biochemical analysis, Cyanobacteria, Satara, *Spirulina subsalsa.***

1. INTRODUCTION

Blue green algae which are now famously called as “Cyanobacteria” are diverse group of Gram negative organism which have originated 3.5 billion years ago. Since then they are serving us by means of their photosynthetic activity, nitrogen fixing ability and by producing number of valuable biomolecules. *Spirulina* is filamentous edible blue green micro alga belonging to family Oscillatoriaceae. It has typical spiral filamentous thallus with typcal blue green colour. It has received much attention all over the world as potential source of food because of nutritional value of its biomass [14]. *Spirulina* is with high protein and fiber content and therefore used as food source [2], [3]. In addition to the high contents of proteins, it is rich in vitamins, polyunsaturated fatty acids and phycocyanin, β carotene and chlorophyll pigments that have been used as food and drink, cosmetic and pharmaceutical colorants [7], [11], [36]. Diversity in its biological and chemical properties have promoted this genus as food for future [5], [6], [20], [29].

There is great interest of scientists all over the world in culturing microalgae and cyanobacteria even though it is estimated that very few algal species have been studied for their physiology and their potential as producers of biocompounds [34]. But cultivation and processing of *Spirulina* is a difficult task. In this present research an attempt has been made to cultivate and biochemical analysis of *Spirulina subsalsa*. Cyanobacteria are very sensitive to fluctuations in environmental conditions such as light, salinity, temperature and nutrient limitation in natural habitats [40]. Growth and cellular composition of *Spirulina* is affected by cultural conditions.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (261-270), Month: July - September 2018, Available at: www.researchpublish.com

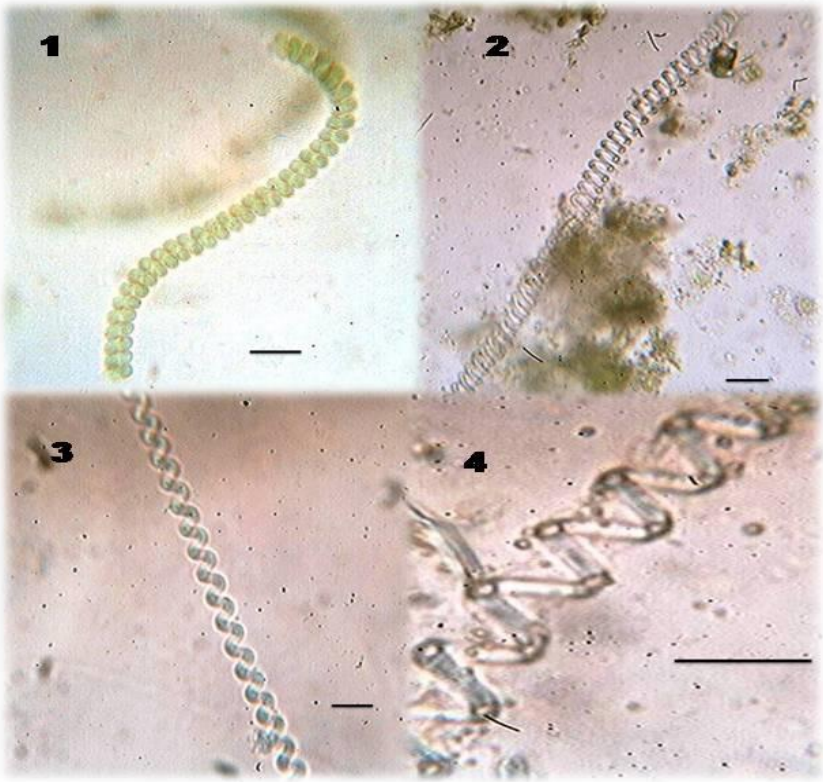
Increasing population and limitations in cultivable land area led the search of new food resources. Therefore, the aim of present work was to cultivate and evaluate the biochemical properties of *Spirulina subsalsa* in order to prove its potential for future use.

2. MATERIALS AND MATHODS

A) Sample collections and isolation:

Cyanobacterial samples were collected from all possible localities of Satara district of Maharashta and brought to the laboratory. Primary observations were made for their identification using standard literature [13]. Filamentous forms were picked up and transferred to sterile distilled water. In order to have the proper isolation the samples were serially diluted and these dilutions were used for the inoculation in BG-11 media [35]. Plates were incubated at a temperature of 22°C±1 under a 16:8 (light: dark) photoperiod at a light intensity of 2000-3000 lux. Culture of *Spirulina* were made unialgal by repeated streaking on agar plates. Forms of *Spirulina* from the cultures were mostly identified upto the species level by using the literature provided [22], [23], [35]. Axenic cultures were maintained in triplicates in 2 L flasks containing BG-11 media [35].

Plate I: Microphotographs of *Spirulina species*



1.*Spirulina subsalsa* Oerst. ex Gomon 2.*Spirulina meneghiniana* Zanard ex Gomont
3.*Spirulina major* Kutz. Ex Gomont 4.*Spirulina gigantea* Schmidle
Bar= 10µm

B) Determination of log phase and harvesting of biomass:

Axenic cultures of *Spirulina* were examined to record their log phase during growth in cultural conditions. Chlorophyll-a was measured as growth index of the cultures after regular interval of time (two days).

Biomass was harvested during their exponential growth phase or log phase aseptically by filtration and the traces of media were removed by frequently washing the culture by sterile distilled water. Algal biomass collected on the filter paper was dried in oven at 45^0C for 36 hrs. This dried powdered mass further used for further biochemical analysis.

C) Biochemical analysis

1. Pigments:

Chlorophyll- a

Chlorophyll-a was determined according to Mackeny [41]. The algal culture was homogenized. 10ml from homogenized culture was taken and centrifuged for 5min at 3000rpm. Algal pellet obtained was suspended in 10ml of 90% acetone. The contents were shaken thoroughly and kept in a refrigerator for 20-24 hrs for complete extraction. After complete extraction the samples were removed from the refrigerator, shaken well and centrifuged at 3000 rpm for 5 min. Supernatant was taken and the volume was made up with 90% acetone, if necessary. The optical density (O.D.) was recorded at 665, 645 and 630 nm using 90% acetone as blank.

Formula used: Chl. a = 11.6(O.D.665) – 1.31(O.D.645) – 0.14(O.D.630)

Carotenoides

Carotenoides were measured according to method of Kirk and Allen [21]. 10 ml homogenized algal suspension was taken and centrifuged at 3000 rpm for 10 min. Then 2-3 ml of acetone (85%) was added to the pellet and grinded in a homogenizer. Homogenized mixture was centrifuged to obtain the supernatant containing pigment. Supernatant was collected and stored at 4^0C. The same process was repeated till the colorless supernatant acetone is obtained. All the fractions of supernatants were pooled. Final known volume (10 ml) was made. The O. D. was recorded at 450nm using 85% acetone as blank. Carotenoides were quantified as mg/ml

Formula used: $\frac{D \times V \times F}{2500 \times 100}$

Phycobiliproteins

Phycobiliproteins were measured according to method of Bennet and Bogorad [8].

Culture was homogenized to make algal suspension.10 ml algal suspension was taken and centrifuged. 10 ml of cell suspension was taken and centrifuged at 4000rpm for 10 min to obtain the pellet. Equal volume (10 ml) of 0.05 M phosphate buffer was added to the pellet. Pellet was subjected to repeated freezing and thawing for the complete extraction of phycobilins from pellet.

The absorbance was recorded at 562 nm, 615 nm and 652 nm. The phycobilins were quantified by using the formulae:

Phycocyanin (mg/mL) = $\frac{A615 - 0.474 - A652}{5.34}$

Allophycocyanin (mg/mL) = $\frac{A652 - 0.208 \times A615}{5.06}$

Phycoerythrin (mg/mL) = $\frac{A562 - [2.41\ (PC) - 0.849\ (APC)]}{9.62}$

2. Total carbohydrate:

50 mg of dried cyanobacterial biomass was hydrolyzed with 2.5N hydrochloric acid at 100ºC for one hour to prepare the extract. The total carbohydrate was determined using sulphuric acid [15].

0.2 ml sample of the extract was transferred to an assay tube containing 1.0 ml of 5% phenol and 5.0 ml of concentrated sulphuric acid. The tubes placed in water bath maintained at 25^0C for 30 minutes. The samples were then analyzed at 488 nm using UV – visible spectrophotometer against the blank and compared with the standard glucose solution.

3. Total protein

The total protein content was determined by Lowry's method [25]. The cyanobacterial proteins were precipitated by hot 6% trichloro acetic acid, extracted with 4.5 ml hot (55^0C) alkaline reagent (i.e., 2% alkaline Na2CO3 and CuSO4 – Na - K tartarate solution) for 3 minutes. The filtrate was collected and the volume was made up to 5.0 ml with alkaline reagent. 0.5 ml of Folin-Ciocalteau reagent was added and mixed rapidly and allowed to stand for 10 minutes at room temperature. After the color development absorbance was recorded at 650nm and compared with the standard calibration curve prepared from bovine serum albumin.

4. Total lipid

Total lipid content was determined according to Bligh and Dyer [9]. 50mg of dried cyanobacterial powder was taken in test tube. To this Chloroform: Methanol solution (1:2 proportion) was added and blended carefully. After blending 10 ml of chloroform was added with equal amount of distilled water and filtered through Whatman filter paper to remove cell debris. Filtrate with two distinct phases of solvents was poured into Buchner funnel to separate the layers. Methanolic layer was aspirated using high temperature. Chloroform layer was collected in pre-weighed bottle which contained lipids. It was dried in an evaporator and final weight was taken. The difference between the initial weight and final weight gave the total lipid content.

5. Estimation of Nitrate reductase (NR) activity

Nitrate reductase (NR) activity was determined according to Lowe and Evans [24]. 10 ml homogenized algal suspension was centrifuged at 4000 rpm for 10 minutes to obtain the pellet. Pellet was washed with sterile water and incubated overnight in BG-11 medium containing 10mM NaNO3. After overnight incubation, one ml of sample was taken and 2 ml of 1% sulphanilamide in 1N HCl was added. 2ml of 0.2% NEDD was added to the reaction mixture after 15 minutes. After development of pink color absorbance was recorded at 540 nm. The values were calibrated against the standard curve using NaNO2. The NR activity was expressed in terms of µ mole NO_2/ml.

6. Total flavonoid content (TFC)

Total flavonoid content (TFC) was determined using $AlCl_3.6H_2O$ [26]. Reaction mixture was prepared by adding 1.5 ml algal extract into 1.5 ml 2 % $AlCl_3.6H_2O$ (In Methanol). Reaction mixture was shaken vigorously and incubated at room temperature for 10 minutes. Samples were prepared in triplicates. Absorbance was taken at 368 nm on UV- Visible spectrophotometer. Results were compared with calibration curve which was prepared using standard solution of Rutin (0.1mg/ml). Total Flavonoid content was calculated in terms of Rutin equivalents (RE)/gm of sample.

7. Total Phenolic content (TPC)

Total Phenolic content (TPC) was determined according to Wolfe *et al.* [43]. Reaction mixture was prepared by adding 0.125 ml algal extract into equal amount of Folin-Ciocalteu reagent. Then 1.25 ml of saturated Na_2CO_3 was added to reaction mixture and shaken vigorously and incubated at room temperature for 90 minutes. Absorbance was taken at 760 nm on UV- Visible spectrophotometer. Results were compared with calibration curve prepared using standard solution of Gallic acid (0.1mg/ml). Total Phenolic content was expressed in terms of Gallic acid equivalents (GAE)/gm of sample.

8. Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) was determined using TAC reagent [33].

Reaction mixture was prepared by adding 0.3 ml algal extract into 3 ml Total Antioxidant Capacity (TAC) reagent. Reaction mixture was shaken vigorously and incubated at room temperature for cooling. Absorbance was taken at 695 nm on UV- Visible spectrophotometer. Results were compared with calibration curve prepared using standard solution of Ascorbic acid (0.1mg/ml). Total Antioxidant Capacity was expressed in terms of equivalents Ascorbic acid per gm of sample.

9. Vitamin C

Vitamin C content was determined according to Omaye [32]. Reaction mixture was prepared by adding 1 ml of algal extract into 0.2 ml of DTC reagent and incubated for 3 hrs. at 37^0C. Then 1.5 ml of 65% sulphuric acid was added and allowed to stand for 30 min. at room temperature. After development of colour absorbance was taken at 520 nm on UV-

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (261-270), Month: July - September 2018, Available at: www.researchpublish.com

Visible spectrophotometer. Results were compared with calibration curve which was prepared using standard solution of Ascorbic acid (0.1mg/ml). Amount of vitamin C was expressed in mg/ gm of sample.

3. OBSERVATIONS

Taxonomic position of genus *Spirulina* Turpin ex. Gomont

According to Desikachary[13].

Division:Cyanophyta

Class: Cyanophyceae Sachs

Order: Nostocales Geitler

Family: Oscillatoriaceae

According to Komarek *et al.* [23].

Division:Cyanophyta

Class: Cyanophyceae Sachs

Order: Spirulinales

Family: Spirulinaceae

1. *Spirulina subsalsa* Oersted ex Gomont (Plate:I; Fig.1)

: Desikachary [13].

Trichome 1-1.5µm broad, blue green, regularly coiled, spiral close to each other, spirals 4.5-5µm broad

Locality : Koregaon-Vasana river, Panchwad-Krishna river (River bank), Kas lake(Bank of lake), Petri (moist soil), Mahuli- Krishna river (River bank)

2. *Spirulina meneghiniana* Zanardini ex Gomont (Plate: I ;Fig. 2)

(Plate :V ; Fig. 3)

: Desikachary [13].

Trichome 1.7µm broad, flexible, spirally coiled, bright blue green, spiral 5-5.8 µm broad, spirals 1.5 µm distant.

Locality : Vaduj –Yeralwadi, Maam-Divad (pond)

3. *Spirulina major* Kutzing ex Gomont (Plate: I ;Fig. 3)

: Desikachary [13].

Trichome 1.4-2µm broad, regularly spirally coiled, bright blue green colored, spiral 3.5-5µm broad, spirals 3-4 µm distant.

Locality : Asale (moist soil along Krishna river bank), Mardhe (Krishna river bank), Kidgaon, Dhamner, Dewapur (Bank of streams)

4. *Spirulina gigantea* Schmidle (Plate : I ; Fig. 4)

: Desikachary [13].

Trichome 3µm broad, regularly spirally coiled, deep blue-green colored, end part conical attenuated, spiral 11-12 µm broad.

Locality : Vaduj -Yeralwadi (pond)

TABLE I: Biochemical characterization of *Spirulina subsalsa*

Sr. No.	Parameter		Content
1	Chl-a		6.510(mg/ml)
2	Carotenoids		3.800(mg/ml)
3	Phycobiliproteins	PE	0.800(mg/ml)
		PC	1.800(mg/ml)
		APC	3.000(mg/ml)
4	Total PCB		5.600(mg/ml)
5	Carbohydrates		8.00(% dry wt.)
6	Proteins		49.00(% dry wt.)

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (261-270), Month: July - September 2018, Available at: www.researchpublish.com

7	**Total lipids**	25.0(% dry wt.)
8	**NR activity**	4.40 µM/ml
9	**Total antioxidant capacity (TAC) AAE**	0.525 mg./g
10	**Total Phenolic content (TPC) GAE**	0.305 mg./g
11	**Total Flavonoid (TFC) equivalent of Rutin**	3.970 mg./g
12	**Vitamin C**	0.452 mg./g

Fig.:1 Pigment composition of *Spirulina subsalsa*

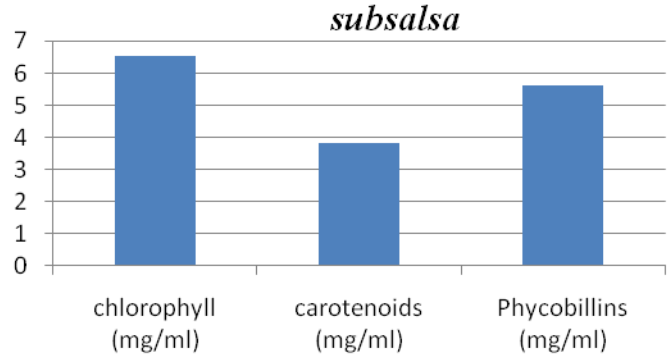


Fig.:2 Carbohydrates, proteis and lipids from *Spirulina subsalsa*

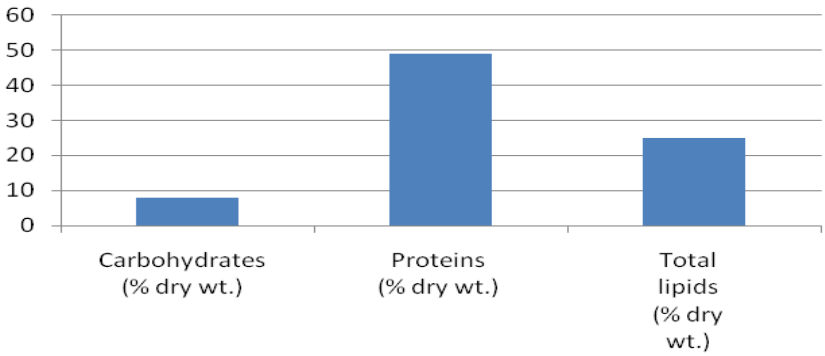
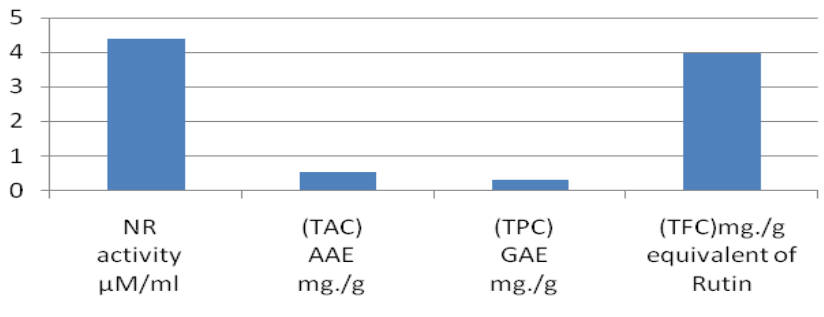


Fig.:3 NR activity, Total antioxident capacity, Total phenolic content, Total flavenoid content from *Spirulina subsalsa*



4. RESULTS AND DISCUSSIONS

Literature survey showed that most of the work was focused on the study of *spirulina platensis* in terms of its culturing and biochemical analysis throughout the world [1], [3], [4], [6], [12], [28], [36]. During our study we have isolated four species of *Spirulina* from the study area viz., *Spirulina subsalsa* Oersted ex Gomont, *Spirulina meneghiniana* Zanardini ex Gomont, *Spirulina major* Kutzing ex Gomont and *Spirulina gigantea* Schmidle. We have cultured *Spirulina subsalsa* in axenic form and carried out its biochemical characterization.

In blue green algae chlorophyll- a is the major light harvesting pigment along with other accessory pigments like carotenoids and phycobillins. These pigments were extracted by using specific organic solvents and quantified in terms of absorbance at particular wavelengths. *Spirulina subsalsa* showed significant amount of chlorophyll- a 6.483 mg/ml. Similar results were recorded in *Spirulina subsalsa* isolated from brackish Lake Faro (Messina, Italy) [40].

The blue green algae are unique photosynthetic organisms in their carotenoids content. Carotenoides protect chlorophyll from photoxidation. β carotenes are most common carotenoid present in blue green algae. Now a days carotenoids are widely used as natural colorant for food, drug and cosmetic products as cyanobacteria are having potential for commercial production of carotenoids [18], [38]. 3.80 mg/ml caroteniod content was recorded in *Spirulina subsalsa.* Our observations differ from observations of Tomaselli [40] who 0.97 to 1.47mg/g of dry wt. It was might be due to difference in climatic conditions.

In *Spirulina subsalsa* phycobilins were measured in terms of Phycoerythin (0.800 mg/ml), allophycocyanin (3.000 mg/ml) and phycocyanin (1.800 mg/ml). Our results coincide with results of [40].

The total amount of carbohydrats recorded in *Spirulina subsalsa* were 7.80 % of dry weight. In *Spirulina platensis* 24% (of dry weight) of Carbohydrates were recorded [1] while in *Spirulina major* 15.56% (of dry weight) carbohydrates were recorded [31].

49.00 % (dry weight) Protein content was recorded in *Spirulina subsalsa.* Maximum amount of protein content was recorded in *Spirulina major* [31]. Gholap and Pingle [17] reported 40.2±1.01%dry weight of proteins in *Spirulina*. 44.1±1.01% dry weight of proteins were reported in *Spirulina* by Mohite [30]. Four strains of Spirulina were analysed for protein content which showed rage from 43-55% of dry weight [16]. Our results are similare to these observations.*Spirulina* is potential protein source and therefore commonly used as food and fodder. Species of *Spirulina* are used as food because of their high protein content. [2],[3].

Total lipid content recorded in *Spirulina subsalsa* was (25.0% dry weight). Ama Moor [1] recorded 30.12% lipids from *Spirulina platensis*.

Spirulina subsalsa showed 4.40 µM/ml NR activity. Jha *et al*., [19] studied induction and inhibition of nitrate reductase in *Spirulina platensis*.

Significant amount of flavonoid was recorded in *Spirulina subsalsa* (3.970 mg/g equivalent of Rutin). Ama Moor [1] recorded total flavonoids (21.2 + 1.18 mg eq. QE/g Ext.) in *Spirulina platensis*.

Amount of phenols was recorded in terms of equivalent of Gallic acid. In *Spirulina subsalsa* 0.305 mg/g equivalent of Gallic acid. According to Ama Moor [1] polyphenols (56.4 + 6.47 mg eq. QE/g Ext. were present in *Spirulina platensis*.

Spirulina subsalsa showed maximum amount of vit C (0.452 mg/ g of vit C). Chakraborty and Bhattacharya [10] recorded Vit C in range from 0.26 to 0.46 mg/g in marine algae. Our results are similar to these results.

Antioxidant capacity in *Spirulina subsalsa* was also recorded (0.525 mg/g equivalent of ascorbic acid). According to Ama Moor [1] total antioxidant capacity in *Spirulina platensis* was 7.5 mg eq. VitC/g Ext.

5. CONCLUSION

This study revealed that *Spirulina subsalsa* has been found with promising biochemical characterization as the major constituents like total carbohydrates, protein and total lipids are significantly higher in *Spirulina subsalsa*. which may be exploited further in future. It also shows presence of vitamins as well as antioxidant activity. According to Vargas *et al*. [42] and Subhashini *et al.*, [37] the biochemical constituents of cyanobacteria depends on the nature of strains, physiological state of culture and environment. Therefore their potential of production of biomolecules can be

manipulated by changing cultural conditions and environmental factors. Therefore in future we can manipulate the biochemical composition of *Spirulina subsalsa* by changing the cultural conditions as per the need of product we are interested. As it is non-toxic, edible blue green algae with many nutritionally important components like carbohydrates, proteins, lipids, vitamins, antioxidants etc. it can become superfood for future. Still more advanced work is needed to improve its yield.

ACKNOWLEDGEMENT

Authors are thankful to Principal, Dr. K. G. Kanade for the encouragement. Our thanks are due to Head of the Department of Botany, for providing necessary facilities.

REFERENCES

[1] Ama Moor V. J., C. A. Pieme, P. C. Nya Biapa, M. E. Ngo Matip, B. Moukette , F. T. Nzufo , P.Nanfack and J. Ngogang, "Chemical Composition Of *Spirulina Platensis* Of Nomayos-Yaounde (Cameroon)", Annals. Food Science and Technology vol.17, no. 2, pp.524-528, 2016.

[2] Anupama P., "Value added food: single cell protein", Biotechnol. Advan. Vol.18, pp. 459–479, 2000.

[3] Anusuya D. , G.Subbulakshmi, D. Madhavi and L. Venkataraman, "Studies on the proteins of mass cultivated blue green alga (*Spirulina platensis*)". J. Agri. Food Chem. Vol.29, pp. 522 – 525,1981.

[4] Baldia, S.F., T.Nishijima, Y. Hata and K. Fukami, "Growth characteristics of a blue–green alga *Spirulina platensis* for nitrogen utilization", Nippon Suisan Gakkaishi, vol, 57, pp. 645–654, 1991

[5] Beeker W., "Microalgae in human and animal" In Richmond A (ed) Handbook of microalgae culture, biotechnology and applied phycology. Blackwell, Oxford, pp.312-352, 2003.

[6] Belay A., "The potential application of *Spirulina* (*Arthospira*) as a nutritional and therapeutic supplement in health management", J. Am. Nutracent. Assoc, vol. 5, pp.27-48,2002.

[7] Belay A., "*Spirulina platensis (Arthrospira*): production and quality assurance". In: Gershwin M.E.; Belay A. (Ed.). Spirulina in human nutrition and health. Taylor & Francis, Portland, USA, pp.2-23, 2008.

[8] Bennet A. and L. Bogorad Complementary chromatic adaptation in a filamentous blue-green algae, The Journal of Cell Biology, vol. 58, pp.419-435,1973.

[9] Bligh E. and W. Dyer, "A rapid method of total lipid extraction and purification", Can J Biochem Physiol, vol. 37, pp. 911-917, 1959.

[10] Chakraborty S. and T. Bhattacharya, "Nutrient composition of marine benthic algae found in the gulf of Kutch coastline, Gujarat, India", Journal of Algal Biomass Utilization, vol. 3, no.1, pp. 32-38, 2012.

[11] Ciferri O.and O.Tiboni, "The biochemistry and industrial potential of *Spirulina*", Ann. Rev. Microbiol., vol. 39, pp. 503-526, 1985.

[12] Cornet J.F., C.G.Dussap and G.Dubertret, "A structured model for simulation of cultures of the cyanobacterium Spirulina platensis in photobioreactors. I. Coupling between light transfer and growth kinetics", Biotechnology and Bioengineering, vol. 40, pp. 817–825, 1992.

[13] Desikachary T.V., "Cyanophyta, ICAR Monographs on Algae", Indian Council of Research, New Delhi, pp. 686, 1959.

[14] Dillion J.C., A.P. Phuc and J.P.Dubacq, **"**Nutritional value of the alga *Spirulina*", World Rev Nutr Diet vol. **77**, pp.32-46, 1995.

[15] Dubois M., Gilles K. , Hamilton, J. , Rebers P. and F. Smith,"Colorimetric method for determination of sugars and related substances", Anal. Chem., vol. 28, pp. 350 – 356, 1956.

[16] Fatma T., R. Sarada and L. Venkataraman, "Evaluation of selected strains of *Spirulina* for their constituents", Phykos, vol. 33: 98-97, 1994.

[17] Gholap D. and S. Pingle, "Estimation of *Spirilina platensis* Nordst. and *Hydrodictyon reticulatum* L. from Ahmednagar", Advances in Plant Sciences, pp.597-598, 2009.

[18] Hirschberg J. and D. Chamovitz, "Carotenoids in cyanobacteria", In The Molecular Biology of Cyanobacteria. Edited by Bryant, D.A. Kluwer Academic Publishers, Dordrecht. pp. 769–823, 1994.

[19] Jha P., Ali A. and N. Raghuram, "Nitrate-induction of nitrate reductase and its inhibition by nitrate and ammonium ions in *Spirulina platensis*", Physiol. Mol. Biol. Plants, vol. 13, no. 2, pp.163-167, 2007.

[20] Khan Z., P. Bhadouria and P.S. Bisen **"**Nutritional and therapeutic potential of *Spirulina*.", Curi Pharm Biotechnol, vol. 6, pp.373-379, 2005.

[21] Kirk J. and R. Allen, "Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione", Biochem. Biophysics Res. J. Canada, vol. 27, pp. 523-530, 1965.

[22] Komarek J., J. Komarkova and H. Kling, " Freshwater algae of North America", Elsevier science (USA), pp.117-196, 2003.

[23] Komárek J., J. Kastovsky, J. Mares and J. R. Johansen, " Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach", Preslia vol.86, pp. 295–335, 2014.

[24] Lowe R. and H. Evans, "Preparation and some properties of a soluble Nitrate reductase from *Rhizobium japonicum"* , Biochim.Biophys. Acta ., vol. 85, pp.377-387, 1964.

[25] Lowry O. , Rosenbrough N. , Farr A. and R. Randall, "Protein measurement with the Folin – phenol reagent",. J. Biol. Chem. , 193, pp. 265 – 275, 1951.

[26] Luximon-Ramma A., T. Bahorun, M. Soobrattee, O. Aruoma, "Antioxidant activities of phenolic, proanthocyanidin and flavonoid components in extracts of *Cassia fistula*", J. Agric. Food. Chem. **,** vol. 58, no.18, pp. 5042-5047, 2002.

[27] Mackeny G., "Absorption of light by chlorophyll solutions", J Biol. Chem., vol. 140, pp. 315-322, 1941.

[28] Madkour F. F.., Kamil A. El-Wahab and Nasr H. S., "Production and nutritive value of Spirulina platensis in reduced cost media", Egyptian Journal of Aquatic Research, vol. 38, pp.51–57, 2012.

[29] Mani U.V., U.M. Iyer, S. A. Dhruy, I. U. Mani and K.S. Sharma, "Therapeutic utility *Spirulina",* In:Gershwin ME. Belay A (eds) *Spiurlina* human nutrition and health. CRC Press. Boca Raton pp.71-100, 2005.

[30] Mohite Y., "Biochemical studies on fresh water algae and their screening for various potentials", Ph. D. thesis, University of Pune, 2007.

[31] Nagle V., N. Mhalsekar and T. Jagtap, "Isolation optimization and characterization of selected cyanophycean members", Indian Journal of Marine Sciences, vol. 39, no.2, pp. 212-218, 2010.

[32] Omaye S., J. Turabull, H. Sanberlich, "Selected methods for the determination of acsorbic acid in animal cells, tissues and fluids", Methods Enzymology, vol. 62, pp. 1-11, 1979.

[33] Prieto P., M. Pineda, M. Anguliar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E", Anal. Biochem. , Vol. 269**,** pp. 337- 341, 1999.

[34] Rasmussen R. and M. Morrissey, "Biotechnology for production of food ingredients", Adv Food Nutr Res., vol. 52, pp. 237-292, 2007.Doi:10.1016/S1043-4526(06)52005-4

[35] Rippka R., J. Deruelles, J. Waterbury, M. Herdman and R. Stanier, "Generic assignments, Strain histories and properties of pure cultures of cyanobacteria", J. Gen. Microb., vol. 111, pp. 1-61, 1979.

[36] Ronda R. and S. Lele, "Culture conditions stimulating high γ-linolenic acid accumulation by *Spirulina platensis*", Brazilian Journal ofMicrobiology, vol. 39, pp. 693-697, 2008.

[37] Subhashini R., K. Kumar and S. Kannaiyan, "Intrinsic antibiotic resistance and biochemical characteristics of *Anabaena azollae* isolated from *Azolla* – cultures", Indian J. Microbiol., vol. 43, pp. 165 -169, 2003.

[38] Takaichi S.and M. Mochimaru, "Carotenoids and carotenogenesis in cyanobacteria: Unique ketocarotenoids and carotenoid glycosides", Cell. Mol. Life Sci., vol. 64, pp. 2607– 2619, 2007.

[39] Tan L., "Bioactive natural products from marine cyanobacteria for drug discovery", Phytochem., vol. 68, pp. 954 – 979, 2007.

[40] Tomaselli L., L. Giovannetti and G. Torzillo, "Physiology of stress response in *Spirulina* spp", In: *Spirulina* algae of life. F. Doumenge, H. Durand Chastel and A. Toulemont (eds.). pp. 65-75, 1993.

[41] Tomaselli L., M. C. Margheri and A. Sacchi, "Effects of light on pigments and photosynthetic activity in a phycoerythrin-rich strain of *Spirulina subsalsa*", Aquat microb Ecol., vol. 9, pp. 27-31, 1995.

[42] Vargas M., Rodriguez H., Moreno J., Olivares H., Del Campo J., Rivas J. and M. Guerrero, "Biochemical composition and fatty acid content of filamentous nitrogen fixing cyanobacteria", J. Phycol., vol.34, pp. 812 – 817, 1998.

[43] Wolfe K., X. Wu, R. Liu, "Antioxidant activity of apple peels", J. Agric. Food Chem. , vol.51, pp. 609-614, 2003.