Research Article [Xalxo *et al.*, 4(9): Sep, 2013]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Effect of antibacterial properties of cyanobacterial *Spirulina platensis*

Raj Kishori Xalxo[1]*, Shweta Sao[1] and Pankaj K. Sahu[2]
1, Department of Life science, Dr. C.V. Raman University, Bilaspur, (CG) - India
2, Department of Botany, Dr. C.V. Raman University, Bilaspur, (CG) - India

Abstract

Present works objective to look for active substances that could be used as antibacterial agents in *Spirulina platensis*. To achieve this target, six different extracts (crude polysaccharide, cell free polysaccharide, hot water extract, methanol soluble pigment, spent medium and phycobilliprotein) from three strains of *Spirulina platensis*, the high salt tolerance mutant strains i.e. the Sodium Chloride resistant mutant (Na-R) and the Lithium Chloride resistant mutant (Li-R) and the Wild type strain was examined. The algal extracts were tested *in vitro* for their antibacterial effects against (*Salmonella, Shigella, Klebsiella, Streptococcus*) using Paper disc diffusion method and concentration from 250-7000 ppm was taken and observed all these bacteria showed inhibition in growth by these extracts and no effect was observed in case of other *Spirulina* extract.

Key words: *Spirulina platensis*, Cyanobacteria, Antibacterial activity, Paper-disc diffusion method, Antimicrobial assay

Introduction

Bacteria have been source for majority of antibiotics and therapeutic compounds. In addition researchers are searching for some new antibiotics/therapeutic compounds from a group of microbes that has been overlooked in the past, the cyanobacteria (Blue green algae). Cyanobacterial, *Spirullina* has emerged as one of the most promising agent for sythesizing potentially new therapeutic compounds. *Spirulina* contains chlorophyll a, like higher plants, botanists classify it as a micro alga belonging to *Cyanophyceae* and chemical composition includes proteins (55-70%), carbohydrates (15-25%) essential fatty acids (18%) vitamins, minerals and pigments like carotenes, chlorophyll a and phycocyanin and phycoerythrin.

The *Spirulina* pigment phycocyanin shows antioxidant activity and it also scanvenges peroxyl radicals. Phycocyanin has been found to protect against hepatotoxins in rats. The mechanism may be via its antioxidant activity. An extract of *Spirulina maxima* also protected against carbon tetrachloride hepatoxicity in rats. The phycocyanin contained in the extract, as well as other antioxidants, probably account for the hepatoprotective effect.

*** Corresponding Author**
E.mail: drshwetasao02@gmail.com, sahu.pankaj1@gmail.com

Most cells mediated immediate – type allergic reactions were found to be inhibited in rats by *Spirulina*. It is speculated that there is substance in Spirulina that may inhibit mast-cell degranulation, possibly by affecting the mast-cell memberane (Kim *et al.* 1998). *Spirulina platensis* extracts have been reported to enhance macrophages function in cats (Quereshi *et al.* 1996). The water extracts of *Spirulina platentsis* have been reported to possess antibacterial property (Singh et al., 2005).

Material and Methods

Source of cyanobacterium (*Spirulina platensis*) & Bacteria

Three strains of *Spirulina platensis*, the high salt tolerant mutant strains i.e. the Sodium Chloride resistant mutant (Na-R) and the Lithium Chloride resistant mutant (Li-R) (lab isolates) and the wild type strain were obtained by Algal Biotechnology Laboratory, Department of Biological Sciences. The bacterial cultures of *Salmonella, Shigella, Klebsiella, Streptococcus* were obtained by Department of Microbiology, Regional Malarial Research Centre, ICMR, Jabalpur, M.P. India,

Composition of growth medium for S*pirulina platensis*

Preparation of medium

Take 4.5 $NaHCO_3$, 0.5 K_2HPO_4, 1.2 $NaNO_3$, 1 K_2SO_4 For preparing 1L growth medium. Each of

macronutient was added to 1L double distilled water and $NaHCO_3$ was added separately. The pH of the medium was adjusted at 11. All chemicals used in CFTRI medium were products of Qualigens (India).

Growth condition and maintenance of culture

All the three strains of *Spirulina platensis* were maintained axenically in C.F.T.R..I medium (Vekataraman,1983) by its routine transfer at a regular interval of 7 days. The axenic cultures were grown photoautotrophically in an air conditioned growth chamber maintained at 25 ± 1°C and illuminated with cool day fluorescent light of 2500 lux intensity for 16h a day. The medium and all the glasswares were sterilized in an autoclave at 15 lbs $inch^{-2}$ pressure (121° C temperature for 15 min.)

Composition of growth medium for Bacteria

5.0 Peptone, 5.0 NaCl, 3.0 Beef extract All the above nutrients were added to 1L of double distilled water. For preparation of solid medium 2% Agar was added to the nutrient broth. pH of the medium was adjusted to 7.0 before strerilization. Medium was autoclaved at 121°C at 15 lb $inch^{-2}$ pressure for 15 minutes.

Composition of Mueller Hinton Broth

Composition of Mueller Hinton Broth are 300.00 gL-1 Beef infusion from, 17.50 gL-1 Casein acid hydrolysate, 1.50 gL-1 Starch. 38.00 gms of above Nutrients were added to 1L of double distilled water. For preparation of solid medium 17.00 gms agar was added to the Mueller Hinton Broth and boiled to dissolve the medium completely. Final pH of the medium at 25°C was adjusted to 7.3±0.2 before sterilization. Medium was autoclaved at 121°C at 15lb $inch^{-2}$ pressure for 15 minutes.

Growth conditions and maintenance of bacterial strains

The bacterial cultures of *Salmonella, Shigella, Klebsiella, Streptococcus* were maintained axenically in Nutrient agar slants at 37°C. Strains were inoculated in sterilized Nutrient broth medium in 100 ml Erlenmeyer Flask and maintained at 37 °C and this culture was used for the assay.

Standardization

Bacterial inoculum: The bacterial inoculum was grown overnight in nutrient broth medium at 37°C. Turbidity of this inoculum was adjusted to 0.5 McFarland Standard, approximately 108 colony forming units [CFU $(ml)^{-1}$].

Antimicrobial test

The antimicrobial activity of *Spirulina* extracts was tested on organisms using the paper disc diffusion method (Pawar *et al*., 1996). The sterile filter paper discs of 4.5 cm diameter were soaked in different extracts of *Spirulina* and placed at different areas on the surface of each inoculated plate. The plates were incubated at 37° for 24 h for antibacterial assay. Antimicrobial activity of each extract against the test bacteria was indicated by growth free "zone of inhibition" near the respective discs.

Preparation of Spent media, Hot water extract and Crude Polysaccharide of *Spirulina platensis* (Bertocchi *et al*. 1990)

Procedure: A known vol (3ml) of 1 month old culture was centrifuged at 3000 xg for 15 min To the pellet water double distilled water was added and incubated in Hot water bath at 100°C for 1 hr and Supernatant used for antimicrobial assay of spent medium. Resulting suspension was centrifuged at 5000 rpm for 15 min. The supernatant was used for antimicrobial assay of Hot Water extract and 3 vol of absolute of ethanol was added. The sample was then centrifuged for 15 min at 5000 rpm by thistThe supernatant was discarded and the pellet recovered and 1 ml D.W. was added to the pellet containing precipitated crude poly saccharide and used for antimicrobial assay.

Extraction of pure cell free polysaccharide (Mouhim *et al*. 1993)

A known volume (3ml) of culture suspension was centrifuged at 5000 rpm for 10 min at 4°C. The pellet was resuspended at 20°C in a low ionic strength 0.05M, pH 8.15 TAPS buffer (3ml) and 0.025M EDTA (3ml). The resulting suspension was heated at 100°C for 20 min and was followed by a second centrifugation at 20000xg for 1½ at 30°C.

The supernatant was used to recover the cell free polymer. 3 ml of 3% of cetytrimethyl ammonim bromide was added to the supernatant. The acidic polysaccharide thus precipitated was recovered by centrifugation at 5000 rpm for 10 min. The acidic polysaccharide was purified by 4 successive cycles of re-solubilization in KCL of decreasing concentration 1.5M, 0.75M, 0.3M) followed by double distilled water. The polysaccharide was reprecipitated by addition of 50% ethanol water and centrifuged at 5000 rpm for 10 min. The pellet of polysaccharide was redissolved in distilled water and use for antimicrobial assay.

Extraction of Methanol soluble pigments (Mackinny, 1941)

A known volume (3ml) of culture was centrifuged at 3000 rpm for 15 min and the supernatant was discarded. The pellet was washed with distilled water and 3 ml of methanol was added and cyclomixed vigorously by a cyclomixer. The sample was then incubated at 60°C in a Hot water bath for 15 min for complete extraction for the pigment. The sample was

then centrifuged at 3000 rpm for 15 min and the supernatant was used for antimicrobial assay.

Extraction of Phycobilliproteins (Bennett and Bogorad, 1973)

Reagents – Phosphate buffer (0.05M) pH 6.8, 1.72g of dipotassium phosphate (K_2HPO_4) and 1.36 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 100 ml of distilled water separately and the pH is set to 6.8 by adding KH_2PO_4 to K_2HPO_4.

Procedure: A known volume (3ml) of homogenous cyanobacterial suspension was centrifuged at 3000 rpm for 10 min.

The pellet was washed thrice with distilled water and to the pellet 3 ml of phosphate buffer was added. The resulting suspension was sonicated using a sonicator ensuring complete breakdown of cell and complete extraction of phycobilliproteins. The sample was then centrifuged at 3000 rpm for 15 min. The supernatant containing the phycobiliproteins was used for antimicrobial assay.

Results and Discussion

Effect of different extracts of (Wild type, Na-R and Li-R) *Spirulina platensis* on Bacteria

The zone of inhibition against *Shigella* produced by phycobilliprotein was 19 mm followed 12 mm by Spent Medium, 11 mm by Crude and cell free polysaccharide, 9 mm by Hot water extract and 8 mm by Methanol soluble pigment. The zone of inhibition against *Klebsiella* produced by phycobilliprotein was 2 mm followed by 10 mm by Hot water extract and cell free polysaccharide, 9 mm in Spent Medium, Methanol soluble pigment and Crude polysaccharide. In the case of Salmonella highest zone of inhibition was produced by Phycobilliprotein i.e 18 mm followed by 13 mm by Crude polysaccharide 11 mm by Spent medium, 10 mm in Hot water extract, 8 mm by cell free polysaccharide and Methanol soluble pigment. The inhibition zone against *Streptococcus* produced by Phycobilliprotein was 18 mm followed by 11 mm zone of inhibition by Spent Medium, 9 mm zone by Hot water extract and Crude polysaccharide, 8 mm in Cell free polysaccharide and 7 mm by Methanol soluble pigment.

Thus the zones produced by phycobilliprotein against all the Bacterial strains were highest followed by the zone of inhibition produced by spent medium followed by the zones produced by the polysaccharide and ethanol extract i.e. the Hot water extract, Crude polysaccharide.

Thus highest zone of inhibition against *Shigella* produced by both crude and cell free polysaccharide followed by the Hot water extract. In *Klebsiella* the Hot water extract and cell free polysaccharide produced by Crude polysaccharide. The zone of inhibition produced by Crude polysaccharide was maximum followed by Hot water extract and cell free polysaccharide against *Salmonella*. The zone of inhibition produced Hot water extract and Crude polysaccharide was same followed by cell free polysaccharide against *Streptococcus.* The zone of inhibition produced was maximum followed by the spent medium, polysaccharide and the lowest zone of inhibition was shown by the methanol soluble extract of *Spirulina.*

On comparing the zone of inhibition exhibited by all the extracts of *Spirulina platensis* (Wilde type) it was concluded that the zone of inhibition exhibited by the phycobilliprotein against all the test bacteria were highest followed by the zones showed by the spent medium. The spent medium containing exometabolites of different cyanobacterial strains have been reported to exhibit a concentration dependent growth inhibition of bacterial and fungal strains (Volk and Fukett, 2006) i.e higher the concentration of exometabolites wide was the zone of inhibition. So it may be due to this fact that though bacterial strains were susceptible the test fungus showed no response towards the spent medium. The Hot water extract, crude and cell free polysaccharide did exhibit antimicrobial property as has been reported by *et al.* 2005 in case of crude polysaccharide.

The zone exhibited by the Hot Water extract was wider than that of the crude and cell free polysaccharide. Potent antibacterial activity of methanol extract has been already reported by Ozdenir *et al.* (2004) and it was reported to be the component which was found to have most potent antimicrobial activity than other volatile component. In the present work the result indicate phycobillins, polysaccharide and the spent medium to be more potent as compared to the volatile component i.e. the methanol soluble pigment.

Acknowledgement

Our sincere thanks to Vice Chancellor Dr. C.V. Raman University, Kargi Road, Kota, Bilaspur (C.G.) for his blessing and inspiration and we are also obliged to the Registrar, Dr. C.V. Raman University, Kargi Road, Kota, Bilaspur.

References

1. Baba, M., Snoeck, R., Pauwels, R., and De Clerq, E., (1988). Sulfated polysaccharides are potent and selective inhibitors of enveloped virus, cytomegalovirus, vascular stomatitis, and human immunodeficiency virus. Antimicrobe Agents Chemother. 32 : 1742 – 1745.

2. Banker, R., and Carmeli, R., (1998). Tenuecyclamides A-D, cyclic hexapeptides from the cyanobacterium Nostoc spongiformae var tenue. J. Nat. Prod. 61 : 1248-1251.
3. Bertocchi, C., Navarini, L., Cesaro, A., and Anastasio, M., (1990). Polysaccharides from Cyanobacteria. Carbohydr, Pc.
4. Bloor, S., and England, R.R., (1989). Antibiotic production by the cyanobacterium Nostoc muscorum. Journal of Applied phycology. 1 : 367 – 372.
5. Borowitzka, M.A., (1988a). Vitamins and fine chemicals from micro-algae, In: Borowitzka, M.A., Cambridge L.J. (Eds.), Micro-algal Biotechnology, Cambridge University Press, Cambridge. Pp.211-217.
6. Borowitzka, M.A., (1988b). Fats, oils hydrocarbons. In : Borowitzka. Cambridge University Press, Cambridge. Pp. 257-287.
7. Borowitzka, M.A., (1995). Microalgae as sources of pharmaceuticals and other biologically active compounds J. Appl. Phycol. 7 : 3-15.
8. Deth, S.K., (1999). Antimicrobial compounds from marine cyanobacteria with special reference to the bioactivity of a purified compound from Oscillatoria laete-virens BDU 20801. Ph.D. thesis Bharathidasan University Thiruchirappalli, India.
9. Gerber, P., Dutcher, D., Adams, V., and Shermann, H., (1958). Protection effect of seaweed extracts for chicken embryos infect with influenza B or mumps. Proc Soc Exp Biol Med. 99 : 590 – 593.
10. Gugliemi, G., Rippka, R., and Marsac, N.T.D., (1993). Main properties that justify the different taxonomic position of Spirulina sp. and Arthrospira sp. among cyanobacteria.In : Doumenge, F., Durand-Chastel, H., Toulemont, A., Eds. Spiruline algae de vie. Bulletin de Institute Oceanographique Monaco. Musee Oceanoraphique Numero special. 12 : 13-23.
11. Harrigan, G.G., Leusch, H., Yoshida, W.Y., Moore, R.E., Nagle, D.G., Paul, J., Mooberry, S.L., Corbett, T.H., and Valeriote, F.A. (1998). Symplostanin 1 : a dolastatin 10 analogue from the marine cyanobacterium Symploca hydnoides. J Nat. Prod. 61 : 1075 – 1077.
12. Harrigan, G.G., Luesch, H., Yoshida, W.Y., Moore, R.E., Nagle, D.G., and Paul, V.J. (1999). Symplostanin 2: a dolastanin 13 analogue from the marine cyanobacterium Symploca hydnoides. J. Nat. Prod. 62: 655-658.
13. Hayashi, K., Hayashi, T., Morita, N., and Kajima, I., (1993). An extract from Spirulina platensis is a selective inhibitor of herpes simplex virus type 1 penetration into HeLa cells. Phytotherapy Res. 7: 76-80.
14. Issa, A.A., (1999). Antibiotic production by the cyanobacteria *Oscillatoria angustissima* and calothrix parientina. Environmental Toxicology and pharmacology. 8: 33-37.
15. Jaki, B., Orjala, J., Heilmann, J., Linden, A., Vogler, B., and Sticker, O., (2000). Novel extracellular diterpenoids with biological activity from the cyanobacterium Nostoc commune. J. Nat. Prod. 63: 339-343.
16. Jaki, B., Orjala, J., and Sticher, O., (1998). New extracellular diterpenoids with antibacterial activity from the cyanobacterium Nostoc commune. In: Symposium papers of the 45th Annual Congress of the Society of Medicinal Plant Research G 51, Vienna.
17. Jaki, B., Orjala, J., and Sticher, O., (1999). A novel extracellular diterpenoids with antibacterial activity from the cyanobacterium Nostoc commune. J. Nat. Prod. 62: 502-503.
18. Joung Han Yim, Sung Jin Kim, Se Hun Ahn, Chong Kyo Lee, Ki Tae Rhie, and Hong Kum Lee (2004) "Antiviral effects of Sulfated Exopolysaccharides from the Marine Microalgae *Gyrodinium impudicum* Strain KGO3, Mar. Biotechnol. 6. 17-25.
19. Kim, H.M., Lee, E.H., Cho, H.H, and Moon, Y.H., (1998). Inhibitory effect of mast cell – mediated immediate type allergic reaction in rats by *Spirulina*. *Biochem Pharmacol*. 55: 1071 -1076.
20. Lau, F., Siedlecki, J., Anleitner, J., Patterson, L., Caplan, P., and Moore, E., (1993). Inhibitory of reverse transcriptase activity by extracts of cultured blue green algae (Cyanophyta). Plant Med. 59 : 148 – 151
21. Mathew, B., Sankaranarayanan, R., Nair, P., Varghee, C., Somanathan, T., Amma, P., Amma, N., and Nair, M., (1995). Evaluation of of chemoprevention of oral cancer with Spirulina fusiformis. Nutr Cancer. 24 : 197-202.
22. Mouhima, F.R., Carnet, J.F., Fournet, B., and Dubertret, G., (1993). Production isolation and prelimina exopolysaccharides of the

Cyanobacterium from Spirulina platensis. Biotechnol. Lett. 15: 567-72.

23. Namikoshi, M. and, Rinehar, K.L., (1996). Bioactive compounds produced by cyanobacteria. J. Ind. Microbiol. 17: 373 – 348.
24. Ozdemir, G., Karabey, N.U., Dalay, M.C., Pazarbasi, S., (2004). Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytotherapy Research. 18 (9): 754 – 757.
25. Pandey, U., and Pandey, J., (2002). Antibacterial properties of Cyanobacteria: A cost – effective and eco-friendly approach to control bacterial leaf spot disease of chilli. Current Science. 82: 3 – 10.
26. Patterson, G.M.L., (1996). Biotechnological applications of cyanobacteria Journal of Scientific and Industrial Research. 55: 669 – 684.
27. Patterson, G.M.L., Larsen, L.K., and Moore, R.E. (1994). Bioactive natural products form blue-green algae. J. Appl. Phycol. 6: 151-157.
28. Quereshi, M.A., and Ali, R.A., (1996). Spirulina platensis exposure enhances macrophage Phagocytic function in Cats. Immunopharmacol Immunotoxicol. 18: 457-463.
29. Salazar, M., Martinez, E., Madrigal, E., Ruiz Le., and Chamorro G., (1998). Subchronic toxicity study in mice fed Spirulina. J. Enthnopharmacol. 62: 235-241.
30. Schwartz, J., and Shklar, G., (1987). Regression of experimental hamster cancer by beta-carotene and algae extracts. J. Oral Maxillofac Surg. 45: 510-515.
31. Singh, S., Ganguly, A., and Baisya, S., (2005). Antibacterial properties of polysaccharide from Spirulina platensis. 46th annual conference of association of microbiologist of India Department of Microbiology Osmania University Hyderabad (AP) India Dec. 8 – 10.
32. Stainier, R.Y., and Van niel, Y. (1962). The concept of a vacterium Arch. Mikrobiol. 42: 17-35.
33. Starr, T.J., Dug, E.F., Church, K.K., and Allen, M.B.,(1962). Antibacterial and antiviral activities of algal extracts. Studies by acridine orange staining. Texas Report on biology and Medicine. 20: 271 – 278.
34. Volk, R.B., and Furkert, F.H., (2006). Anti-algal antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacterial during growth. Microbiological Research. 161: 180 – 186.

Table 1: Effect of different extract of (Wilde type) *Spirulina platensis* on Bacteria

Spirulina platensis Extracts	Inhibition zone diameter (mm)			
	Shigella	*Klebsiella*	*Salmonella*	*Streptococcus*
Hot Water Extract	9mm	10mm	10mm	9mm
Crude Polysaccharide	11mm	9mm	13mm	9mm
Cell Free Polysaccharide	11mm	10mm	8mm	8mm
Methanol Soluble Pigment	8mm	9mm	8mm	7mm
Spent Medium	12mm	9mm	11mm	11mm
Phycoblliprotein	19mm	22mm	18mm	18mm

Table 2: Effect of different extracts of (LiR) *Spirulina platensis* on Bacteria

Spirulina platensis Extracts	Inhibition zone diameter (mm)			
	Shigella	*Klebsiella*	*Salmonella*	*Streptococcus*
Hot Water Extract	9mm	9mm	11mm	8mm
Methanol Soluble Pigment	9mm	10mm	9mm	9mm
Spent Medium	10mm	15mm	22mm	11mm

Table 3: Effect of different extract of (LiR) *Spirulina platensis* on Bacteria

Spirulina platensis extracts	Inhibition zone diameter (mm)			
	Shigella	*Klebsiella*	*Salmonella*	*Streptococcus*
Hot Water Extract	9mm	8mm	9mm	8mm
Methanol Soluble Pigment	9mm	10mm	9mm	9mm
Spent Medium	11mm	10mm	10mm	11mm

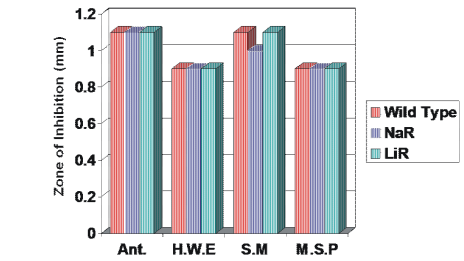

Fig.8: Effect of Hot Water Extract, Spent media and Methnol Soluble Pigement of (wild type, NaR, LiR) *Spirulina platensis* on *Shigella*

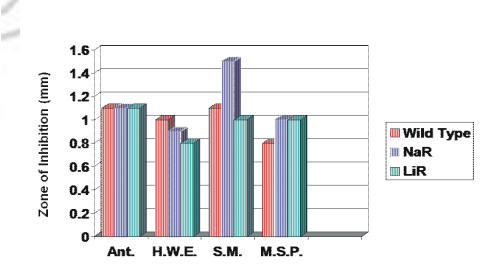

Fig.9: Effect of Hot Water Extract, Spent media and Methnol Soluble Pigement of (wild type, NaR, LiR) *Spirulina platensis* on *Klebsiella*

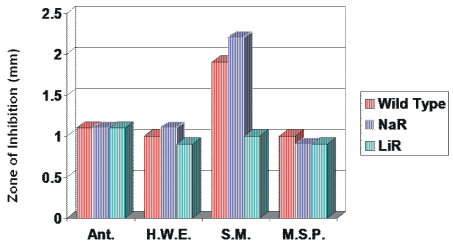

Fig.10: Effect of Hot Water Extract, Spent media and Methnol Soluble Pigement of (Wild type, NaR, LiR) *Spirulina platensis* on *Salmonella*

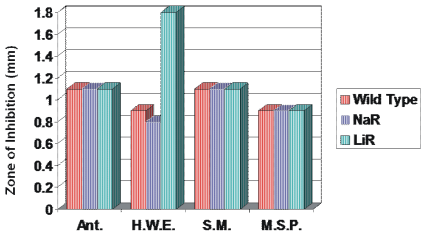

Fig.11: Effect of Hot Water Extract, Spent media and Methnol Soluble Pigement of (Wild type, NaR, LiR) *Spirulina platensis* on *Streptococcus*

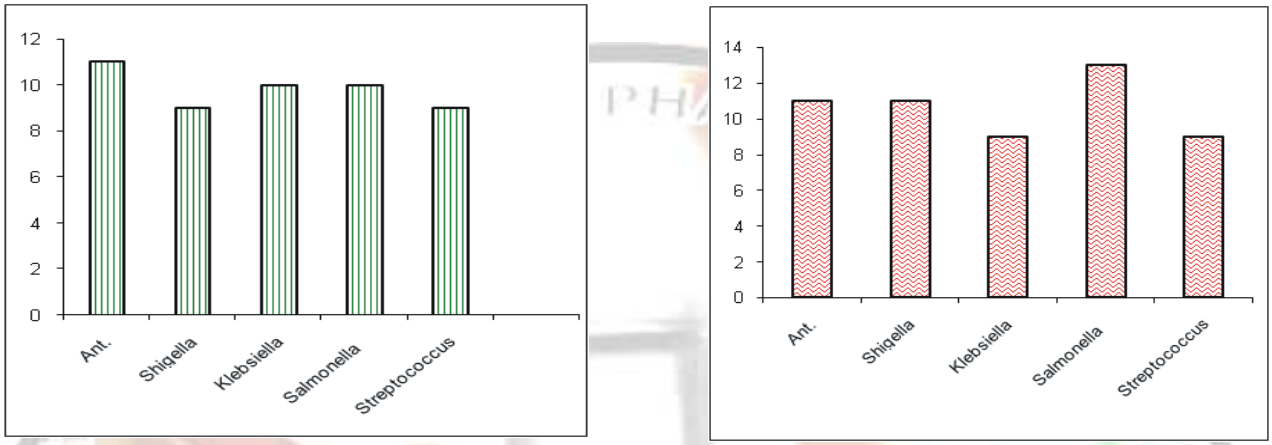

Fig.3: Effect of Crude Polysaccharide of (wild type) *Spirulina platensis* on all test bacteria

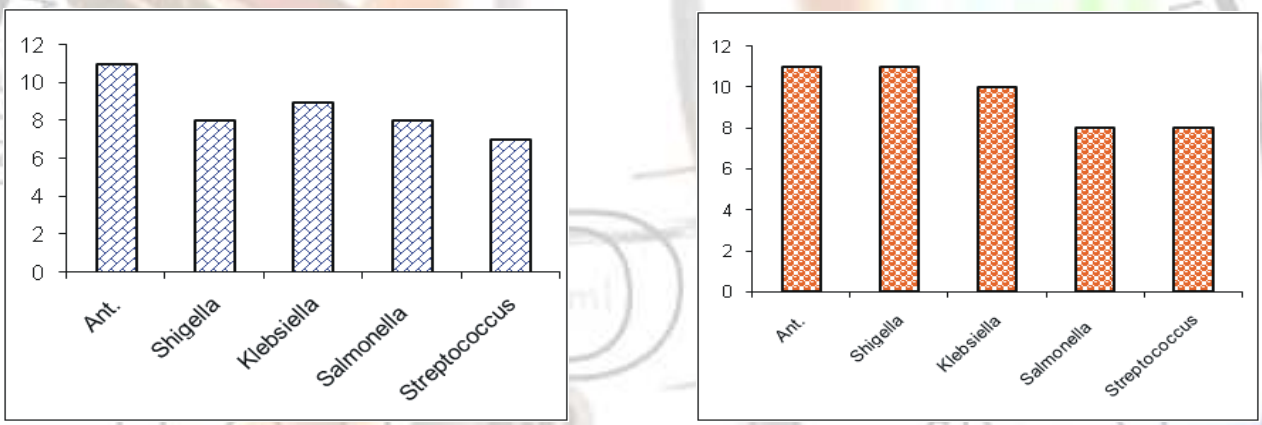

Fig. 4a & b Effect of methanol soluble pigment and Cell free polysaccharide of (wild type) *Spirulina platensis* on all test bacteria

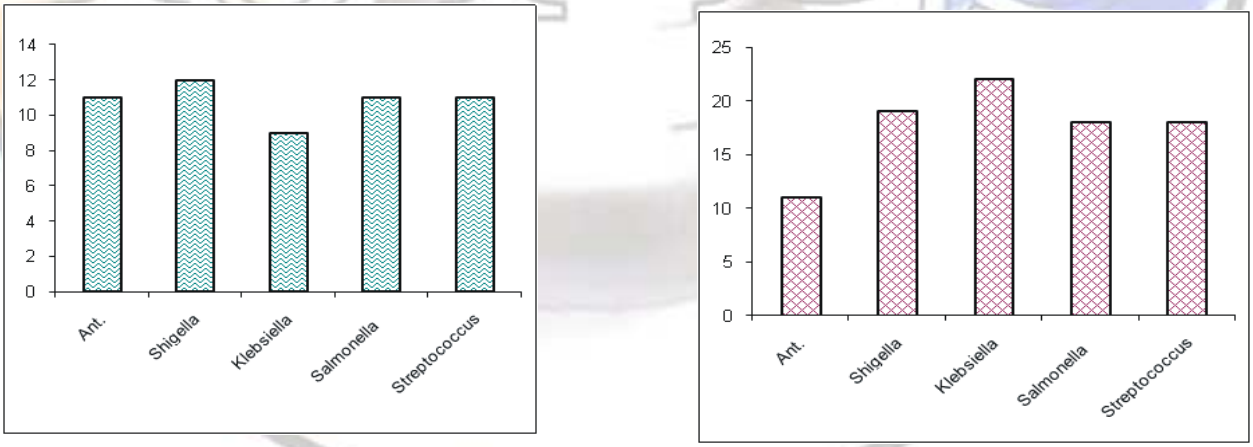

Fig.6 a & b. Effect of Spent Medium and Phycobilliprotein of (wild type) *Spirulina platensis* on all test bacteria