International Journal of Current Science Research
www.drbgrpublications.in

IP Indexing - Impact factor – 3.5
Volume: 3; Issue: 10; October-2017; pp 1417-1425. ISSN: 2454-5422

# Antibacterial activity of *Spirulina platensis* grown using *Sargassum wightii as a* fertilizer medium

N.Sinthiya[1*] and A.Selvin Samuel[2]

[1]Assistant Professor, Department of Botany, Arignar Anna College, Aralvoimozhi, India.
[2]Associate Professor, Department of Botany, St.John's College, Palayamkottai, India.
*Corresponding author email: sinthmony1214@gmail.com

## Abstract

*Spirulina platensis*, a blue green alga is an edible, microscopic, multicellular cyanobacterium, attracted attention to many scientists due to its beneficial medicinal applications. *S. platensis* or its extracts are having several biological activities like anticancer, antiviral, antioxidant and antimicrobial activities.In the present study, antibacterial activity of *S.* supplemented *S. platensis* solvent extracts was investigated against pathogenic bacteria. The antibacterial effect of ethanol, methanol, petroleum ether and extract of *S.platensis* showed the antibacterial activities against different bacterial strains (*Klebsiella pneumoniae, Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi, Bacillus cereus*) were tested, using disc diffusion method. Methanolic extract of *Spirulina platensis* showed maximum antibacterial activity of 15mm against *E. coli* and a minimum activity of 9.0mm against *S.aureus*. Ethanolic extract of *Spirulina platensis*also showed the highest biological activity of 13mm against *Klebsiella pneumoniae*, minimum ctivity of 9.0 mm against *S.aureus.* The finding in this study reveals that antimicrobial activity of *Spirulina platensis* was highly effective in methanol the rest of the solvents showed varying degree of inhibition. *Spirulina platensis* showed maximum zone of inhibition against all the bacterial isolates**.**

**Keywords:** *Spirulina platensis,* Antibacterial activity, cyanobacterium, *Sargassum*

## Introduction

*Spirulina platensis* are filamentous, blue-green algae are photoautotrophic microorganisms have been used as human food for many years because of their high protein content (35–65%) and nutritional value largely distributed in nature. This planktonic photosynthetic filamentous cyanobacterium is recognizable by the main morphological feature of the genus, i.e. the arrangement of multicellular cylindrical trichomes in an open left-hand helix along the entire length of the filaments that forms massive populations in tropical and subtropical bodies of water which have high levels of carbonate and bicarbonate, and alkaline pH values up to 11 (Vonshak, 1997). *S.platensis* currently mass produced as a monoculture in outdoor cultivation systems, wherein the growth medium utilized forms an important input and accounts for a major share of the costs involved in *S.platensis* production. *S.platensis* as many other cyanobacteria species have the potential to produce a large number of antimicrobial substances, so they are considered as suitable organisms for exploitation as bio control agents of plant pathogenic bacteria

The chemical constituents of *S.platensis* shows rich source of proteins, vitamins, minerals and polyunsaturated fatty acids (Miranda *et al.,* 1998; Estrada, 2001). *S. platensis* extract is known to have antioxidant activity, due to its higher content of phenolic compounds (Wu, L.C., *et al.,* 2005). Microalgae make an extensive range of chemically active metabolites in their environs, potentially to protect themselves against the other organisms. These dynamic metabolites also identified as biogenic compounds that are formed by numerous species of marine macro and microalgae and have antibacterial and antifungal activities which are efficient in the avoidance of fouling and have other likely uses in therapeutics (Bhadury, 2004; Smit, 2004).

*S.platensis* or its extracts show therapeutic properties, such as the ability to prevent the incidence of cancers, decrease blood cholesterol levels, stimulate the immunological system, reduce the nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation (Belay *et al.,* 1993; Abd El-Baky, *et al.,* 2006). Preventing disease with drug or chemicals are able to induce resistance in bacteria. The decreased efficiency and resistance of pathogen to antibiotics has necessitated to combate serious bacterial infections. Antimicrobials and vaccines play a major role in the prevention, control and even eradication of infectious diseases.

Recently more attention has been given to study its therapeutic effects, which include reduction of cholesterol and nephrotoxicity by heavy metals, anticancer properties, protection against radiation, and enhancement of the immune system (Belay *et al.,* 1994*). S. platensis* also possesses other biological functions such as antiviral, antibacterial, and antifungal activities (Khan *et al.,* 2005). Some *S. platensis* species exhibit antibacterial activity (Ozdemir, *et al.,*2001*).*Various active substances with antibacterial, antiviral, fungicide, enzyme inhibiting, Immune suppressive and cytotoxic and algicide activity have been isolated from cyanobacterial biomass. The defense mechanism against antibiotics is widely present in bacteria and became a world health problem. (DeivyClementino de Lima *et al.,*2005).

*S. platensis* as many other cyanobacteria species have the potential to produce a large number of antimicrobial substances, so they are considered as suitable organisms for exploitation as biocontrol agents of plant pathogenic bacteria and fungi (Kulik, 1995). Thus, there is an urgent need to develop alternative biodegradable agents, which could be free from side effects. Therefore the algal extracts having wide range of antimicrobials effects can be employed safely to prevent microbial attacks and invasions.This search prompted the exploration of natural algal product that could be non-toxic antimicrobial agents with microbial toxic properties and interest to antibacterial activity of the *S. platensis.* The aim of this study is to examine the antibacterial activities in extracts of *S. platensis* grown using *S. wightii* bio fertilizer.

## Materials and Methods

### Antibacterial activity of *S.platensis*

### Preparation of test organisms

*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Bacillus cereus* were collected from Scadder laboratories, Nagrecoil, Kanyakumari District and conformed by conventional microbiology procedure. Stock cultures of different bacteria were grown in nutrient broth at 30 $^{o}$ C and were sub-cultured and maintained in nutrient broth at 4 $^{o}$C. Before swabbing, each culture was diluted (1:10) with sterile nutrient broth.

**Antibacterial assay**

The antibacterial activity was determined by the paper disc diffusion method (Bauer *et al*. 1966). A suspension of the organism was added to sterile nutrient agar medium at 45 °C. The mixture was transferred to sterile petriplates and allowed to solidify. Sterile disc of diameter 5mm (made from Whatman No 1 filter paper previously sterilized in autoclave) was dipped in test solution of each extract prepared by dissolving separately in respective solvents. The sterile disc containing test solution of the plant extract was placed over the seeded agar plates in such a way that there is no overlapping of zone of inhibition. Standards and blank were placed on the surface of the agar plate. The antibiotic amikacin (30 g/disc) was used as standard for bacteria to compare its effect on test organism with the plant extracts. The plates were kept at room temperature for 2hours to allow diffusion of the test solution in to the agar; they were incubated for 24 hours at 37 °C. After the incubation period was over, the plates were observed and Zone of the inhibition was measured in millimetres (mm).

## Results and Discussion

*S. platensis* is a planktonic photosynthetic filamentous cyanobacterium that forms massive populations in tropical and subtropical bodies of water which have high levels of carbonate and bicarbonate, and alkaline pH values of up to 11. The properties of secondary metabolites in nature are not completely understood Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetic importance. Recently, there has been an increasing interest in cyanobacteria as a potential source for new drugs (Glombitza K.W and Koch, 1989; Schwartz *et al.,* 1990).

*S. platensis* has been studied because of its therapeutic properties and the presence of bioactive compounds (Belay *et al*., 1993). The zone of inhibition of *S. platensis* extracts against bacteria was ranged between 10 mm to 20 mm algae have provided a source of inspiration for novel drug compounds for large contributions to human health and well being. Algal compounds are of great interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agent. The algal derived medicines are widely used because they are relatively safer than the synthetic substitute and they are easily available and cheaper

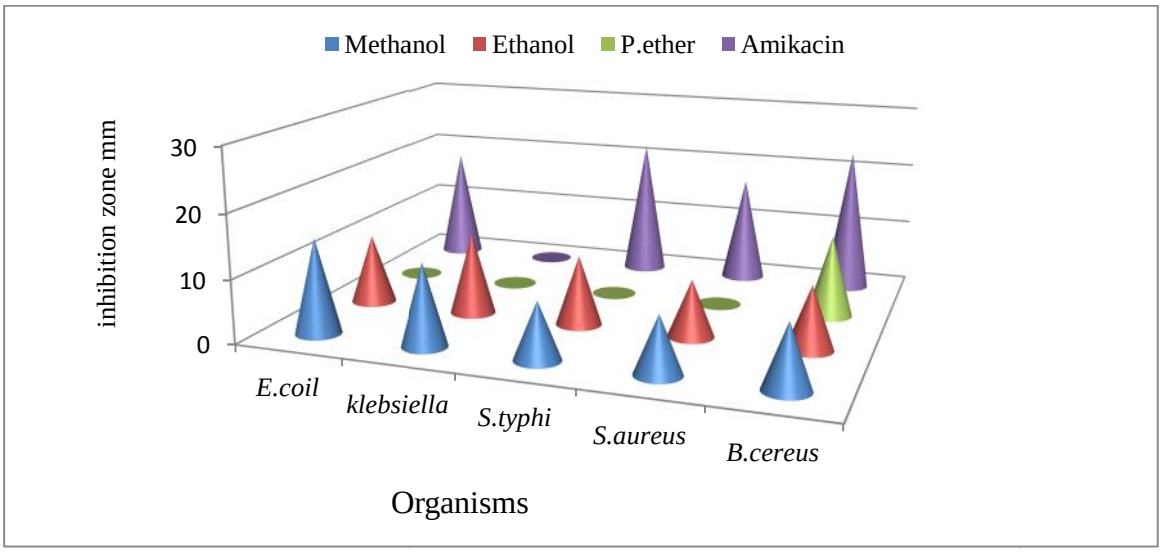
The antibacterial activity of three crude extracts (methanol, ethanol and petroleum ether) of *S. platensis* grown using *S. wightii* bio fertilizer against five bacterial pathogens were determined by paper disc diffusion method and the results were tabulated in Table 1, and their zone of inhibition compared with standard antibiotic amikacin. It is clear from the tables that the diameter of the inhibition zone depends mainly on the types of algal strains, type of solvent used and tested microbes. Among the three different crude extracts assayed, *Klebsiella pneumoniae* antibacterial activities were observed only in methanol and ethanolic extracts of *S.platensis* grown using *S.wightii* bio fertilizer. Methanolic extract of *S. platensis* showed maximum antimicrobial activity of 15.0mm against *E. coli*, followed by 13.0mm activity in and a minimum activity of 9.0mm against *S.aureus*. Ethanolic extract of *Spirulina platensis* also showed the highest biological activity of 13.0 mm against *Klebsiella pneumoniae*, minimum activity of 9.0mm against *S.aureus.* In the present study, antimicrobial activity of *S.platensis*, the methanolic extract of *S.platensis* showed maximum activity against bacterial pathogens when compared to the other solvent extracts.

**Table 1: Antibacterial activity of different solvent extracts of *S.wightii* supplemented *S.platensis***

| Test organism | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | **Methanol** | **Ethanol** | **petroleum ether** | **Control (Amikacin)** |
| *E. coli* | 15 | 11 | 0 | 18 |
| *K. pneumonia* | 13 | 13 | 0 | R |
| *S. typhi* | 0 | 11 | 0 | 22 |
| *S. aureus* | 9 | 9 | 0 | 17 |
| *B.cereus* | 10 | 10 | 13 | 23 |

In *S. platensis*, among the 2 crude extracts showing antibacterial activity, highest was observed in methanolic extracts. Ethanolic extracts shows activity against all the pathogens tested. Petroleum ether extract of *S. platensis* grown using *S.wightii* bio fertilizer was fail to show activity against the selected five pathogens.

**Fig.1 Antibacterial activity of *S.platensis* cultured in Zarrouk's medium supplemented with *S.wightii***




Many investigations mentioned that the methanol extracts of *Nostoc muscorum* revealed antibacterial activity on *Sclerotinia sclerotiorum* by Ishida *et al*., (1997). Also the methanolic extract of a blue green alga has been investigated by Kumar *et al*., (2006) for *in vitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus* and *Rhizopus nigricans* using agar cup diffusion method. The antimicrobial activity of methanolic extract of *S. platensis* was also explained by Demule *et al*., (1996) due to the presence of gamma- linolenic acid and compound was also present in the methanol extract.The minimum inhibition was observed in *S. aureus* (9mm) and *Escherichia coli* (9mm) in methanolic extract. High antimicrobial activity of phenolic compounds extracted with methanol from *S. platensis* against Gram positive *Staphylococcus aureus* (Vinay Kumar *et al*., 2013).

## Conclusion

Antibacterial activity of different crude extracts were examined against five different human pathogens by the paper disc diffusion method. Among the three different extracts examined, the antimicrobial activity determined in terms of inhibition zone diameter and antibacterial activity varied in different solvent extracts. The maximum microbicidal activities observed in the crude methanolic extract of *S. wightii* supplemented *S. platensis* than ethanol and petroleum ether, variability in the antibacterial activity may be due to the presence of active biomolecules and

combined effect of *S. wightii* and *S. platensis.* This review of the study have revealed that combined effect of *S. wightii* and *S. platensis.*

## References

Abd El-Baky, H.H., El Baz, F.K., El-Baroty, G.S., 2006. Over-production of Lipid Rich in g-Linolenic Acid by Blue Green Alga *Spirulina maxima* and its Inhibitory Effect on Carcinoma Cells. Ad. Food Sci., 4: 206-212.

Wu, L.C., J.A.A. Ho, M.C. Shieh and W. Lu, 2005. Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* extracts. *Journal of Agriculture and Food Chemistry*, 53(10): 4207-4212.

Schwartz RE, Hirsch CF, Sesin DF (1990). Pharmaceuticals from cultured algae. *Journal of Industrial Microbiology*; 5: 113–124.

Vonshak A, 1997 ed. *Spirulina platensis (Arthrospira): Physiology, Cell-Biology and Biotechnology*; 233.

Demule MCZ, Decaire GZ, Decano MS (1996). Bioactive substances from S*pirulina platensis. Int. J. Exp.*, 58: 93-9

Ozdemir, G., Dolay, M., Kücükakyüzk, K., Pazarba B., Yilmaz, M., 2001. *J. Fish. Aquat. Sci.*,18(1): 161-166.6.

Ishida K, Matsuda H, Murakami M, Yamaguchi K (1997). Kawaguchipeptin B. and Antibacterial cyclic undecapeptide from the cyanobacterium *Microcystis aeruginosa. J. Nat. Prod*., 60: 724-726.

Kumar P, Angadi S, Vidyasagar G (2006). Antimicrobial activity of blue green and green algae. *Ind. J. Pharm. Sci*., 68: 647-648.

Vinay Kumar, Prem SaranTirumala, Abha Singh, A.K. Bhatnagar , J.N. Shrivastava , Natural Compounds from Algae and *Spirulina platensis* and its Antimicrobial Activity *Indo Global Journal of Pharmaceutical Sciences,* 2013; 3(3): 212-223.

Khan M, Ather A, Thompson K, Gambari R (2005). Extracts and molecules from medicinal plants against herpes simplex viruses. *Antiviral Res*.; 67: 107-19.

Bhadury P, Wright PC. 2004. Exploitation of marine algae: biogenic compounds for potential antifouling applications. *Planta*; 219(4):561-78.

Miranda MS, Cintra RG, Barros SBM, Filho JM. Antioxidant activity of the microalga *Spirulina maxima. Brazilian Journal of Medical and Biological Research*; 1998. 31: 1075–1079.

Belay, A., Ota, Y., Miyakawa, K., Shimamatsu, H., 1993. Current knowledge on potentialhealth benefits of *Spirulina. J. Appl. Phycol.,* 5: 235-241.

Belay A, Ota Y, Miyakawa K, Shimamatsu H. 1994. Production of high quality *Spirulina* at Earthrise Farms. In: Phang *et al.,* eds. *Algal Biotechnology in the Asia-Pacific Region.* University of Malaya. 92-102.

DeivyClementino de Lima, PaulaAlvarezAbreu, Cý´ cero Carlos de Freitas, Dilvani Oliveira Santos, RodrigoOliveira Borges, Tereza Cristina dos Santos, LúcioMendes Cabral, CarlosR. Rodrigues and Helena, 2005. Carla Castro: *AlternatMed*., 2: 39-47.

Ozdemir, G., Dolay, M., Kücükakyüzk, K., Pazarba B., Yilmaz, M., 2001. *J.Fish. Aquat. Sci., 18(1): 161-166.*

Kulik, M.M., 1995.The potential for using cyanobacteria (blue green algae) and in the biological control of plant pathogenic bacteria and fungi. *Eur. J. Plant Path*., 101(6): 585-599.

Chessbrough, M., 2000. Medical laboratory manual for Tropical countries, Linacre House, *Jordan Hill, Oxford.*

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol*.,45(4): 493-496.

Glombitza KW, Koch M. 1989. Secondary metabolites of pharmaceutical potential. In: Cresswell RC, Rees TAV, Shah N, eds. *Algal and Cyanobacterial Biotechnology*. 161–238.

Smit AJ, (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review Phycol. Mental retardation and developmental disabilities research reviews. 16:245-62.

Estrada JE, Bescós P, Villar Del Fresno AM (2001). Antioxidant activity of different fractions of *Spirulina platensis* protean extract. *Il Farmaco*; 56: 497–500.