Antibacterial activity of *Spirulina platensis* supplemented with *Sargassum wightii*, panchagavya andbanana peel extract

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Abstract **-** *Spirulina platensis*, a cyanobacterium, attracted attention to many scientists due to its beneficial medicinal applications. *Spirulina* or its extracts are having several biological activities like anticancer, antiviral, antioxidant and antimicrobial activities. In the present study, antibacterial activity of *Spirulina platensis* solvent extracts was investigated against pathogenic bacteria. The antibacterial effect of ethanol, methanol, petroleum ether and extract of *Spirulina platensis* showed the antibacterial activities against different bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Escherichia* coli were tested, using disc diffusion method. Methanolic extract of *Spirulina platensis* showed maximum anti bacteriall activity of 15.0 mm against *E. coli* and a minimum activity of 9.0 mm against *S. aureus* and *B. subtilis*. Ethanolic extract of *Spirulina platensis* also showed the highest biological activity of 13.0 mm against *Klebsiella pneumoniae*), minimum activity of 9.0 mm against S. *aureus* and *B. subtilis*. 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**.**

Key words: *Spirulina platensis*, Antibacterial activity, *Sargassum*, bio fertilizer

I. INTRODUCTION

Microalgae grow in soil unfit for agriculture and livestock and in lakes or ponds located in inhospitable lands, such as deserts, which are usually unsuitable for generating any kind of food. Microalgae can double their biomass in a period of 3-5 days, achieving high yields (Chisti, 2007). After harvesting and drying of the biomass, the final state of the product is a powder. According to the chemical composition of microalgae, the biomass may have several applications. This alga is being widely studied, not only for nutritional reasons but also for its reported medicinal properties (Hirahashi *et al.*, 2002; Subhashini *et al.*, 2004), antimicrobial activities (Demule *et al.*, 1996; Ozdemir *et al.*, 2004) as well as to inhibit the replication of several viruses, such as Herpes simplex and HIV-1.

The conventional treatment of fungal diseases is limited in comparison with antibiotic therapy for bacterial infection. The majority of clinically used antifungal have various drawbacks in terms of toxicity, efficacy, cost and their frequent use has led to the emergence of resistant strains. For centuries it has been known that the *Spirulina platensis* is a very promising source of anti-bacterial, anti-fungal, anticancer, antiviral and anti-plasmodial activities (Ozdemir *et al.*, 2004). Thus, there is an urgent need to develop alternative biodegradable agents, which could be free from side effects. This search prompted the exploration of natural algal product that could be non-toxic antimicrobial agents with microbial toxic properties and interest to antimicrobial activity and its structural characterization of bioactive constituents. The *in vivo* and *in vitro* studies indicate that the *Spirulina* supplement is an antioxidant agent but is mostly a pro-inflammatory agent. It enhances antioxidant activity and promotes the production of antibody and cytokines in both healthy animal models and disease models (Bolanho *et al.*, 2014; Deng and Chow, 2010). Efforts were made by several workers in India and abroad to test bioactive substances from *Spirulina*. Considering these, one of the objectives of the present work is examining the antibacterial activity of the microalgae *Spirulina platensis.*

II. REVIEW OF LITERATURE

Antibacterial activity means the substance which kills or inhibits the growth of bacteria such as *E.*

coli, Pseudomonas, Bacillus etc. Antibacterial drugs either kill microbes is known as a Bcteriocidal. The history of antimicrobials begins after the observations of Pasteur. The discovery of antimicrobials by Fleming like penicillin and tetracycline covered the way for better health for millions of people around the world. Before thousands of years ago, human has known about the advantage of drugs from nature. Plant extracts, for the treatment of various diseases, were extremely regarded by the ancient civilizations (Grabley and Thiericke, 1999). Even today, many plant materials remain play an important role for treating illnesses, including infectious diseases, and many of these plant materials have been investigated for novel drugs or templates for the production of new therapeutic agents. Plants produce about 7000 different pharmaceutically and important compounds and a number of top selling drugs of present time which helps to treat many infectious diseases, e.g., quinine, artemisinin, taxol etc., (Tshibangu *et al*., 2002). There are more than 120 plant derived drugs approved worldwide, and they come from only 95 plant species. Out of the 250000 species of flowering plants, only 5000 plants have pharmaceutical activity (Lewington, 1990). A number of investigations show an important role of plants to produce several antibacterial compounds and antibiotics. Cyanobacteria have drawn much attention as prospective and rich source of biologically active constituents and have been identified as one of the most promising groups of organisms to be able of producing bioactive compounds. Screening of cyanobacteria for antibiotics and other pharmacologically active compounds have received considerable attention (Borowitzka, 1995).

The search for cyanobacteria with antimicrobial activity has gained importance in recent years due to growing worldwide concern about alarming increase in the rate of infection by antibiotic-resistant micro-organisms. Among cyanobacteria, *Spirulina platensis* is gaining more and more attention not only for the food aspect but also for the development of potential pharmaceuticals (Quoc & Pasuan, 1996). Several studies have focused on physiological properties of some valuable antiviral or antioxidant compounds in blue green alga *Spirulina* (Ozdemir, *et al*.,2004, Sudha *et al.*, 2006.). Among algal species, *Spirulina* has been reported to prevent oxidative damage by scavenging free radicals and active oxygen, and hence can indirectly reduce cancer formation in human body. In this respect, the increased consumption of foods characterized by free radical scavenging activity, leads up to a doubling of protection against many common types of cancer formulation (Cooke, 2002). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural source many based on their uses in traditional medicine. The main aim of the present work was to study the antibaterial activity of *Spirulina.*

III. MATERIALS AND METHODS

A. Preparation of test organisms.

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

B. 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 2 hours 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 (plate - 1, 2, 3).

IV. RESULT AND DISCUSSION

The present *in vitro* study focused on determining the antibacterial activity of the efficacy of different solvents methanol, ethanol and petroleum ether extracts of *S. platensis* cultured in Zarrouk's medium supplemented with of *Sargassum wightii, panchagavya* and *banana* peel extract against standard strains of *E. coli, K. pneumoniae, S. typhi, A. aureus* and *B. cereus.*

The antimicrobial properties of the methanol, ethanol and petroleum ether extracts of *Sargassum wightii* were evaluated by paper disc diffusion method against different microbial isolates. The antibacterial activity of different solvent extracts of *Sargassum wightii* against the pathogenic bacteria showed varied levels of

inhibition. As shown in Table 2 among the solvent extracts tested, methanol extract had a broad spectrum of activity against all the bacteria tested and showed the highest zones of inhibition against *E. coli* (15.0 mm), *K. pneumoniae* (13.0 mm), *A. aureus* (15.0 mm) and *B. cereus* (16.0 mm). The least zone of inhibition was observed with the petroleum ether extract which showed an inhibition of 13.0 mm against *B.cereus*and did not show any inhibition against *E. coli*, *K. pneumoniae*, *S. typhi*, and *A. aureus*. The moderate zone of inhibition was observed with the ethanol extracts of *S. wightii* which exhibited an inhibition of *E. coli* (11.0 mm), *K. pneumoniae* (13.0 mm), *A. aureus* (11.0 mm), *S.aureus* (9.0 mm)and *B. cereus* (16.0 mm).

The efficacy of various solvent extracts of *S. platensis* cultured in Zarrouk's medium supplemented with Fertilizer *Panchagavya* against the pathogenic bacteria showed varied levels of inhibition. Among the various extracts, maximum *in vitro* inhibition of the tested bacteria *E. coli, K. pneumoniae, S. typhi, A. aureus* and *B. cereus* was achieved in methanol extract with zones of inhibition of 13.0 mm, 12.0 mm, 10.0 mm, 10.0 mm, and 16.0 mm respectively (Table 3).Ethanol extract showed an inhibition of 13.0 mm against *E. coli*, 12.0 mm against *K. pneumonia*, 10.0 mm against *A. aureus* and 16.0 mm against *B. cereus*. As with other solvent extracts tested, a zone of inhibition greater than 16.0 mm could not be achieved with any of the bacteria. The least inhibition was observed with the methanol and ethanol extract of *S. platensis*

Table 1- Antibacterial activity of *S. platensis* cultured in Zarrouk's medium

Microorganisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Petroleum ether	Amikacin
E. coli	**13±0.30**	**11±0.31**	**-**	**21±0.13**
K. pneumonia	**11±0.14**	**12±0.13**	**-**	**R**
S. typhi	**13±0.25**	**12±0.12**	**-**	**24±0.15**
S. aureus	**9±0.36**	**9±011**	**-**	**18±0.14**
B. cereus	**16±0.20**	**15±0.19**	**-**	**22±0.16**

Table 2 - Antibacterial activity of *S. platensis* supplemented *S. wightii*

Microorganisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Petroleum ether	Amikacin
E. coli	**15±0.26**	**11±0.3**	**-**	**21±0.18**
K. pneumoniae	**13±0.2**	**13±0.15**	**-**	**R**
S. typhi	**-**	**11±0.17**	**-**	**24±0.15**
S. aureus	**15±0.11**	**9±0.26**	**-**	**18±0.16**
B. cereus	**16±0.15**	**13±0.43**	**13±0.10**	**22±0.17**

against *S. typhi* and *A. aureus.* Petroleum ether extract of *S. platensis* did not show any inhibition against *E. coli, K. pneumoniae, S. typhi, A. aureus* and *B. cereus.*

Antibacterial activity of *S. platensis* cultured in Zarrouk's medium supplemented with Banana peel extract in different solvent extracts against the pathogenic bacteria. The highest antibacterial activity as indicated by the zone of inhibition was achieved with Methanol extract which showed inhibition zones of 12.0 mm, 9.0 mm, 8.0 mm, and 8.0 mm against *E. coli, K. pneumoniae* and *B. cereus* respectively. Among the bacteria tested, *E. coli*, and *K. pneumoniae* showed maximum susceptibility to *S. platensis* methanol extract. *S. aureus* and *B. cereus* showed

Table 3 - Antibacterial activity of *S. platensis* supplemented with panchagavya

Microorganisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Petroleum ether	Amikacin
E. coli	**13±0.10**	**13±0.15**	**-**	**21±0.14**
K. pneumoniae	**12±0.45**	**12±0.30**	**-**	**R**
S. typhi	**10±0.05**	**-**	**-**	**24±0.18**
S. aureus	**10±0.37**	**10±0.28**	**-**	**18±0.23**
B. cereus	**16±0.15**	**16±0.45**	**-**	**22±0.21**

Plate: 1 **Plate: 2** **Plate: 3**

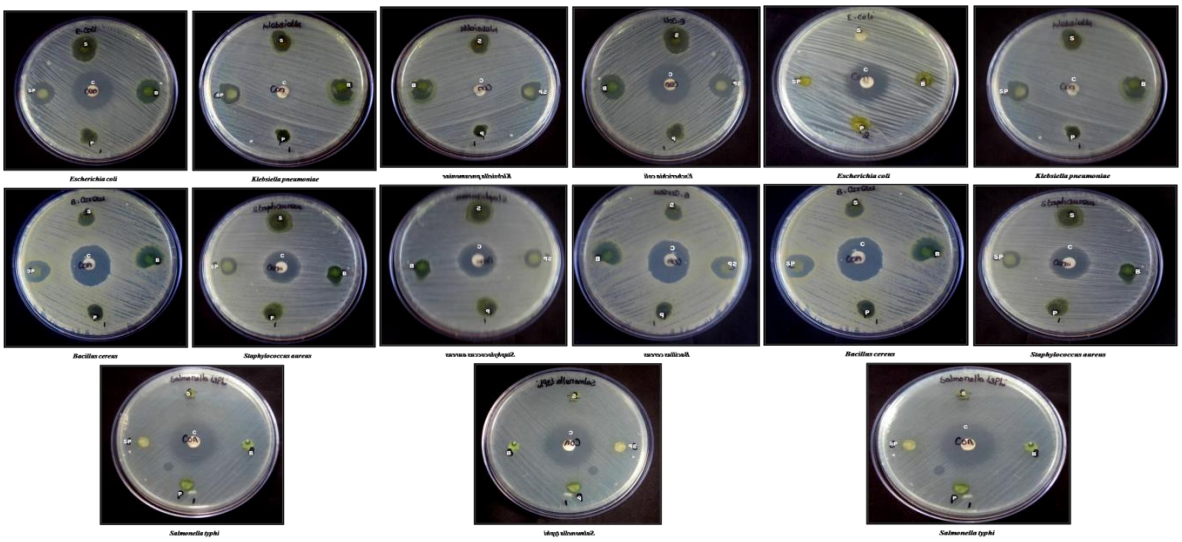

Plate: 1 Antibacterial activity of *S. platensis* from the methanol extracts
(In plate C - Control, S - *S. wightii*, B - Banana, P - *Panchagavya*, SP - *S. platensis*)

Plate: 2 Antibacterial activity of *S. platensis* from the ethanol extract
(In plate C - Control, S - *S. wightii*, B - Banana, P - *Panchagavya*, SP - *S. platensis*)

Plate: 3 Antibacterial activity of *S. platensis* from the petroleum ether extract
(In plate C - Control, S - *S. wightii*, B - Banana, P - *Panchagavya*, SP - *S. platensis*)

the least antibacterial activity. *S. typhi* did not show any inhibition against *S. platensis* methanol extract. Petroleum ether extract of *S. platensis* had no activity against the pathogenic bacteria (Table 4).

Table 4 - Antibacterial activity of *S. platensis* supplemented with banana peel extract

Microorganisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Petroleum ether	Amikacin
E. coli	**12±0.15**	**13±0.2**	**-**	**21±0.12**
K. pneumoniae	**9±0.40**	**13±0.17**	**-**	**R**
S. typhi	**-**	**-**	**-**	**24±0.18**
S. aureus	**8±0.36**	**12±0.25**	**-**	**18±0.13**
B. cereus	**8±0.11**	**14±0.36**	**-**	**22±0.17**

The inhibition values of methanol, ethanol and petroleum extract of *S. platensis* cultured in Zarrouk's medium *against* the tested five bacteria are represented in Table1. Maximum antibacterial activity was observed against *B. cereus* followed by *S. typhi, E. coli, K. pneumoniae* and *S. aureus* with an inhibition of 16.0 mm, 13.0 mm, 13.0 mm, 11.0 mm and 9.0 mm, respectively. Ethanol extract revealed a minimum antibacterial activity against *E. coli* (11.0 mm), *K. pneumonia* (12.0 mm), *S. typhi* (12.0 mm), *A. aureus* (9.0 mm) and *B. cereus* (15.0 mm). Zero growth inhibition was exhibited by petroleum ether extract.

V. SUMMARY AND CONCLUTION

One of the ongoing problems scientists and medical workers face in the fight against infectious diseases is the development of resistance to the agents used to control them. There has been a remarkable progress in the prevention, control and even eradication of infectious diseases with improved hygiene and development of antimicrobials and vaccines. However, infectious diseases still remain a leading cause of global disease burden with high morbidity and

mortality, especially in the developing world. Furthermore, there have been threats of new diseases during the past three decades due to the evolution and adaptation of microbes and the re-emergence of old diseases due to the development of antimicrobial resistance and the capacity to spread to new geographic areas. The impact of the emerging and re-emerging diseases in India has been tremendous at the socioeconomic and public health levels. Their control requires continuing surveillance, research and training, better diagnostic facilities and improved public health system. Emerging and re-emerging zoonotic diseases, food borne and waterborne diseases and diseases caused by multi-resistant organisms constitute the major threats in India (Chugh,2008). The search for cyanobacteria with antimicrobial activity has gained importance in recent years due to growing worldwide concern about alarming increase in the rate of infection by antibiotic-resistant microorganisms. Biologically active substances were proved to be extracted by Cyanobacteria. The present study concluded that organic fertilizers can be used as a good source of nutritive media for *Spirulina* culture at domestic level. Active ingredients present in *S. platensis* have diverse biological activity. Identification of these active ingredients present in *S. platensis* may be interesting topic to study antimicrobial activity of *S. platensis*

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