Antimicrobial Properties of *Spirulina Platensis* Extract Against Certain Bacterial Pathogens

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ABSTRACT

The microalga, *Spirulina platensis* (Single Cell Protein) has been used by human because of its nutritional and possibly medicinal effects. It contains high protein content, vitamins (A, D, E, K, and B complex vitamins), beta-carotene, manganese, zinc, copper, iron, selenium, and gamma linolenic acid. Numerous studies reported that *Spirulina* contains biological properties such as immuno modulation, antioxidant, anticancer, antimicrobial, and probiotic effects. The aim of the present study is to analyze the antibacterial activity of *Spirulina platensis* extract of methanol, ethanol, acetone and hexane against certain human bacterial pathogens *viz*., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Vibrio vulnificus* by paper disc diffusion and agar well diffusion methods. The different extract of *S. platensis* concentrations at 10 µg were used to analyze the antibacterial activity in human bacterial pathogens. In the present study, two different diffusion techniques like paper disc and agar wells were used to find effect of *S. platensis* against bacterial pathogens. Methanol extract of *S. platensis* showed highest inhibition zone against bacterial pathogens compare to other extracts in both techniques.

Key words – *Spirulina platensis*, antibacterial activity, bacterial pathogens.

Introduction:

Spirulina platensis is a blue green alga which comes under the family cyanobacterium. It is a bluish green colored, spiral shaped, microscopic, multicellular, edible belonging to the class Cyanophyta. It is known for the rich sources of protein, vitamin etc. which is present in the algae. *Spirulina* algae exist in various types of habitats like sea water, fresh water, brackish water, waters from industries and domestic uses etc. *Spirulina* contains 50-70% of protein which is higher than other food substances. In recent years, the antimicrobial activity of cyanobacteria has gained importance due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Borowitzka, 1995). Cyanobacteria found to be a rich source for various products for commercial and pharmaceutical interest. It also contains primary metabolites such as proteins, fatty acids, vitamins, pigments (Borowitzka, 1988a) and various secondary metabolites with different bioactivities such as antifungal, antiviral, antibiotic, and other properties (Patterson *et.al*., 1994). *Spirulina platensis* or its extract show therapeutic properties, such as the ability to prevent cancers, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation (Belay et. al., 1993). The aim of the present

study is to analyze the antibacterial activity of *Spirulina platensis* extract of methanol, ethanol, acetone and hexane against human bacterial pathogens *viz*., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Vibrio vulnificus*

Materials and Methods:

Algal source:

The *Spirulina platensis* culture which is used in this research was obtained from CAS in Marine Biology, Annamalai University, Parangipettai, Chidambaram and was maintained in Zarrouk's medium at 30°C. Samples were then sun dried and ground in pulverizer to get coarse powder. Subsequently, the powdered samples were stored in refrigerator.

Extraction of *S. platensis*

Sun dried *S. platensis* sample was extracted by soxhlet apparatus using four different solvents (methanol. ethanol, acetone and hexane) separately at the ratio of 0.5:10 w/v. 50 g of dried Spirulina packed separately in Soxhlet apparatus containing 300 ml of solvents (methanol, chloroform, acetone and hexane) and extracted continuously for 72 hrs. The resulting extracellular extracts of *S. platensis* from these different solvents were covered with aluminum foil and stored at 40°C until dried. Dried extracellular extracts were diluted in various concentration levels using dimethyl sulfoxide (DMSO), such as 2, 4, 6, 8, and 10 µg.

Collection of bacterial pathogens

In vitro antibacterial studies were carried out against four different bacterial pathogens, *viz., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Vibrio vulnificus* which were obtained from the RMMCH, Annamalai University, Annamalai Nagar, Chidambaram, India. Bacterial cultures were emulsified in normal saline.

Maintenance of Test culture:

The bacterial isolates were cultured and maintained on Nutrient agar slants and stored in refrigerator.

Bacterial inoculum preparation:

Inoculum was prepared by inoculating a loopful of bacterial culture in Nutrient broth and incubated at 37°C 3-5 hours till a moderate turbidity is developed.

Paper Disc Preparation:

Preparation of *Spirulina platensis* discs for Antibacterial activity:

Using sterile Whatman No.1 filter paper 6 mm diameter *Spirulina* discs were prepared. The *Spirulina platensis* extract (5 mg/ml) obtained using solvents *viz*., methanol, ethanol, hexane and acetone were mixed with 1ml of 5% DMSO. The discs were impregnate with 10µl of different solvent extracts to check the antibacterial activity.

Paper Disc diffusion method:

The antibacterial activity of *Spirulina platensis* extracts was determined by disc diffusion method proposed by Bauer *et al.*,1966. Petri plates were poured using Mueller Hinton Agar and allowed to solidify. The plates were used for the susceptibility test for bacteria. Plates were dried, and 0.1 ml of the inoculums was poured on to the plate and distributed uniformly. After pouring the inoculums the plates were kept for drying. After drying the discs with the extracts were placed on the plate with forceps and gently pressed to contact with agar. The plates were incubated at 37ºC for 24 hours. The zone of the inhibition is observed and measured in millimeters.

Agar well Diffusion method:

Agar well diffusion technique was determined by following the method of Shammuga *et al.*, 2004. Different concentration of methanol, ethanol, acetone and hexane extracts of *Spirulina platensis* were tested against bacterial pathogens. Mueller Hinton agar were poured in sterile petri plates and kept for solidification. Using a cork borer wells were made in the Mueller Hinton agar and the algal extracts of different solvent extracts were poured in the respective wells. After pouring of extracts the plates were kept for incubation. After incubation period the zone of inhibition were measured, and the values are tabulated.

Results and discussion:

Paper disc diffusion method:

The antimicrobial activity in terms of inhibition zone exhibited by the *S. platensis* extracted from methanol, ethanol, hexane and acetone against bacterial pathogens viz., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Vibrio vulnificus*are shown in Fig.1.The effect of *S. platensis* extracts on bacterial pathogens was studied by using disc diffusion method. The results showed that methanol extracts at 10µg concentration recorded higher inhibition zone in *E.coli* of 24.0 mm followed by *Staphylococcus aureus* (21.0 mm) and *P.aeruginosa* (20.0 mm) and least was observed in *V.vulnificus* (18.0 mm). In ethanol extract the maximum zone of inhibition was observed in *S.aureus* (20.0 mm), followed by *E.coli* (18.0 mm), *V.vulnificus* (17.0 mm) and *P.aeruginosa* (15.0mm). In hexane extract *V.vulnificus* augmented maximum inhibition zone of 19.0 mm, followed by *S.aureus* (18.0 mm), *P.aeruginosa* 17.0 mm, *E.coli* 15.0 mm respectively. The least values of inhibition zone were noticed in acetone extract of S. platensis against all bacterial pathogens. Among the different extracts of *S. platensis*, the methanol extract performed higher potential of inhibition zone towards bacterial pathogens followed by ethanol, hexane and acetone extracts. Species like *E. coli, S. aureus*and *P. aeruginosa* were highly susceptible to the methanol extracts whereas moderate susceptibility noticed in *S. aureus* and *E. coli* in ethanol extract.

Agar well diffusion method:

In vitro study of antimicrobial properties of *S. platensis* extracts from methanol, ethanol, hexane and acetone against bacterial pathogens was carried out by adopting agar well diffusion technique. The results of present study are shown in fig.2. Among the different extracts of *S. platensis*, methanol extract showed maximum zone of inhibition compare to other extracts against the bacterial pathogens. Bacterial pathogen E.*coli* exhibited higher inhibition zone (26.0 mm), followed by *S.aureus* (24.0 mm), *P.aeruginosa* (23.0 mm), *V.vulnificus* (21.0 mm) respectively. Next to methanol, hexane extract showed maximum antibacterial activity against all bacterial pathogens. The zone of inhibition for *S.aureus* (21.0 mm), *E.coli* (19.0 mm),

P.aeruginosa (18.0 mm) and *V.vulnificus* (17.0 mm) were noticed in hexane extracts of *S. platensis*. Ethanol extract of *S. platensis* showed antibacterial activity next to hexane. Whereas acetone extract exhibited least antimicrobial activity.

Discussion:

Spirulina platensis is one of the most important microalgae showed antimicrobial activity against many pathogenic bacteria and fungi (Kumar *et. al*., 2011). Extracts from *S. platensis* inhibited the growth of *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, S. typhi,* and *Klebsiella pneumoniae*. They used hexane, ethyl acetate, dichloromethane, and methanol to obtain the phenolic extracts, and the methanolic extracts exhibited maximum activity (Kaushik and Chauhan, 2008). Parisi*et al., (*2008) also found high antimicrobial activity of phenolic compounds in methanol extracts from *S. platensis* against *S. aureus.* Bloor and England (1991) reported that extracellular metabolites produced by *Nostoc muscorum* inhibited the growth of *Bacillus circulans*. *Spirulina* has been studied because of its therapeutic properties and the presence of bioactive compounds (Belay*et. al*.,1993). Agustini *et al.,* (2015) analyzed the proximate composition of phytochemicals and reported that dried samples of *Spirulina* contain high protein and ash content when compared with fresh samples. The methanolic extract of a blue-green alga has been investigated (Kumar *et. al.,*2006) for *in vitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, E. coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus,* and *Rhizopus nigricans* using agar well diffusion method with best activity. In the present study also, methanol extracts showed maximum inhibition zone against bacterial pathogens *viz., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Vibrio vulnificus.*

Conclusion:

Based on the results which occurred on the research it shows the antimicrobial properties of *S. platensis* against pathogenic microorganisms. Among the different diffusion techniques, agar wells diffusion technique performed better in antimicrobial properties against bacterial pathogens and methanol extract of *S. platensis* shows highest inhibition zone against bacterial pathogens compare to other extracts in both techniques. It was concluded that, methanol extract of *S. platensis* could be effectively used to control the bacterial pathogens for in vitro study.

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Fig.1 Antibacterial activity of *Spirulina platensis* extracts (Paper disc diffusion method)

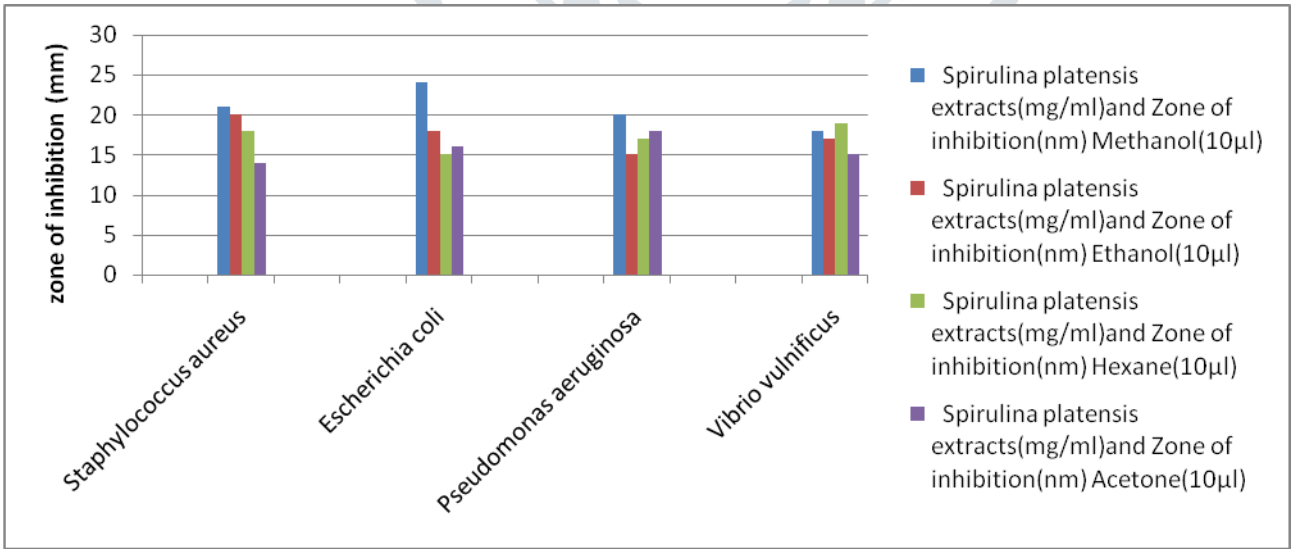

Fig.:2 Antibacterial activity of *Spirulina platensis* extracts (Agar well diffusion method)