

## ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACTS OF THE *SPIRULINA PLATENSIS* AGAINST *VIBRIO FLUVIALIS*

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### ABSTRACT

The aim of the present study was to evaluate antibacterial activity of the crude extracts of *Spirulina platensis* against a marine pathogen. Crude algal extracts were prepared in water, ethanol and acetone with different concentrations and tested against *Vibrio fluvialis*. Highest activity was observed in the water extracts and the least in acetone. Aqueous extracts showed highest zone of inhibition of 19mm in diameter followed by ethanol extract and acetone extract which revealed zone of inhibition of 15mm and 09mm in diameter respectively. Average minimum inhibitory concentration of water, ethanol and acetone extract had shown 250µg/ml, 500µg/ml and 750µg/ml concentration respectively.

**Key Words:** *Spirulina platensis*, *Vibrio fluvialis*, Antibacterial activity, Minimum inhibitory concentration, Water extract, Ethanol extracts.

### INTRODUCTION

*Spirulina* or *Arthrospira* is a microscopic and filamentous blue-green alga (syn. *Corallina rubens* L., family Corallinaceae, Rhodophyta) commonly distributed in Greece (Nigrita, Serres), Japan, India, United States and Spain [1]. It has attracted attention of many scientists due to its beneficial medicinal applications.

*Spirulina* or its extracts have been investigated for treatment of many diseases like diabetes, hypercholesterolemia and atherosclerosis [2-4] as well as in reducing the body weight in obese human subjects [5]. It has been well documented that *Spirulina* exhibits anti-inflammatory properties by inhibiting the release of histamine from the mast cells [4,5]. The active component of aqueous extract of *S. platensis* (calcium spirulan) showed to inhibit *in vitro* replication of several enveloped viruses including Herpes simplex type I, human cytomegalovirus, measles and mumps virus, influenza A virus and human immunodeficiency virus-1 virus (HIV-1). Another more recent study showed that an aqueous extract of *S. platensis* inhibited HIV-1 replication in human T-cells, peripheral blood mononuclear cells and Langerhan cells [6].

*Vibrio fluvialis* is a gram-negative, rod shaped bacterium commonly found in the aquatic environments and seafood. This organism has been reported as an emerging pathogen associated with cholera-like diarrhea in India and China. It is also associated with a life-threatening septicemia [7,8].

In our present study we have investigated the antimicrobial activity of spirulina extracts against this emerging marine pathogen i.e. *Vibrio fluvialis*.

### MATERIALS AND METHODS

#### *Spirulina platensis* and chemicals:

Powder form of SP was purchased from Siri Farms, Kothapet, Hyderabad, Telanagana-500035, India. It is a spray dried powder, standard in quality and a part of bulk production by the industry. All other chemicals used in this experiment were of analytical grade.

#### Test microorganism

#### Identification of the marine bacterial isolate

*Vibrio fluvialis* was isolated from diarrheal fecal samples from patients admitted in the Malla Reddy Institute of Medical

Sciences, Telangana, India. The organism was identified and confirmed by our clinical microbiologist.

For identification, diluted samples were inoculated into the sterile Thiosulphate Citrate Bile Sucrose (TCBS) agar plates and incubated at 37°C for 24 hours. The colonies thus obtained were subjected to series of tests (such as staining, biochemical tests) for the strain identity.

#### Preparation of Inoculum

Culture of test organisms were used as inoculum. Loop full of test microorganism was taken from the 24 hrs old slant and transferred to a flask containing sterilized nutrient broth and allowed to grow at 37°C. The 18 hr culture was then plated on a nutrient agar plate to study the morphological character. The cultures from nutrient broth were centrifuged and a suspension of cells were made with the sterile saline (10%). This culture suspension was used for further studies.

#### Antibacterial activity assay:

#### Preparation of assay medium

Readymade dehydrated medium supplied by Hi Media was used for testing the antibacterial activity of algal extracts. 3.8 grams of the dehydrated Mueller Hinton agar medium was taken into a conical flask containing 100 ml of distilled water and heated to boiling as per the instructions given by manufacturer. Flask mouth was plugged with cotton and sterilized by autoclaving at 15 lb/sq. inch pressure at 121°C for 20 min.

#### Seeding of culture

To a 20ml of sterile Mueller Hinton agar medium maintained at 40°C in tubes, 0.2 ml of the culture suspension was added. The tubes were mixed thoroughly and poured into sterile plates and care was taken to form a uniform layer. Three different extracts were added into the wells prepared using a sterile cork borer and incubated for 24 hours at 37°C.

#### Determination of MIC:

Five different concentrations of each extract were prepared. 10 ml of sterile nutrient broth was taken into a series of five test tubes and labelled accordingly. 1ml of the extract was added into the respective tube followed by 0.2ml of the inoculum and incubated.

Both positive and negative controls were included. The MIC was measured based on the turbidity obtained after the incubation.

**Controls for the test**

Blank inoculated agar plate with extract was taken as a positive control to check the compatibility of the media constituents with the organism and for the absence of an inhibitory substance. As per I.P the assay medium selected must support the growth of the microorganisms. Agar plate without drug and inoculum was taken as negative control in order to check the sterility of the medium.

**Preparation of *Spirulina platensis* extracts:**

Ten grams of spray dried spirulina powder was added into the conical flasks containing 150ml of respective solvents (water, ethanol and acetone). The extracts were heated in the hot air oven for a period of three hours with intermittent cooling and shaking to avoid overheating. The resultants extracts were then filtered and dried at their respective boiling points of the solvents in a rotary evaporator.

**Phytochemical analysis[9]**

The crude extracts were subjected separately to a preliminary phytochemical tests using standard methods.

**RESULTS AND DISCUSSION**

The phytochemical analysis showed the presence of biologically active compounds like carbohydrates, phenols, triterpenoids, steroids, flavonoids and saponins. The extracts showed the presence of these phytochemicals effectively increased the antibiotic efficacy of *S. platensis*.

**TABLE 1: SUMMARY OF PHYTOCHEMICAL SCREENING**

Chemical constituents	Tests	Algal extract		
		Water	Methanol	Acetone
Alkaloid	Mayer's reagent	-	-	-
	Hager's reagent	-	-	+
	Wagner's reagent	-	-	+
Carbohydrate	Mohlisch's reagent	+	+	-
	Fehling's test	+	+	+
Glycosides	Legal's test	+	+	-
Flavonoid	Shinoda test	+	+	+
Tannin	Ferric chloride test	-	-	-
	Lead acetate test	-	-	-
Saponin	Foam test	+	+	+
Steroids	Lieberman test	+	+	-

**TABLE 2: AVERAGE ZONE OF INHIBITION AGAINST DIFFERENT EXTRACTS OF *SPIRULINA PLATENSIS* AGAINST *VIBRIO FLUVIALIS***

Extracts	Zone of inhibition in diameter
Water extract	19 mm
Ethanol extract	15mm
Acetone extract	9mm

**TABLE 2: AVERAGE MINIMUM INHIBITORY CONCENTRATION OF *SPIRULINA PLATENSIS* EXTRACTS AGAINST *VIBRIO FLUVIALIS* AT 540NM**

Extracts	Concentration of extracts in µg/ml				
	125µg/m	250µg/m	500µg/m	750µg/m	1000 µg /ml
Water extract	+	-	-	-	-
Ethanol extract	++	+	-	-	-
Acetone extract	++	++	+	-	+

- = no growth; + = light growth; ++ = moderate growth.

*Vibrio fluvialis* isolates produced yellow color colonies on TCBS medium. Then the strain was identified through series of sub culturing and analyzing enzymatic properties. It showed a positive reaction to indole production, ONPG (O-nitrophenyl-beta-D-galactosidase), oxidase test and fermentation of glucose, lactose, sucrose, d-mannitol, l-arabinose, maltose, trehalose, d-galactose, and d-galacturonate. Negative findings include lysine decarboxylase test, Voges-Proskauer etc. Characterization of isolates showed gram negative rod shaped halophilic motile bacterium as *Vibrio fluvialis*. Finally the strain identity was confirmed by rapid detection tests and sequencing method.

MIC of water extract, ethanol extract and acetone extract had shown to 250µg/ml, 500µg/ml and 750µg/ml of concentration respectively. The ability of test substance (plant extract) to inhibit bacterial growth was confirmed by the appearance of zone of inhibition around the well containing the test solution after a specified incubation period. The negative control was checked for the absence of growth, thereby indicating the sterility of the medium. The remaining plates were examined for the presence or absence of growth. The positive control without algal extract was checked to ensure that the test strain was capable of showing adequate growth in the medium. In reading the end points, a faint haze of growth of a single colony was evident for antimicrobial activity. A dense film of growth or more than one colony was considered as evidence that the algal extract failed to inhibit the growth.

All the three extracts were found to exhibit inhibitory activity against *Vibrio fluvialis*. The results of MIC and antibacterial activity are summarized in Table 2 & 3. Water extract gave the highest zone of inhibition of 19 mm in diameter followed by ethanol extract with zone of inhibition of 15 mm in diameter and acetone extract provided the lowest zone of inhibition of 9 mm.

Antibacterial activity has been proposed in a number of marine algae which were collected from the coastal areas. The maximum antibacterial activity was reported in the class of Rhodophyceae (80%) followed by the Chlorophyceae (62.5%) and the Phaeophyceae (61.9%) [10]. *Spirulina* is one among them. Antimicrobial compounds found in cyanobacterial exudates include polyphenols, fatty acids, glycolipids, terpenoids, alkaloids and a variety of yet to be described as bacteriocins. Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects. The antimicrobial substances involved may target various kinds of micro-organisms, prokaryotes as well as eukaryotes [10].

Kaushik and Chauhan [11] reported that the extracts of *Spirulina platensis* inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* in different concentrations. It also showed antifungal activity against was fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida tropicalis*, *Candida albicans* and *Candida glabrata*). Vinay Kumar et al. examined the algal extracts *in vitro* for their antibacterial effects against (*Staphylococcus aureus* and *Salmonella typhimurium*) using Agar well diffusion method and Paper disc diffusion method with concentration from 250 ppm upto 7000 ppm was taken and observed all these bacteria showed inhibition in growth by these extracts [12].

Mechanisms of anticancer, antiviral and antimicrobial effects of *Spirulina* are due to its content of endonuclease (which repair damaged DNA), calcium sulfated polysaccharide (which inhibits *in vitro* replication of viruses) and fatty acids (specially high content of  $\gamma$ -linolenic acid), respectively [13]. It was hypothesized that lipids kill microorganisms by leading to disruption of the cellular membrane as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration [14]. This can probably be explained by the strong fabric of the cell wall of Gram-positive bacteria, which maintain their structure in spite of substantial hydrostatic turgor pressure within the bacteria.

## CONCLUSION

This observation may be of practical importance especially in the treatment of secondary infections of intestinal tract where bacteria act as an opportunistic organism. It can therefore be concluded that natural products of marine origin can be exploited for the search of natural pesticides as well as for medicinal products [15].

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