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Comparative study of different strains of *Spirulina platensis* (Geiltler) against some human pathogens

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Abstract

The concept of biological control for health maintenance has received widespread attention during the last few years. In most African countries, traditional phytomedicines are used to control the disease. Various algae are known for their various biological activities. In present study, four different strains of *Spirulina platensis* (Jal Mahal Lake, Ramgarh Lake, Dayalbagh, Rajkot) were isolated from different habitats and tested with three different concentrations prepared in four different solvent extracts (N-hexane, Chloroform, Acetone, Methanol) to check the antimicrobial activity of microbes (*Microsporum canis* MTCC-3270, *M. fulvum* MTCC-7675, *Candida albicans* MTCC-227) and bacteria (*Salmonella typhimurium* MTCC-TA 98, *Staphylococcus aureus* MTCC-96) by using Agar-well diffusion method. The finding in this study reveals that antimicrobial activity of Jal Mahal Lake strain was highly effective in two solvents Acetone and Methanol. The rest of the strains and solvents showed varying degree of inhibition.

Keywords: Antimicrobial, *Spirulina platensis*, Agar-well diffusion method

Introduction

Pharmaceutical drug discoveries, for past 140 years depended largely on the process of empirical screening of large number of pure compounds. Algal organisms are rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry (Ely *et al.*, 2004, Febles *et al.*, 1995, Tuney *et al.*, 2006).

Cyanobacteria are a very old group of organisms and represent relics of the oldest photoautotrophic vegetation in the world that occur in fresh water, marine and terrestrial habitats. 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 has recently received considerable attention (Borowitzka, 1995). The search for cyanobacteria with antimicrobial activity has gained importance in recent years due to growing world wide concern about alarming increase in the rate of infection by antibiotic-resistant micro-organisms. Various active substances with antibacterial, antiviral, fungicide, enzyme inhibiting, immunosuppressive and cytotoxic and algicide activity have been isolated from cyanobacterial biomass (Knubel *et. al.*, 1990; Mule *et. al*., 1991; Gerwork *et. al*., 1994; Jaki *et. al*., 1999). Microalgae, such as *Ochromonas* sp., *Prymnesium parvum*, a number of blue green algae produce toxins that may have potential pharmaceutical application (Katircioglu *et al*., 2006). 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).

Spirulina platensis produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources,

many based on their uses in traditional medicine The aim of the present work was to study the antimicrobial activity of cell extracts of various strains of *Spirulina platensis*

MATERIAL AND METHODS

Collection of sample

S. platensis strains were isolated from four different habitats:

(i) *S. platensis* Jal Mahal Lake strain (Jaipur)

(ii) *S. platensis* Ramgarh Lake strain (Jaipur)

(iii) *S. platensis* Dayalbagh strain (Agra)

(iv) *S. platensis* Rajkot strain (Ahmedabad), and maintained in CFTRI medium at 500 lux intensity of light

The Targeted microbes:

The micro-orgainsms used in antimicrobial assays were supplied by Institute of Microbial Technology (IMTECH). The species employed include pathogenic Gram-positive bacteria (*Staphylococcus aureus* MTCC-96), Gram-negative bacteria (*Salmonella typhimurium* MTCC-TA98), fungi (*Microsporum canis* MTCC-3270, *M. fulvum* MTCC-7675, *Candida albicans* MTCC-227).

Agar-well diffusion method:

Antimicrobial activity of different solvent strains of Spirulina platensis were performend by agar well diffusion method as described by (Shanmuga et al., 2002). Briefly, 1×105 spores/ml of different bacteria was prepared and 0.2 ml spore suspension was spread over the agar surface of the plates. The plates were placed at 27±2°C for 30 min in order to make the agar surface dry. Different conc. of the algal extract was added into the well with the help of sterilized micropipette. The plates were kept in an upright position in an incubator until the extracts diffused in the agar at least for 3-4 hr. These plates were then inverted and further incubated at 27°c for 3-5days.The plates were observed for zone of inhibition (mm) around the wells.

Extraction of *S. platensis*:
Following Khan (1988), different solvent extracts (N-Hexane, chloroform, acetone, methanoll) were prepared. Dry algal mass (lg/15ml) was extracted for 24 hr. in different solvent. All the extracts were preserved at 4°C. Antifungal activity of microalgae was tested with control (Pure solvent).

Results and Discussion

The results obtained from the present study concerning the biological activity of the antimicrobial agents present in *Spirulina platensis* strains against different pathogenic bacteria and fungi used in the study. 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.

The main objective of the work was to evaluate and compare the ability of different strains of *Spirulina platensis* from four different localities of India to find the most effective strains which produce bioactive compounds of potential therapeutic interest. The results of antimicrobial activity against tested pathogen and tabulated Tables from Tables 1 to 4.

In the case of Jal Mahal Lake strain maximum inhibition was observed in Microsporum canis (23mm) in methanol solvent extract followed by Microsporum fulvum and Salmonella typhimurium (21mm) in the same solvent. The minimum inhibition was observed in chloroform extract in Candida albicans and M. fulvum (9mm). The control used was the pure solvent found to be ineffective against all the tested pathogens. Table-1

In the case of Rajkot strain, maximum inhibition was observed in M. fulvum (19mm) in methanol extract followed by 16mm in acetone solvent in M. fulvum. The minimum inhibition was observed in S. aureus (10mm) in acetone extract. In the case of bacteria this strain showed very less effectiveness. Table-2

In the case of Dayalbagh strain, maximum inhibition observed in Acetonic extract of M. fulvum and minimum inhibition (8mm) N-Hexane in S. aureus.Table-3

Finally in the Ramgarh strain, maximum inhibition observed M.canis (19mm) in methanol solvent and minimum inhibition (9mm) in N-Hexane extract against S. typhimurium, M.canis. The minimum inhibition of (9mm) was also observed in chloroform extract of S.aureus and Acetonic extract of C.albicans.Table-4

Table- 1: Zone of inhibition of N-Hexane, Chloroform, Acetone, Methanol extract of Jal Mahal Lake strain of *Spirulina platensis* against different microbes

Name of the Organisms	Solvents	xZone of Inhibition (mm)			
		25µl	50µl	100µl	C (Solvent)
Salmonella typhimurium	N-Hexane	R	R	12	R
	Chloroform	R	R	15	R
	Acetone	R	13	16	R
	Methanol	R	15	19	R
Staphylococcus aureus	N-Hexane	R	R	11	R
	Chloroform	R	R	13	R
	Acetone	R	16	20	R
	Methanol	R	17	21	R
Candida albicans	N-Hexane	R	R	11	R
	Chloroform	R	9	14	R
	Acetone	R	14	15	R
	Methanol	R	12	19	R
Microsporum canis	N-Hexane	R	R	13	R
	Chloroform	R	11	15	R
	Acetone	R	13	20	R
	Methanol	R	16	23	R
Microsporum fulvum	N-Hexane	R	R	12	R
	Chloroform	R	9	17	R
	Acetone	R	16	20	R
	Methanol	R	14	21	R

R=Resistant

Table- 2: Zone of inhibition of N-Hexane, Chloroform, Acetone, Methanol extract of Rajkot Lake strain of *Spirulina platensis* against different microbes

Name of the Organisms	Solvents	Zone of Inhibition (mm)			
		25µl	50µl	100µl	C (Solvent)
Salmonella typhimurium	N-Hexane	R	R	R	R
	Chloroform	R	R	R	R
	Acetone	R	11	13	R
	Methanol	R	R	14	R
Staphylococcus aureus	N-Hexane	R	R	R	R
	Chloroform	R	R	R	R
	Acetone	R	R	10	R
	Methanol	R	R	13	R
Candida albicans	N-Hexane	R	R	R	R
	Chloroform	R	R	11	R
	Acetone	R	R	13	R
	Methanol	R	10	15	R
Microsporum canis	N-Hexane	R	R	R	R
	Chloroform	R	R	12	R
	Acetone	R	11	15	R
	Methanol	R	13	14	R
Microsporum fulvum	N-Hexane	R	R	R	R
	Chloroform	R	10	12	R
	Acetone	R	10	16	R
	Methanol	R	9	19	R

Table- 3: Zone of inhibition of N-Hexane, Chloroform, Acetone, Methanol extract of Dayalbagh strain of *Spirulina platensis* against different microbes

Name of the Organisms	Solvents	Zone of Inhibition (mm)			
		25µl	50µl	100µl	C (Solvent)
Salmonella typhimurium	N-Hexane	R	R	11	R
	Chloroform	R	R	13	R
	Acetone	R	14	16	R
	Methanol	R	15	17	R
Staphylococcus aureus	N-Hexane	R	R	8	R
	Chloroform	R	9	11	R
	Acetone	R	R	16	R
	Methanol	R	12	15	R
Candida albicans	N-Hexane	R	R	9	R
	Chloroform	R	10	14	R
	Acetone	R	12	17	R
	Methanol	R	14	15	R
Microsporum canis	N-Hexane	R	R	11	R
	Chloroform	R	9	17	R
	Acetone	R	14	16	R
	Methanol	R	11	20	R
Microsporum fulvum	N-Hexane	R	R	13	R
	Chloroform	R	11	16	R
	Acetone	R	16	23	R
	Methanol	R	13	19	R

Table- 4: Zone of inhibition of N-Hexane, Chloroform, Acetone, Methanol extract of Ramgarh strain of *Spirulina platensis* against different microbes

Name of the Organisms	Solvents	Zone of Inhibition (mm)			
		25µl	50µl	100µl	C (Solvent)
Salmonella typhimurium	N-Hexane	R	R	9	R
	Chloroform	R	R	11	R
	Acetone	R	13	17	R
	Methanol	R	11	15	R
Staphylococcus aureus	N-Hexane	R	R	R	R
	Chloroform	R	9	13	R
	Acetone	R	10	16	R
	Methanol	R	13	17	R
Candida albicans	N-Hexane	R	R	11	R
	Chloroform	R	R	15	R
	Acetone	R	9	13	R
	Methanol	R	13	17	R
Microsporum canis	N-Hexane	R	R	9	11
	Chloroform	R	12	14	R
	Acetone	R	15	17	R
	Methanol	R	12	19	R
Microsporum fulvum	N-Hexane	R	R	14	R
	Chloroform	R	11	15	R
	Acetone	R	13	16	R
	Methanol	R	12	20	R

There were varied results in all the strain concerning inhibition of the microbes. In the present study it was observed that Acetone and Methanol was found to be the best organic solvent for extracting the effective antimicrobial material from algal strains used in the experiment. The result exhibited by chloroform and N-Hexane was less effective then that by methanol and acetone. The best halo zone produced was in the extract of Jal Mahal lake strain of *S.platensis*. Similar results were obtained by different seaweeds collected from the Vedalai coast, Gulf of Mannar, Tamil nadu wherein, some commonly occurring green algae *Codium adherens*, *Ulva reticulata* and *Halimeda tuna* were evaluated for antibacterial activity (Kulandaivel et. al., 2009). Many investigations mentioned that the methanol extracts of *Nostoc muscorum* revealed antibacterial activity on *Sclerotinia sclerotiorum* (De Mule et al.,

1991: Ishida et al.,1997) also the methanolic extract of a blue green alga has been investigated by (kumar . et al., 2006) for *invitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, E. coli, Pseudomonas aeruginosa, Aspergillus niger, A. flavus* and *Rhizopus nigricans* using agar cup diffusion method

Present investigations is contradictory with the results of Kaushik et al, 2008 because N hexane showed inhibition against *S. aureus* whereas in our findings N-hexane showed no inhibition, it may be due to the production of bioactive compounds due to the seasonal effects, organic solvents used for extraction of bioactive compounds and difference in assay methods and supported by (Raina et al., 2008) according to them extracts of methanol, acetone showed inhibition against *S. aureus* and *C. albicans*. The antibacterial and antifungal activities of the extracts of marine algae were found to exhibit seasonal variations . Marine algae are a rich source of fatty acids and antioxidants and the overall antimicrobial activity assessed from the above results indicates the presence of active constituents in the extractions of algae which can be exploited for the production of lead molecules which are of use in pharmaceutical industry

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