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Original Article

SCREENING FOR ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF *SPIRULINA PLATENSIS*

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Abstract: Various organic and aqueous extracts of Spirulina platensis were screened for their antibacterial activities. The extracts were tested against different species of human pathogenic bacteria by the agar-solid diffusion method. Water extract of Spirulina platensis showed maximum antimicrobial activity of 18.0mm against Klebsiella pneumoniae (NCIM2063) and a minimum activity of 10.0mm against Proteus vulgaris (NCIM2027). All the tested microorganisms were resistant to methanol, ethanol and propanol extracts except Pseudomonas aeruginosa (NCIM2076) and Escherichia coli (NCIM2065) which exhibited a least inhibition zone of 7.0 and 8.0 mm respectively in propanol. Acetone extract of Spirulina platensis also showed the highest biological activity of 17.0 mm against Klebsiella pneumoniae (NCIM2063), moderate activity of 11.0 mm against Salmonella typhi (NCIM2080), and 10.0 mm against Pseudomonas aeruginosa (NCIM2076), Escherichia coli (NCIM2065) and Staphylococcus aureus (NCIM2079). Sequential extract of Spirulina exhibited maximum antimicrobial activity. Inhibition zone of 25.3 mm was observed for Klebsiella pneumoniae and 16.0 mm for Proteus vulgaris. FTIR Spectrum of sequential extract showed strong bands at 1047,1383,1640,2361, and 3450 cm^{-1}.

Key words: Antibacterial activity, *Spirulina platensis*

INTRODUCTION

The search for plants with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms [1]. Although extremely effective, antibiotics are able to induce resistance in bacteria. For 450 years, bacterial resistance has been the main factor responsible for the increase of morbidity, mortality and health care costs of bacterial infections. The defense mechanism against antibiotics is widely present in bacteria (e.g. *Pseudomonas, Klebsiella, Enterobacter, Acinetobacter, Salmonella, Staphylococcus, Enterococcus and Streptococcus*) and became a world health problem [2]. However, there has been a rising interest of researchers for natural products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades [3-6]. Several algal species contain natural bioactive compounds that act as potent antimicrobial agents [7,8]. *Spirulina species*, for example, have some valuable antiviral and antioxidant compounds [7,8].

The cyanobacterium *Spirulina platensis*, a blue green micro alga, has been used since ancient times as a source of food because of its high nutritional value [9]. It is rich in nutrients such as proteins, vitamins, minerals, carbohydrates, and γ-linolenic acid. In recent years it is gaining more and more attention, not only due to its food value but also for the development of potential pharmaceuticals [10]. Nevertheless, there are a few reports on the biological

activities of this primitive plant. Therefore, an effort is made in the present investigation to evaluate the antimicrobial activity of *Spirulina platensis* against gram positive and gram negative bacteria.

MATERIALS AND METHODS

Collection of *Spirulina platensis*: The algae (AJ401120) was purchased from Antenna Trust, Madurai.

Preparation of *Spirulina* extracts: Freshly dried *Spirulina platensis*, was mixed with acetone, ethanol,methanol,petroleum ether and diethyl ether(150ml solvent/100g of *Spirulina platensis*) in soxhlet apparatus and extracted for 60 minutes. The extracts were filtered and the solvent was removed using rotary evaporator.Sequential extraction was performed with all solvents in the order, water,acetone,ethanol,methanol andpetroleum ether. The extracts were stored in an airtight glass bottles in a refrigerator. For antimicrobial activity, extracts obtained with organic solvents and water extracts were prepared at a concentration of 100 mg/ mL^1(freeze dried material/mL of solvent).

Microorganisms tested: Strains of human pathogenic microorganisms used in this study were as follow: *Klebsiella pneumoniae* (NCIM2063), *Shigella shigae* (NCIM2064), *Pseudomonas aeruginosa* (NCIM2076), *Escherichia coli* (NCIM-2065), *Staphylococcus aureus* (NCIM2079), *Proteus vulgaris* (NCIM2027) *and Salmonella typhi* (NCIM2080). All these strains were colleted from National Chemical Laboratory,Pune- and maintained in ultra low temperature freezer-(-80ºC).

Preparation of 24 hours pure culture: A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these were streaked on to the appropriate culture slants and incubated at 37 ºC for 24 hours.

Standardization of micro-organisms: Each of the 24 hour old pure culture was suspended in a Roux bottle containing 5 ml of physiological saline. Each suspension of microorganism was standardized to 25% transmittance at 560 nm using an Ultraviolet (UV) visible spectrophotometer.

Antimicrobial activity using disc diffusion method (11): Antimicrobial activity was checked by disc gel diffusion method. The cultures were grown in nutrient broth and incubated at 37 °C for 24 h. After incubation period is over, the O.D. of the culture was adjusted to 0.1 with sterile nutrient broth. 20 ml molten Mueller-Hinton agar medium was poured into sterile petri plates and allowed to solidify. The discs (8 mm diameter) impregnated with 100 µg of respective extracts/ml were placed on the surface of the petri plates seeded with 0.1 ml of microbial suspension (5 x 10^5 CFU/ml). Soon afterwards the plates were kept at 10 ^{0}C for 30 min. After it normalized to room temperature the plates were incubated at 37 ^{0}C for 24 h. After incubation period the zone of inhibition was measured.

Measurement of zone of inhibition (ZIH): The zones of inhibition of the tested microorganisms by the extracts were measured using a Fisher-Lilly antibiotic zone reader model 290 (U.S.A). The antimicrobial activities were determined by the ratio of the ZIH diameters of the extracts to that of the standard antibiotic ciprofloxacin 5µg/disc in the same petri dish, where in a higher ratio indicates a more potent extract.

FTIR Spectrum of sequential extract of *Spirulina platensis*: The FT-IR spectra of the sequential extract of *Spirulina platensis* was recorded on a Bomem Michelson MB100 FT-IR, equipped with a laser source, KBr beam splitter and DTGS detector. The instrument was set at 10 scans at 4cm ± 1 resolution with cosine apodisation in the mid- IR region: 400 - 4,000cm±1, using Bomem Easy (ver 1.5) and Matrix Plotter software. All the samples were examined as KBr diskettes. The spectra was smoothened with a factor of 4 on a scale of 1-16, where 1 was least and 16 was maximum smoothening.

Data and statistical analysis: Data are expressed as mean ± standard deviation (SD) of triplicates. Two-way ANOVA was used to analyze the effect of different solvents on antimicrobial activity. Tukey-Krammer multiple comparison test was used to assess the significance among the extracts.

RESULTS AND DISCUSSION

The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol and water [12-14]. Acetone, although not a very commonly used solvent, has been used by a number

of workers [15,16]. The results obtained from the present study concerning the antimicrobial activity of *Spirulina platensis* extracted with different solvents against different species of bacteria are recorded in table 1. It is clear from study that the diameter of the inhibition zone depends mainly on the type of the solvent used and the tested bacteria.

Water extract of *Spirulina platensis* showed maximum antimicrobial activity of 18.0 mm against *Klebsiella pneumoniae* (NCIM2063) and a minimum activity of 10.0 mm against *Proteus vulgaris (*NCIM202*).* All the tested microorganisms were resistant to methanol, ethanol and propanol extracts except *Pseudomonas aeruginosa* (NCIM-2076) and *Escherichia coli* (NCIM2065) which exhibited a least inhibition zone of 7.0 and 8.0 mm respectively in propanol. Acetone extract of *Spirulina platensis* also gave the highest biological activity of 17.0 mm against *Klebsiella pneumoniae* (NCIM2063), moderate activity of 11.0 mm against *Salmonella typhi* (NCIM2080), and 10.0 mm againest *Pseudomonas aeruginosa* (NCIM2076), *Escherichia coli* (NCIM2065) and *Staphylococcus aureus* (NCIM2079). Aqueous acetone was better at extracting total phenolics than aqueous methanol.

Extracts of *Spirulina platensis,* obtained by different solvents exhibited different degrees of antimicrobial activity on both Gram-positive and Gram-negative organisms [17, 18]. While diethyl ether and petroleum ether were the best organic solvents for extracting the antibacterial agents from *Spirulina platensis* in the present evaluation. Statistically the effects of the two diethyl ether and petroleum ether were insignificant. Comparatively diethyl ether showed a marked activity against *Klebsiella pneumoniae* (NCIM2063) followed by *Shigella shigae*(NCIM2064) exhibiting 20.3 mm and 20.1 mm of inhibition zone. The same results were also reported by other workers [19-21].

Most antimicrobial active components that have been identified are not water soluble and organic solvent extracts have been found to be more potent [22]. Some of the simplest bioactive chemicals consist of a single substituted phenolics ring. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity [23]. Extracts from organic solvents have been found to show more consistent antimicrobial activity as compared to water extracts [22]. The intensity of the inhibitory effects was variable between the extracts. The ratio of inhibition zone between the test extract and the reference standard ciprofloxacin 5µg/ disc is represented in figure 1

From the figure 1 it is inferred that alcoholic extract was the least toxic and the diethyl ether extract was the one with more pronounced inhibitory effect. In terms of antibacterial activity, our results fit into the picture that antibacterial activity of cyanobacteria is directed against both Gram-positive and Gram negative bacteria**.** Other studies [9,19] showed that most Gram-negative bacteria are resistant to toxic agents in the environment due to the barrier of lipopolysaccharides of their outer membrane. The present results are in contrast with the previous reports [24]. The difference in the results in toxicity against Gram-positive and Gram-negative bacteria also indicates that the mechanism of toxicity involves different functions in the two types of cells namely different permeability to the cyanobacteria compounds [25].

Antimicrobially active lipids and active fatty acids are present in a high concentration in this alga [26]. It was hypothesised that lipids kill microorganisms by leading to disruption of the cellular membrane [27] 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. 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 [28]. The difference in the susceptibility of *E. Coli* might be due to the differences in the outer membrane or the cell wall of bacteria [29]. The external leaflet of the outer membrane of Entero bacteriacae, such as *E. coli* that lives in the rectum, an environment rich in hydrophobic compounds, is almost entirely composed of lipopolysaccharides and proteins. These bacteria have a hydrophilic surface because of the side chains of lipopolysaccharides, and thus hydrophobic molecules, like lipids, have difficulty in entering the bilayer [28]. This could have contributed to the comparatively less susceptibility of *E. Coli* than other organisms. Sequential extract of *Spirulina* exhibited maximum antimicrobial activity. Inhibition zone of 25.3 mm observed for

Table 1. Antimicrobial activity o*f Spirulina platensis* extracted with different solvents (diameter of inhibition zone in mm). *R Resistance

S.No	Microorganism	Water	Methanol	Ethanol	Propanol	Acetone	Petroleum ether	Diethyl ether	Sequential extract
1	*Klebsiella pneumoniae* (NCIM2063)	18	R	R	R	17	20	20.3	25.3
2	*Shigella shigae* (NCIM2064)	16	R	R	R	16	23	20.1	23.1
3	*Pseudomonas aeruginosa* (NCIM2076)	12	R	R	7	10	11.6	11.9	16.9
4	*Escherichia coli* (NCIM2065)	12	R	R	8	10	13.9	13.3	18.3
5	*Staphylococcus aureus* (NCIM2079)	12	R	R	R	10	13	13.4	18.4
6	*Proteus vulgaris* (NCIM2027)	10	R	R	R	9	11	11	16
7	*Salmonella typhi* (NCIM2080)	13	R	R	R	11	16	16.2	21.2

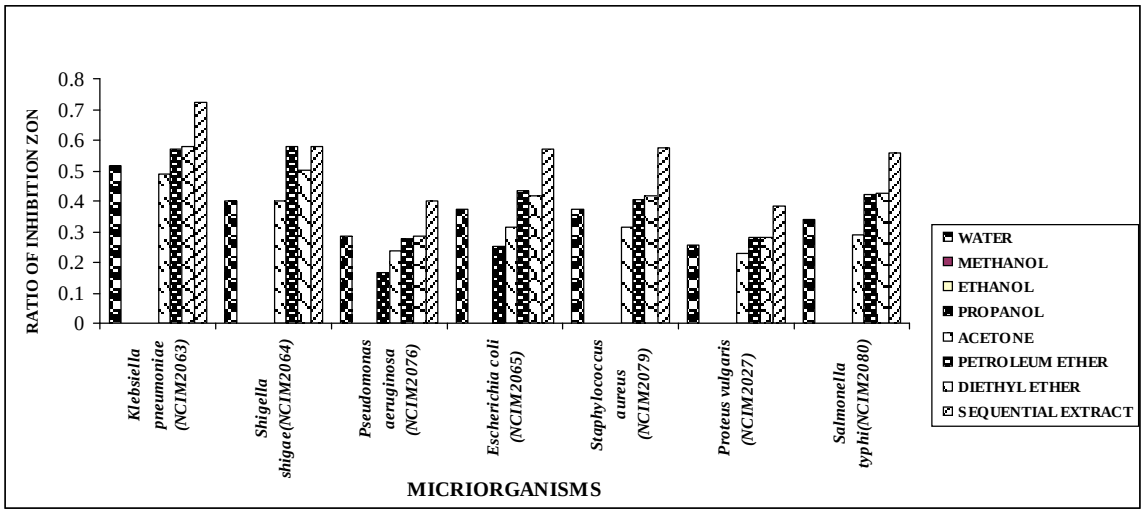

Fig. 1: Antimicrobial activity of *Spirulina platensis* expressed as the ratio of inhibition zone between test extract and reference standred.

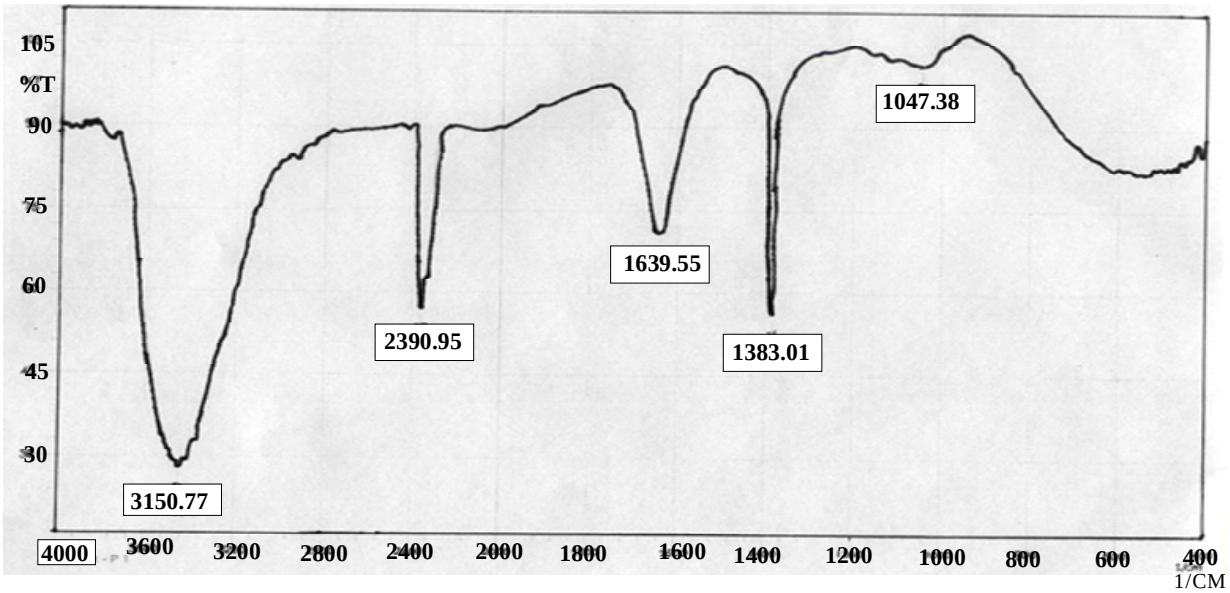

Fig. 2: FTIR Spectrum of sequentially extracted *Spirulina platensis*

Klebsiella pneumoniae and 16.0 mm for *Proteus vulgaris*. The enhanced antimicrobial activity expressed in sequential extraction might be due to the fact that both hydrophobic and hydrophilic bioactive compounds were extracted. When extraction was done with any one solvent bioactive compounds soluble in the respective solvent only be extracted.

The characteristic FT-IR Spectrum of sequentially extracted *Spirulina platensis* is illustrated in figure 2.It showed strong bands at 1047,1383,1640,2361, and 3450 cm-1. Peaks at 3450 cm^{-1} is due to -NH stretching of secondary amide. C=O stretching is responsible for peak at 1640 cm^{-1}. The bands at around 1088 and 1047 cm^{-1} are attributed to the C–O–P stretching and phosphorylated hydroxyl group [29]. This suggests the presence of small peptides which could have bactericidal activity.

The results obtained in the present study suggest that *Spirulina platensis* has antibactericidal activity against pathogenic bacteria. An improved knowledge of the composition, analysis, and properties of *Spirulina platensis* with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application of this blue green algae.

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