ORIGINAL RESEARCH PAPER

Botany

EVALUATION OF ANTIBACTERIAL ACTIVITY OF A CYANOBACTERIUM SCYTONEMA SCHMIDTII GOM.

KEY WORD: Scytonema schmidtii, Antibacterial Activity, BG-11, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa

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ABSTRACT

Scytonema schmidtii, a cyanobacterium was isolated from the collected soil samples from different locations of Ahmednagar district of Maharashtra state (India). Identification was carried out using morphological variation and taxonomical approaches according to Desikachary (1959) and Prescott (1962) .The axenic culture of *Scytonema schmidtii* was obtained by using the method recommended by Bolch and Blackburn (1996). The isolated *Scytonema schmidtii* was grown autotropically in BG-11 medium as described by Rippka *et al*., (1979) and incubated at 30±2°c. After 25 days, biomass was harvested by filtration through double layered muslin cloth and dried using air blower. The biomass of this *Scytonema schmidtii* species was used for the assessment of antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Micrococcus flavus* and *Proteus mirabilis*. The antibacterial activity was studied by disc diffusion method (Anonymous, 1996). Methanol extract of *Scytonema schmidtii* showed the activity against all the tested bacterial strains. Maximum zone of inhibition (23±1.9 mm) was recorded with methanol extract of *Scytonema schmidtii.*

INTRODUCTION

An infectious disease is one of the reasons for increasing number of deaths in developing countries and world-wide. They hold the second position after heart diseases. The search for antibiotics began in the late 1800s; the scientist began to devote time for searching the drugs that would kill the disease-causing bacteria. The goal of such research was to find so called 'magic bullet or wonder drug' that would destroy microbes without toxicity to the person taking that drug. Today, most of the diseases are caused by pathogens that can be cured with the help of available antibiotics. Still there is need to explore and develop new effective antibiotics against microbial pathogens because of resistance mechanism of the target organism. Taking this into consideration, there has been a global attention towards finding new chemicals, which led to the development of structure, which either directly or after some modifications can be used for development of new drugs.

The interests in the cyanobacteria, as generators of pharmacologically active and industrially important compounds have been stimulated by the recent years (Singh *et al*., 2002). Therefore, an optimized production of relevant compounds under controlled conditions is conceivable (Kulik, 1995).

The first partly identified antimicrobial compound isolated from algae was obtained from unicellular green algae particularly, chlorella which contained a 'chlorellin' that exhibited inhibitory activity against both gram-positve and gram-negative bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Pratt *et al*., 1994). A pronounced reduction of gram-positive bacteria in lakes during the occurrence of cyanobacterial water-blooms was reported and the production of antibacterial substances may be one reason for this phenomenon.

Cyanobacteria are known to be able to survive under all kinds of environmental conditions, terrestrial, saline water and freshwater, and even under extremely competitive environments; moreover, they are exposed to a wide variety of predators and to microbial pathogens, such as bacteria, viruses, and fungi. Their flexible metabolism underlies both their adaptation to a diversity of growth conditions and habitats and their capacity to respond to different environmental stresses and nutrients sources. This versatility can explain the diversity and the number of chemical compounds that have been isolated from them (Falaise, *et al* 2016, Shah *et al* 2017)

Secondary metabolites from cyanobacteria have been reported to have pharmaceutical potential belonging to a wide range of structural classes like alkaloids, aromatic compounds, peptides, terpenes, etc. all of which exhibit some biological activity (Konig and wright, 1993). They are known to produce a wide variety of toxins which include 40 % lipopeptides. According to Burja *et al*., (2001) and Singh *et al*., (2001) the cyanobacterial lipopeptides include different compounds like cytotoxic (41%), antitumor (13%), antiviral (4%), antibiotics (12%) and 18% activity includes antimalerial, antimycotics, multi-drug resistance reversers, anti-feedant, herbicides and immunosuppressive agents. Isolation of bioactive compounds from cyanobacteria is done with two objectives. One is to discover new compounds for pharmaceutical, agricultural or biocontrol application. The other is to better understand the interactions of individual organisms within their natural communities. For each of these purposes, there is a need to screen new culturable organisms to understand the frequency and distribution of bioactive strains. There are numerous review articles about marine, freshwater, and terrestrial cyanobacteria, belonging to different families, as a source of antibacterial molecules. The present work describes the results of screening of *Scytonema schmidtii* against pathogenic bacteria.

MATERIALS AND METHODS

Collection, isolation and identification of cyanobacteria

Scytonema schmidtii was isolated from the collected soil samples from different locations. The isolated *Scytonema schmidtii* was grown in BG-11 medium as described by Rippka *et al*., (1979) and incubated at 30±2°C. Identification was carried out using morphological variation and taxonomical approaches according to Desikachary (1959) and Prescott (1962). *Scytonema schmidtii* was cultured in BG-11 culture medium for large scale biomass production. After 25 days biomass was harvested by filtration through double layered muslin cloth and dried using air blower. The biomass of *Scytonema schmidtii* was used for the assessment of antibacterial activity.

Extraction Procedure

Five g of finely powdered biomass was successively extracted in 50 ml of hexane, chloroform and methanol by using soxhlet app aratus at 40°C for 24 h. The filtered extract was concentrated in vacuo at 40°C. Final volume of the extract was made 1ml with respective solvents.

Standard antibiotic

Standard antibiotic disc (10 µg/ml streptomycin) used in the present study was procured from Hi Media (India). These

discs were kept on the nutrient agar media containing known volume of the bacteria.

Test organisms

Pure cultures of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Micrococcus flavus* and *Proteus mirabilis* were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune and used for antibacterial assay. Cultures were maintained according to guidelines of NCIM, NCL, Pune.

Preparation of culture medium

The chemicals required to prepare the nutrient agar media was procured from Hi-media Laboratories; Pune (India). Composition of the medium is as follow.

ingreadiant	gL^{-1}
peptone	10.00
Beef	10.00
Nacl	5.00
Agar	20.00

pH was adjusted to 7.5 using 0.1 N HCL or 0.1 N NaOH on standardized pH meter. The culture medium was sterilized in an autoclave at 1.06 kg cm^{-2} pressure for 20 minutes. Required appliances like Petri-dishes, conical flasks, forceps, Pipettes, etc. were also sterilized in an autoclave at 1.06 kg cm^{-2} pressure for 30 minutes.

Preparation of Inoculum

The gram positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus flavus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) were pre-cultured in nutrient broth for overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610}nm).

Antibacterial assay

Antibacterial assay was carried out by agar diffusion assay (Anonymous, 1996). Paper discs (Whatman No. 41) of 6.4 mm diameter were prepared and sterilized in autoclave. The 10 ml molten nutrient agar medium was allowed to cool to 45°C and to it 20 µl bacterial cultures at a concentration of approximately 1.5 X 10^8 colony forming unit (CFU) was added and poured in sterile petri-dish. This was allowed to solidify and then individual plates were marked for the organism inoculated. The bundles of discs (Four disc together) were prepared consisting 400µg/ml of extracts. Solvent was allowed to evaporate. After solidification, the discs were placed in petri plates at equal distance. By the same method, for each organism duplicate plate, standard plates and control plates (solvent) were prepared. For standard plates, antimicrobial substance streptomycin (10 g/ml) was used. The plates were incubated at 4°C for 8 hours to allow the diffusion of the samples. After that the plates were incubated at 37° C for 24 hours. After 24 hours, the diameter of the zone of inhibition was measured to the nearest mm. Depending on diameter of the zone of inhibition; activity of test extract was compared with standard. All the tests were performed under sterile conditions and repeated for three times.

Determination of minimum inhibitory concentration (MIC)

Crude extracts of biomass of *Scytonema schmidtii* were screened for antibacterial activity against Gram positive and Gram negative bacteria using the micro broth dilution techniques (Sahm and Washington, 1991). Dilution of the crude extracts was prepared in nutrient broth ranging from 1 to 400 g ml^{-1} in dimethylsulphoxide (DMSO). The extract solutions were serially diluted in 96 well plates. Bacteria at a concentration of approximately 1.5 x10^8 colony forming units (CFUs) ml^{-1} were added to each well. Plates were then incubated at 37°C for 24 hours, and the final MIC was determined as the lowest concentration turbidity (by measuring absorbance at 600nm). Streptomycin was used as positive control, and DMSO was used as a negative control.

Results and Discussion

The antibacterial activity of *Scytonema schmidtii* was studied by disc diffusion method (Anonymous, 1996). Judging by the size of inhibition zone, the results of antibacterial activity of methanol extract of different cyanobacteria at 400µg/ml concentration against gram positive and gram negative bacteria are given in Table IV-1. Streptomycin was used as a positive control. The antibacterial activity of the methanol extracts showed varying magnitudes of inhibition patterns with standard positive control depending on the susceptibility of the tested microorganism. Methanol extract of *Scytonema schmidtii* showed the activity against all the tested bacterial strains. Maximum zone of inhibition (23±1.9 mm) was recorded with methanol extract .

Chloroform extract of *S. schmidtii* showed the activity against all the tested bacteria except *E .coli.* Chloroform extract of *Scytonema schmidtii* showed more pronounced activity against *Micrococcus flavus*.

Hexane extract of *Scytonema schmidtii* was moderately effective against *B. subtilis*, *S.aureus*, *M. flavus* and *P. mirabilis* at 400µg/ml concentration.

Antibiotics are the most important weapons in fighting bacterial infections and have greatly benefited the health-related problems of human life. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine.

The antibacterial activity of *Scytonema schmidtii* extracts were examined against six pathogenic bacteria. The extraction was carried out using hexane, chloroform, methanol and water. Out of six bacterial strains tested, five showed inhibition activity to all the extracts. The highest activity in terms of effective zone of inhibition (23 mm) was observed in *Scytonema schmidtii* .The analysis of methanol extract of *S. schmidtii* showed a significant level of inhibition against *B. subtilis*. On the other hand, comparatively less activity was observed in chloroform extract of *S. schmidtii.* It is understandable that methanol extract is more potent, showing a higher degree of antimicrobial activity to pathogen in comparison to other extract. Rosell *et al.*, (1987) and Moreau *et al.*, (1988) also reported that the methanolic extraction yields higher antimicrobial activity than hexane and other solvents, whereas others reported that chloroform is better than methanol and benzene (Febles *et al.*, 1995). It is clear that organic solvents provide higher efficiency in the extraction of compounds for antimicrobial activity when compared to the water based methods (Asthana *et al.*, 2006; Stensvik *et al.*, 1998).

Table-Antibacterial activity of different extracts of *Scytonema schmidtii* at 400µg/ml concentration against gram positive and gram negative bacteria.

Bacterium	Diameter of effective zone of inhibition (mm)				
	Methanol extract	Chloroform extract	Hexane extract	Aqueous (water) Extract	Streptomycin (10 µg/ml)

Escherichia coli	11±2.1	-	-	-	17±2.4
Bacillus subtilis	23±1.9	14±1.3	11±1.7	-	25±1.3
Staphylococcus aureus	19±2.5	12±1.1	9±1.3	-	23±1.7
Pseudomonas aeruginosa	12±1.6	10±1.4	-	-	22±1.6
micrococcus flavus	16±1.6	18±1.8	16±2.1	-	20±1.2
Proteus mirabilis	19±2.2	13±1.9	13±1.8	-	19±1.2

Cannell *et al*., (1988) screened organic solvent extracts of different cyanobacteria for their antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and found activity in five cyanobacterial cultures.

In chloroform extract, the maximum activity in terms of effective zone of inhibition (18 mm) was recorded in *Scytonema schmidtii* against *Micrococcus flavus*. Chloroform extract of *Scytonema schmidtii* did not show any activity against *E. coli*. This means that the compound responsible for the antibacterial activity may be least in concentration. The chloroform extract was found less effective as compared to methanolic extract.

The hexane extract was observed less effective against the tested bacteria as compared to methanolic and chloroform extract of cyanobacterial biomass. In hexane extract, there was no activity against *E. coli* up to 400µg/ml concentration.

Falch *et al*., (1995) made successive extractions with solvents of increasing polarity i. e. petroleum ether, dichloromethane, ethyl acetate, methanol, etc. These extracts showed different antibacterial effects in bioautographic assay with *B. subtilis*, *E. coli* and *Micrococcus luteus*. According to our experimental results, methanol caused better effect than chloroform and hexane against gram positive and gram negative bacteria.

Screening procedures gave some indication about the nature of compound involved in antibacterial activity of *Scytonema schmidtii* which gave positive results. During this study the best antibacterial metabolite producing strains *Scytonema schmidtii* showed varied spectra of activity, inhibiting the growth of bacteria (*B. subtilis*, *S. aureus*, *P. mirbilis)*. The presented results are consistent with finding of others that cyanobacteria can be a rich source of biologically active compounds (Tan , 2007; Skulberg, 2000; Piccardi *et al*., 2000; Zorica *et al*., 2008).

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