Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [9] August 2021 : 127-131

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Screening of a Cyanobacterium *Westiellopsis prolifica* Janet. for Antibacterial Activity

Janardhan Namdeo Nehul
Department of Botany
Dada PatilRajale College of Science, Adinathnagar Tal-PathardiDist-Ahmednagar-414505(MS)India .
Email: jnnehul@gmail.com

ABSTRACT
Acyanobacterium,Westiellopsis prolifica 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.The axenic culture of Westiellopsis prolifica was obtained in the laboratory. The isolated Westiellopsis prolifica was grown autotropicallyin BG-11 medium 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 Westiellopsis prolifica 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. Methanol extract of Westiellopsis prolifica showed the activity against all the tested bacterial strains. Maximum zone of inhibition (13±1.6 mm) was recorded with methanol extract of Westiellopsis prolifica against Bacillus subtilis. The MIC for all the bacteria was in the range of 256– >400 µg/ml.
Key Words-*Westiellopsis prolifica, Antibacterial Activity,BG-11, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*

Received 21.06.2021 Revised 11.07.2021 Accepted 21.08.2021

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 [1]. Therefore, an optimized production of relevant compounds under controlled conditions is conceivable [2].

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* [3]. 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 [4,5].

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 [6].They are known to produce a wide variety of toxins which include 40 % lipopeptides. 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 [7,8]. 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 *Westiellopsis prolifica* against pathogenic bacteria.

MATERIAL AND METHODS

Collection, isolation and identification of cyanobacteria

Westiellopsis prolifica was isolated from the collected soil samples from different locations. The isolated *Westiellopsis prolifica* was grown in BG-11 medium and incubated at 30±2°C [9]. Identification was carried out using morphological variation and taxonomical approaches.[10,11]. *Westiellopsis prolifica* 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 *Westiellopsis prolifica* was used for the assessment of antibacterial activity.

Extraction procedure

Five g of finely powdered biomass of *Westiellopsisprolifica* was successively extracted in 50 ml of hexane, chloroform , methanol and water by using soxhlet apparatus at 40^{o}C for 24 h. The filtered extract was concentrated in vacuo at 40^{o}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.

Ingredient	**g L^{-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. 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 (10µg/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 biomassof*Westiellopsisprolifica*were screened for antibacterial activity against Gram positive and Gram negative bacteria using the micro broth dilution techniques [12]. Dilution of the crude extracts was prepared in nutrient broth ranging from 1 to 400 µg 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 *Westiellopsi s prolifica* was studied by disc diffusion method. Judging by the size of inhibition zone, the results of antibacterial activity of different extracts of *Westiellopsisprolifica*at 400µg/ml concentration against gram positive and gram negative bacteria are given in Table-1. Streptomycin was used as a positive control.The antibacterial activity of *Westiellopsisprolifica*extracts were examined against six pathogenic bacteria. The extraction was carried out using hexane, chloroform, methanol and water. Out of six bacterial strains tested, some bacteria showed inhibition activity to all the extracts. The highest activity in terms of effective zone of inhibition (13±1.6 mm) was observed in *Westiellopsis prolifica* against *Bacillus subtilis*.The analysis of methanol extract of *Westiellopsisprolifica* showed a significant level of inhibition against *Bacillus subtilis.* On the other hand, comparatively less activity was observed in chloroform extract of *Westiellopsis prolifica.*

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 *Westiellopsisprolifica*showed the activity against all the tested bacterial strains. Maximum zone of inhibition (13±1.6.4mm) was recorded with methanol extract against *Bacillus subtilis*.Chloroform extract of *Westiellopsis prolifica* showed the activity against *Bacillus subtilis, Staphylococcus aureus* and *Proteus mirabilis.* Chloroform extract showed more pronounced activity against *Bacillus subtilis*. Hexane extract was found effective against*all bacteria* at 400µg/ml concentration except *Escherichia coli*and *Pseudomonas aeruginosa.* Aqueous extract did not show activity against any bacterium.

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.

It is understandable that methanol extract is more potent, showing a higher degree of antimicrobial activity to pathogen in comparison to other extract. Some researchers also reported that the methanolic extraction yields higher antimicrobial activity than hexane and other solvents[13,14] , whereas others reported that chloroform is better than methanol and benzene [15]. It is clear that organic solvents provide higher efficiency in the extraction of compounds for antimicrobial activity when compared to the water based methods [16,17]. Organic solvent extracts of different cyanobacteria have been screened for their antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and found activity in five cyanobacterial cultures [18].

In chloroform extract, the maximum activity in terms of effective zone of inhibition (8±1.3 mm) was recorded in *Westiellopsis prolifica* against *Bacillus subtilis*. Chloroform extract of *Westiellopsis prolifica* showed moderate activity against all tested bacteria. 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 lesseffective against the tested bacteria as compared to methanolic and chloroform extract of *Westiellopsisprolifica*biomass. In hexane extract, there was no activity against *E. coli*and *Pseudomonas aeruginosa*up to 400µg/ml concentration.

There was a 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 *Micrococcusluteus* [19]. According to our experimental results, methanol caused better effect than chloroform and hexane against gram positive and gram negative bacteria.

Minimum inhibitory concentration (MIC)

The lowest concentration of compound that failed to show any visible macroscopic growth was considered as its MIC. The MIC values for a given isolate were either identical, or within one dilution. In the present study, the extracts of biomass were prepared in methanol, chloroform, hexane, and water and studied against different gram positive and gram negative bacteria. The MIC was found to vary from solvent to solvent of *Westiellopsis prolifica*. The MIC for all the bacteria was in the range of 256–>400 µg/ml. The methanol extract of *Westiellopsis prolifica* showed lower MIC of 256µg/ml against *Micrococcus flavus* and *Proteus mirabilis*.Chloroform extract showed maximum activity at MIC 325µg/ml.Higher value of MIC was shown in hexane and lower in methanol extracts. The MIC of hexane extract was in between 325 - >400 µg/ml. Aqueous extract of *Westiellopsis prolifica* did not show any activity at any MIC upto 400 µg/ml.

Screening procedures gave some indication about the nature of compound involved in antibacterial activity of *Westiellopsis prolifica* which gave positive results. During this study the best antibacterial metabolite producing strains *Westiellopsis prolifica* showed varied spectra of activity, inhibiting the growth of bacteria (*B. subtilis*, *S. aureus Proteus mirabilis)*. The presented results are consistent with finding of others that cyanobacteria can be a rich source of biologically active compounds [20,21,22,23].

Table-1.Antibacterial activity of different extracts of *Westiellopsis prolifica* 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	-	-	-	-	17±2.4
Bacillus subtilis	13±1.6	8±1.3	4±1.2	-	25±1.3
Staphylococcus aureus	9 ±1.5	6±0.7	5±1.4	-	23±1.7
Pseudomonas aeruginosa	-	-	-	-	22±1.6
Micrococcus flavus	11±1.2	-	6±1.5	-	20±1.2
Proteus mirabilis	12±1.2	7±1.2	3±0.92	-	19±1.2

Table -2: Minimum inhibitory concentration (MIC) of different extracts of *Westiellopsis prolifica* against tested pathogenic bacteria. Concentration of extracts is expressed in terms of µg/ml.

Bacterium	Concentration of extracts in µg/ml.			
	Methanol extract	Chloroform extract	Hexane extract	Aqueous(water) Extract
Escherichia coli	>400	>400	>400	>400
Bacillus subtilis	325	325	400	>400
Staphylococcus aureus	325	325	400	>400
Pseudomonas aeruginosa	>400	>400	>400	>400
Micrococcus flavus	256	>400	325	>400
Proteus mirabilis	256	325	400	>400

CONCLUSION

Out of six bacterial strains tested, some bacteria showed inhibition activity to all the extracts. The highest activity in terms of effective zone of inhibition (13±1.6 mm) was observed in *Westiellopsis prolifica* against *Bacillus subtilis*.The analysis of methanol extract of *Westiellopsis prolifica* showed a significant level of inhibition against *Bacillus subtilis*. Chloroform extract of *Westiellopsis prolifica* showed moderate activity against all tested bacteria.The hexane extract was observed less effective against the tested bacteria as compared to methanolic and chloroform extract of *Westiellopsis prolifica* biomass. Aqueous extract of *Westiellopsis prolifica* did not show any activity at any MIC upto 400 µg/ml.

REFERENCES

1. Singh DP, Tyagi MB, Kumar A, Prasuna EG (2002) Bioactive Secondary Metabolites from cyanobacteria. Pg.275-292. In Algological research in India. Edt. N. Anand, Pbl. Bsmps Dehradun. India.

2. Kulik MM (1995) The potential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur. J. Pl. Pathol. 101: 585-599.
3. Pratt R, Daniels TC, Eiler JJ, Gunnison JB, Kumler WD, Oneto JF, Strait LA, Spoehr HA, Hardin GJ, Milner HW, Smith JHC, Strain HH (1994) Chlorellin, an Antibiotic substance from *Chlorella*. Science 99: 351-352.
4. Falaise, C.; François, C.; Travers, M.-A.; Morga, B.; Haure, J.; Tremblay, R.; Turcotte, F.; Pasetto, P.; Gastineau, R.; Hardivillier, Y.; et al.(2016) Antimicrobial Compounds from Eukaryotic Microalgae against Human Pathogens and Diseases in Aquaculture. Mar. Drugs , 14, 159.
5. Shah, S.; Akhter, N.; Auckloo, B.; Khan, I.; Lu, Y.; Wang, K.; Wu, B.; Guo, Y.-W. (2017) Structural diversity, biological properties and applications of natural products from cyanobacteria. A review. Mar. Drugs , 15, 354.
6. Konig GM, Wright AD (1993) Algal secondary metabolites and their pharmaceutical potential. In Kinghorn AD, Balandrin MF (eds), Human medicinal agents from plants. ACS Washington D.C. pp. 276–293 .
7. Burja AM, Banaigs, EB, Abou-Mansour BJG and Wright PC (2001) Marine cyanobacteria- a prolific source of natural products. Tetrahedron 57: 9347-9377.
8. Singh PK, Dhar DW, Pabbi S, Prasanna R, Arora A(2001) *Recent Advances in the Exploitation of Blue-green algae and Azolla.* National centre for conservation and utilization of Blue-green algae. Indian Agricultural Research Institute, Venus Printers and Publishers, New Delhi. 1-137.
9. Rippka R, Deruelles J, Waterbury JB, Herd man M, Stainer RY (1979) Generic assignments. Strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111: 1-61.
10. Desikachary TV (1959) A monograph on Cyanophyta, ICAR Publication, New Delhi.1-686.
11. [11]. Prescott GW (1951) Algae of the Western Great Lakes Area. Publ. Otto Koeltz Science Publishers, Koeningstein. 1-935.
12. Sahm DH, JA Washington (1991) Antibacterial susceptibility tests: Dilution methods. InBalows A, Hausler WJ, Herrmann KL, Isenberg HD, Shamody HJ (eds.) Manual of clinical microbiology. American Society for Microbiology, Washington DC.
13. Rosell KG, Srivstava LM (1987) Fatty acid as antimicrobial substances in brown algae. Hydrobiologia 151/152: 471-475.
14. Moreau J, Pesando D, Bernard P (1988) Seasonal variations in the production of antifungal substances by some Dictyoles (brown algae) from French Mediterranean coast. Hydrobiology 162: 157-162 Piccardi R, Frosini A, Tredici MR, Margheri MC (2000) Bioactivity in free-living and symbiotic *Cyanobacteria* of the genus *Nostoc*. J. Appl. Phycol.12: 543-547.
15. Febles CL, Arias A, Gill-Rodriguez MC (1995) *In vitro* study of antibacterial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). Anuario del EstudiosCanarios. 34: 181-192.
16. Asthana RK, Srivastava A, Singh AP, Deepali, Singh SP, Nath G, Srivastava R, Srivastava BS (2006) Identification of an antimicrobial entity from the cyanobacterium *Fischerella*sp. isolated from bark of *Azadiractaindica* (Neem) tree. J. Appl. Phycol. 18: 33-39.
17. Stensvik, Skulberg, OM, Underdal B, Hormazabal V (1998) Antibacterial properties of extracts from selected plaktonic freshwater cyanobacteria-a comparative study of bacterial bioassays. The Society for Applied Microbiology. 84: 1117-1124.
18. Cannell RJP, Owsianka AM, Walker JM (1988) Results of a large scale screening programme to detect antibacterial activity from fresh water Algae. Br. Phycol. J. 23: 41-44.
19. Falch PS, Konig GM, Wright AD (1995) Biological activities of cyanobacteria: Evaluation of bacteria and pure compounds. Planta Med. 61:321-328.
20. Tan TL (2007) Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry 7: 954-979.
21. Skulberg OM (2000) Microalgae as a source of bioactive molecules – experience from cyanophyte research. J. Appl. Phycol.12: 341-348.
22. Piccardi R, Frosini A, Tredici MR, MargheriMC . Bioactivity in free-living and symbiotic *Cyanobacteria* of the genus *Nostoc*. J. Appl. Phycol. 2000;12: 543-547.
23. Zorica S, Dragana C, Jelica S, Maja K, Dejan S (2008) Antibacterial, antifungal and cytotoxic activity of terrestrial cyanobacterial strains from Serbia Sci China Ser C-Life Sci. 51: 941-947.

CITATION OF THIS ARTICLE

J N Nehul. Screening of a Cyanobacterium *Westiellopsis prolifica* Janet. for Antibacterial Activity . Bull. Env.Pharmacol. Life Sci., Vol10[9] August 2021 : 127-131