Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 5, 2015

Original Article

ANTIBACTERIAL ACTIVITIES OF *SCYTONEMA HOFMAN* EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

SANGEETA PANIGRAHI, ASHOK KUMAR SETHI[1], LAKSHMI KUMARI SAMAD

[1]Department of Botany, [2]Department of Microbiology, College of Basic Science and Humanities, OUAT, Bhubaneswar, Odisha, India
Email: aksethi2008@gmail.com

Received: 15 Nov 2014 Revised and Accepted: 16 Dec 2014

ABSTRACT

Objective: The present study is focused, to evaluate the effectiveness of organic solvent or aqueous extracts of *Scytonema hofman* against some human bacterial pathogens.

Methods: The aqueous and organic solvent extracts of *Scytonema hofman* were analyzed for their antibacterial property against some of the human bacterial pathogens by agar well diffusion technique followed by determination MIC value.

Results: The results of the present study revealed that chloroform extracts of *Scytonema hofman* showed a maximum inhibition zones against *Escherichia coli* (17.9 mm) followed by *Klebsiella pneumonia* (14.3 mm) and *Pseudomonas aeruginosa* (11.6 mm) but less effective against *Staphylococcus aureus* (4.8 mm). Similar result was also observed in case of ethanolic extract but having fewer inhibition zones when compared with chloroform extract. The aqueous extract was found to be insignificant along with other five organic solvent against all the tested human bacterial pathogens. The MIC values of chloroform extract were found to be 31.25 µg/ml against *Escherichia coli* and *Pseudomonas aeruginosa*, whereas in case of *Klebsiella pneumonia* the value was found to be 250 µg/ml.

Conclusion: The chloroform extract of *Scytonema hofman* was found to be the most effective antibacterial property against all the tested bacterial pathogens.

Keywords: *Scytonema hofman*, MIC value

INTRODUCTION

Now a day a number of microbial secondary metabolites like, hormones, antibiotics, toxins, pheromones, enzymes, etc. play an immense role in the pharmaceutical industry for the development of newer drugs by replacing drugs originated from synthetic formulation. Actinomycetes, bacteria and fungi are the principal producer of novel drugs by employing advance techniques but among these Actinomycete stands first [1].

The arbitrary use of conventional drugs and genetic mutation leads to immersing of latest drug resistant microorganisms, which going to be a threat for human as well as for animals. These all lead to search for novel drugs and perhaps the new sources because there are still a large number of microbes lagging behind which may produce good and potent bioactive compound. In this respects cyanobacterial origin has most important because in the microbial diversity only 1-10% of cultured bacteria has been explored and the natural products from cyanobacteria, actinomycetes and uncultured bacteria are likely to offer newer source of antibiotics [2, 3].

Cyanobacteria formerly referred to as blue green algae having a diversified and ubiquitous group of prokaryotes with several unifying features. They are rich sources of structurally novel and biologically active metabolites which are shown to exhibit antibacterial [4], antifungal, anticancer or cytotoxic [5], antimalarial [6] and other pharmacological activities. The chloroform extracts of *Scytonema br1* isolated from wall and Terrace, Konark temple, Puri, Odisha showed significant antialgal activity against Anabaena BT2 [7]. The lipophilic extract of the cultured terrestrial blue green algae *Scytonema pseodohofmanni* Bharadwaja contains five novel macrolides such as Scytophytins A, B, C, D and E which exhibit cytotoxicity and antifungal activity [8].

The findings of several workers related to bioactive potential of some Cyanobacteria species put step stone for the current investigation. So the present investigation mostly focused on to evaluate the antibacterial properties of organic and aqueous extracts of *Scytonema hofman* by employing the standard technique. This study will also hopefully expose new frontiers on the current applications of the algal extract.

MATERIALS AND METHODS

Mass culture of algae

The present studied Cyaobacterial spp *Scytonema hofman* was collected from Department of Botany, College of Basic Science & Humanities, OUAT, and Bhubaneswar where it was maintained in axenic condition. The collected alga was purified and transferred for their mass production to the 250 ml conical flask containing nitrogen-free BG-11 medium [9] and incubated at 25±20 °C under a continuous light intensity of 7.5 W/m2 up to 25 d for mass production.

Preparation of cyanobacterial extracts

The axenic *Scytonema hofman* culture was centrifuged at 5000 rpm at 100 °C for 20 min and the pellet was collected followed by blot dried with filter paper. Aqueous and organic solvent fractions (acetone, chloroform, DMSO, diethyl ether, ethanol, hexane and methanol respectively) were prepared by transferring one gm of the blot dried sample to the homogenizer containing 10 ml of different solvents and homogenized. The resultant mixture again centrifuged at 5000 rpm for 20 min and the supernatant was collected, preserved at 4°C for further analysis [10].

Collection of test pathogenic organism

For the present investigation, four common human bacterial pathogens were procured from an Institute of Microbial Technology (IMTECH), Chandigarh, India. Among four bacterial strains, three belongs to gram-negative bacteria such as. *Escherichia coli* (MTCC-723), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC–741) and one was gram-positive *Staphylococcus aureus* (MTCC-902). The lyophilized vials of the pathogenic microorganisms were revived and maintained in Nutrient Agar slants for further study.

Selection of standard antibiotic

The antibiotics such as Ciprofloxacin, Gatifloxacin, Gentamicin, Streptomycin, Cefixime, Cefotaxime, Amoxicillin and Chloramphenicol were used to test the sensitivity of bacterial pathogens by standard disc diffusion method [11]. The potency of each antibiotic was 10 mg per disc. The antibiotic which showed the best inhibition zone against all the four bacterial pathogens was selected as positive control.

Evaluation of antibacterial activity

Four pathogenic bacteria were used to study antibacterial potency of different cyanobacterial extracts by using:

a) Agar well diffusion technique [12],

b) Determination of minimal inhibitory concentrations (MICs) of organic solvent extract which show the best result in agar well diffusion technique, by employing tube dilution technique [13].

RESULTS

The antibacterial potency of different extracts from *Scytonema hofman* against four bacterial pathogens was investigated, and the findings were somewhat presentable. All the pathogens were sensitive to organic solvent fraction of *Scytonema hofman* except DMSO and aqueous extract. Chloroform extracts of *Scytonema hofman* showed the maximum inhibition zone against *E. Coli* 17.9±0.8 mm, *K. pneumonia* 14.3±0.6 mm and *P. aeruginosa* 11.6±0.2 mm respectively, while least inhibition zone was observed in case of *S. aureus* 4.8±0.4 mm. A similar pattern result was observed in case of Ethanol fraction but having some changes in zone size which is depicted in table number-1. The Acetone and Hexane fractions from *Scytonema hofman* showed the inhibition zones against all pathogens (table No: 1 & 2), but the result was immaterial when compared with control (Acetone, Hexane). Diethyl Ether and Methanol fractions were found to be less effective against *K. pneumonia*, *S. aureus*, *E. coli* while *P. aeruginosa* was found to be sensitive to Diethyl Ether, Methanol fractions, showed an inhibition zone of 14.6±0.2 mm and 4.3±0.5 mm respectively (table No: 2). The positive control used in this study was Ciprofloxacin which showed best inhibition zone against all four test pathogens. The antibacterial property of all the organic extracts when compared with ciprofloxacin, it was found that of all six organic solvent fraction *Scytonema hofman* showed fewer inhibition zones than ciprofloxacin but chloroform extract was more effective than that of ciprofloxacin 16.5±0.2 mm in the case of *E. coli* 17.9±0.8 mm. In the present context chloroform fraction of the studied Cyanobacteria spp showed the best antibacterial property against all most all pathogens for which this fraction was taken into account to study its MIC value. The MIC value of Chloroform fraction was determined by using the tube dilution technique.

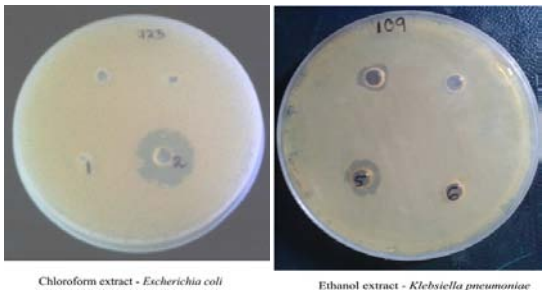

Fig. 1: Antibacterial activity of Chloroform and Ethanol extracts

Fig. 2: Different fractions of *Scytonema hofman*

The MIC values of chloroform extract were found to be 31.25 µg/ml against *E. coli*, whereas in case of *K. pneumoniae* and *P. aeruginosa* the value was fond to be 250 µg/ml and 31.25 µg/ml respectively.

Table 1: Antibacterial activity of different extracts of *Scytonema hofman-bangii*

Test pathogen	Solvent								Ciprofloxacin
	Aqueous		Organic						
	Water		Acetone		Chloroform		Ethanol		
	Control	Extract	Control	Extract	Control	Extract	Control	Extract	
E. coli (MTCC–723)	-	-	2.5±0.5	3.0±0.4	4.5±0.2	17.9±0.8	5.4±1.0	8.0±0.3	16.5±0.2
K. pneumoniae (MTCC–109)	-	-	4.5±0.2	4.0±0.5	3.2±0.5	14.3±0.6	6.3±0.9	13.0±0.6	19.8±0.8
P. aeruginosa (MTCC–741)	-	-	3.0±0.5	5.0±0.3	2.4±0.8	11.6±0.2	5.6±0.8	11.2±0.8	15.0±0.4
S. aureus (MTCC–902)	-	-	4.2±0.9	5.0±0.1	3.5±0.2	4.8±0.4	4.5±0.7	6.0±1.5	18.5±0.2

Values are mean inhibition zone in (mm)±S. D of three replicas.

Table 2: Antibacterial activity of organic extracts of *Scytonema hofman-bangii*

Test Pathogen	Solvent								Ciprofloxacin
	Organic								
	DMSO		Diethyl ether		Hexane		Methanol		
	Control	Extract	Control	Extract	Control	Extract	Control	Extract	
E. coli (MTCC–723)	-	-	-	-	5.0±0.6	6.0±0.7	-	-	16.5±0.2
K. pneumoniae (MTCC–109)	-	-	-	-	4.5±0.4	5.5±0.9	-		19.8±0.8
P. aeruginosa (MTCC–741)	-	-	4.0±0.1	14.6±0.2	2.6±0.9	4.0±0.5	1.8±0.6	4.3±0.5	15.0±0.4
S. aureus (MTCC–902)	-	-	-	-	3.5±0.4	5.5±0.2	-	-	18.5±0.2

Values are mean inhibition zone in (mm)±S. D of three replicas.

DISCUSSION

The growth of microorganisms yields some of the secondary metabolites of different natures which may accumulate within the cells or excreted into the medium to support the growth or inhibition of the self or non self originated. The antimicrobials production ability of several microorganisms may be significant not only act as a defensive tools for them but also as a good source of novel bioactive compounds from pharmaceutical point of view. Like other microorganisms a number of Cyanobacterial spp exhibit antimicrobial property which were reported by several workers [14-16].

The bioactive potency of organic extracts was more than that of aqueous extracts [17] which was proven in the present investigation. The present studied test pathogens were more sensitive towards organic solvent fractions of *Scytonema hofman* than aqueous extracts. Organic solvent extracts of *Scytonema hofman* affects the growth of both gram positive organisms (*S. aureus*) and gram negative (*E. coli*, *K. pneumonia*, *P. aeruginosa*) bacterial species but the effect was species specific as well as type of extracting solvent used which was also reported by workers [18], that the antibacterial and antiviral property of some marine microalgae extracts on pathogens varies from species to species due to variation in cell wall composition of tested species and the genetic material harbored within them.

The findings of some workers suggest that, antibacterial property of different organic solvent fractions from *Scytonema spp* was more in case of gram positive bacteria (*S. epidermidis*) than that of gram negative one. They found that antibacterial property *Scytonema spp* somewhat depends the polarity of an organic solvent used for extraction of secondary metabolites from tested cynobacterial spp, less polar solvent gives better result (Hexane) than that of high polar solvents (Chloroform) [19]. The organic solvent extracted biomass from *Scytonema spp* was tested for their antibacterial property after reconstitute with DMSO and the findings confirmed that gram positive (*S. aurous*) microorganisms were more sensitive than that of gram negative bacteria (*E. coli, K. pneumonia, P. aeruginosa and P. mirabilis*) towards cyanobacterial biomass [20]. The present findings concluded that the antibacterial property of *Scytonema hofman* extracts were more effective against gram negative bacterial pathogens such as, chloroform extract exhibit an inhibition zone against *Escherichia coli* followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* but less inhibition zone against gram positive pathogen, *Staphylococcus aureus*. Similar result was also observed in case of Ethanol extracts having the slight variation in zone size which is depicted in table no-1. DMSO, Acetone and Hexane extract were found to be least effective against these test pathogens but diethyl ether extract showed an inhibition zone against *Pseudomonas aeruginosa*. The variation in results of present work with other literatures may be due to, the strain of the bacteria used that could affect the results significantly or the degree solubility of bioactive compounds in different solvent systems [21]. Some gram negative bacteria were more susceptible than that of gram positive towards ethyl acetate and chloroform extract of two cyanobacterial spp (*Anabaena sp, Oscillatoria sp.*) [22], this type result goes in harmony with our findings. The antibacterial activity of the organic solvent fraction was compared with standard antibiotic (ciprofloxacin) to demonstrate the potency and found that the effect of ciprofloxacin was more than that of all six organic solvent cyanobacterial extracts with an exception found in case of chloroform. The chloroform extract was more effective than that ciprofloxacin in the case of *E. coli,* these findings some way similar with the reports that effect of cyanobacterial extracts on *E. coli* and *Staphylococcus aureus* was more than that of standard antibiotic [23].

As chloroform extract was more active against all tested pathogen, so it was subjected for determination of minimum inhibitory concentration by standard protocols [13]. Active crude chloroform extract was diluted to determine the MIC values against all the four pathogens keeping ciprofloxacin as positive control. Chloroform extract showed best minimum inhibitory concentration value against (31.25 µg/ml) *Escherichia coli*, *Pseudomonas aeruginosa* and in the case of *K. pneumoniae* the value was found to be 250 µg/ml. The above findings go in harmony with the findings of other workers stated that, highest MIC values of DMSO extract *Scytonema sp* was observed in case of Methicillin Resistant *Staphylococcus aureus*(64 µg/ml) followed by *Escherichia coli*, *Pseudomonas aeruginosa* (128 µg/ml) [20].

CONCLUSION

Several natural products still to be explored for the growth and development of human civilization. From the previous studies in addition to the current results, it could be concluded that a number of cyanobacterial species represents a new source of antibacterial formulation with stable and biologically active compounds. Therefore a scientific data base needs to be established for their use in new antibiotic production and further work need to be carried out to isolate, purify and characterize the active compound responsible for antibacterial, antifungal or antiviral of the cyanobacterial species. Also additional work is encouraged to elucidate the possible mechanism of action of these extracts.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Harvey AL. Natural products in drug discovery. Drug Discovery Today 2008;19/20(13):894-901.
2. Cowan DA. Microbial genomes: the untapped resource. Trends in Biotechnol 2000;18:14-6.
3. Clardy J, Fischbach MA, Walsh CT. New antibiotics from bacterial natural products. Nat Biotechnol 2006;24:1541–50.
4. Ghasemi Y, Yazdi MT, Shokravi SH, Soltani N, Zarrini G. Antifungal and antibacterial activity of paddy-fields cyanobacteria from the north of Iran. J Sci I R Iran 2003;14:203-9.
5. Kwan JC, Teplitski M, Gunasekara SP, Paul VJ, Luesch H. Isolation and biological evaluation of 8-epi-malyngamide c from the floridian marine cyanobacterium, *Lyngbya majuscule*. J Nat Prod 2010;73:463-6.
6. Linington RG, Gonza`lezuren`a JL, Romero I, Barri`a EO, Gerwick WH, Venturamides A. Antimalarial constituents of the panamanian marine cyanobacterium *Oscillatoria* sp. J Nat Prod 2007;70:397-401.
7. Yadav S, Sinha RP, Tyagi MB. Antimicobial activity of some cynobacteria. Int J Pharm Pharm Sci 2012;4 Suppl 3:631-5.
8. Ishibashi M, Moore RE, Patterson GML, Xu C, Clardy J. J Org Chem 1986;51:5300-6.
9. Rippka R, Deruelles J, Water bury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 1979;3:1-61.
10. Gonzalez Del Val, Plates G, Basilio A. Screening of antimicrobial activities in red-green and brown macroalgae from gran canaria (Canary Island, Spain). Int Microbiol 2001;4:35-40.
11. Baur WA, Kirby MMW, Sherris CJ, Turch M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:494–6.
12. Cruickshank R, Duguide G, Marmion B, Swain RHA. 12th ed. Vol. 2. Medical microbiology. Churchill Livingston, New York; 1975. p. 358-9.
13. Begum S, Usmni S, Siddigui B, Saeed S, Farnaz S, Ali Khan K, *et al.* Chemistry and biological activity of a tryptamine and B-carboline series of bases. Arzneim-Forch Drug Res 1996;46:1163-8.
14. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic and found as a selective cytotoxic substance in a marine red alga. Anticancer Res 2002;22:2587-90.
15. Østensvik Ø, Skulberg OM, Underdal B, Hormazabal V. Antibacterial properties of extracts from selected planktonic freshwater cyanobacteria-a comparative study of bacterial bioassays. J Appl Microbiol 1998;84:1117-24.
16. Tan LT. Bioactive natural products from marine cyanobacteria for drug discovery. Phytochem 2007;68:954-79.
17. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibactrial activity of some medicinal plant extracts. J Nat Med 2008;62:259-62.

18. Caccamese S, Azzolina R, Furnari G, Cormaci M, Grasso S. Antimicrobial and antiviral activities of some marine algae from eastern Sicily. Bot Mar 1981;1:365-7.
19. Zeeshan Md, Shazia S, Biswas D, Farooqui A, Arif MJ. Antibacterial and free radical scavenging potential of some cyanobacterial strains and their growth characteristics. J Chem Pharm Res 2011;3(2):472-8.
20. Bharat N, Irshad Md, M Rizvi Alam Md, Fatma T. Antimicrobial and cytotoxic activities of cyanobacteria. Int J Innovative Res Sci Eng Tech 2013;2(9):4328-43.
21. Philip K, Sinniah SK, Muniandy S. Antimicrobial peptides in aqueous and ethanol extracts from microbial, plant and fermented sources. Biotechnol 2009;1:1-16.
22. Khair HM, El-Kas YH. Active substance from some blue green algal species used as antimicrobial agents. Afr J Biotechnol 2010;9(19):2789-800.
23. Helen DY, Appavoob RM, Parthipana B. Antibiotic activity of Cyanobacteria isolated from salt pans of kanyakumari district (South India) against human pathogenic bacteria. Int J Curr Sci 2014;11:32-9.