# Evaluation of Nostoc commune for potential antibacterial activity and UV-HPLC analysis of methanol extract

P Kaushik, A Chauhan, G Chauhan, P Goyal

**Citation**

P Kaushik, A Chauhan, G Chauhan, P Goyal. *Evaluation of Nostoc commune for potential antibacterial activity and UV-HPLC analysis of methanol extract*. The Internet Journal of Microbiology. 2007 Volume 5 Number 1.

**Abstract**

In vitro antibacterial activity of aqueous and organic extracts of Nostoc commune were evaluated against Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis, Bacillus cereus and Bacillus pumilus) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumoniae). The methanol extract showed more potent activity than other organic and aqueous extracts. However, culture supernatant found to be inactive against all the test organisms. No inhibitory effect was found against Klebsiella pneumoniae and Salmonella typhi. Gram-positive bacteria were found to be more susceptible as compared to Gram-negative bacteria. The broth microdilution assay gave minimum inhibitory concentrations values ranging from 1 to 512 µg/ml. MICs of methanol extract were 128 µg/ml against Staphylococcus aureus, 512 µg/ml against E. coli, 256 µg/ml against Bacillus subtilis and Bacillus cereus whereas it was more than 512 µg/ml against Bacillus pumilus . This extracts was further analysed by UV-Vis spectroscopy and HPLC, which reveals the presence of one or more active compound(s) in the extract.

## INTRODUCTION

Cyanobacteria (blue-green algae) are a group of extraordinary diverse Gram-negative prokaryotes that originated 3.5 billion years ago. The medicinal and nutrient qualities of cyanobacteria were first appreciated as early as 1500 BC, when Nostoc species were used to treat gout, fistula and several forms of cancer (Liu and Chen, 2003). Their diversity ranges from unicellular to multicellular, coccoid to branched filaments. They exist in almost all conceivable habitats. Most species of cyanobacteria are free-living, freshwater, marine or terrestrial: planktonic, or benthic; and comprise major components of microbial mats. Some cyanobacterial species are thermophilic and growing in hot springs. A few cyanobacteria are symbionts of liverworts, water ferns and cycads; while a number of them are found as the phototrophic component of lichens. 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 microorganisms. Biologically active substances were proved to be extracted by cyanobacteria. Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial (Falch et al., 1995; Mundt et al., 2001; Kaushik and Chauhan, 2008), antifungal (MacMillan et al., 2002), cytotoxic (Luesch et al., 2000), algaecide (Papke et al., 1997), immunosuppressive (Koehn et al., 1992), and antiviral activities (Hayashi and Hayashi, 1996). This study was designed to evaluate the antibacterial activity of different extracts of Nostoc commune.

## MATERIAL AND METHODS

### ORGANISM AND GROWTH CONDITIONS

Nostoc commune used in the study was collected from National Center for Conservation and Utilization of Blue-green Algae, Indian Agricultural Research Institute, New Delhi (India). The culture was grown in BG-11 growth medium (Stanier et al.,1971) containing ($gL^{-1}$) $MgSO_4$.7H2O (0.75), $NaNO_3$ (1.5), $CaCl_2.2H_2O$ (0.036), $K_2HPO_4.3H_2O$ (0.04), $Na_2$ EDTA (0.001), $Na_2CO_3$ (0.02), Ferric ammonium citrate (0.006), citric acid (0.006) as macronutrients along with 1 ml of micronutrients ($gL^{-1}$) $H_3BO_3$ (2.86), $ZnSO_4.7H_2O$ (0.22), $MnCl_2.4H_2O$ (1.81), $CuSO_4.5H_2O$ (0.08), $Na_2MoO_4.2H_2O$ (0.39) and $CO(NO_3)_2.6H_2O$ (0.049) under rotatory conditions and illuminated continuously at a light intensity of 3500 LUX.

### PREPARATION OF EXTRACTS

At the stationary phase of growth (25 days), the culture was harvested and spent media was collected after filtering the biomass. The collected biomass was dried in hot air oven at 60 ° C for one h and then placed in solvent. Left the mixture for few hours at room temperature and then sonicated for 10 min followed by centrifugation at 4000 rpm for 10 min. After centrifugation, supernatant was collected in a preweighed test tube and then concentrated under nitrogen until completely dry, again weighed and then resuspended in the appropriate solvent to make the solution of known concentration for the antibacterial assay. Same procedure was adopted for the preparation of aqueous extract.

## TEST MICROORGANISMS

The microorganisms used in antibacterial assay were collected from Institute of Microbial Technology (IMTECH), Chandigarh, India .The species employed include four Gram-positive bacteria (Staphylococcus aureus MTCC-740, Bacillus subtilis MTCC-736, Bacillus cereus MTCC-430, Bacillus pumilus MTCC-1607) and four Gram-negative bacteria (Escherichia coli MTCC-739, Pseudomonas aeruginosa MTCC-741, Salmonella typhi MTCC-733 and Klebsiella pneumoniae MTCC-139). The bacterial strain were inoculated on Tryptone Soya Agar (TSA) and incubated for 24 h at 30°C then suspended in saline solution 0.85% NaCl and adjusted to yield approximately 1.0 x $10^8$-1.0 x $10^9$ cfu/ml by using spectrophotometer (25% transmittance at 530 nm). Media component were purchased from Hi Media, Mumbai, India. All the chemicals used were of analytical grade.

## EVALUATION OF ANTIBACTERIAL ACTIVITY

In vitro antibacterial activity of different crude extracts of Nostoc commune was evaluated using the agar well diffusion assay (Perez et al., 1990). 100 µl of adjusted culture was mixed with 100 ml of Muller Hinton Agar (MHA) and poured 25 ml each into sterile petridishes (90 mm), this was allowed to solidify and then individual plates were marked for the organisms inoculated. After solidification plates were punched to make the well of 6 mm diameter with the help of sterile cork borer. 100 µl of the respective cyanobacterial extracts were pipetted into the well in assay plates (Kaushik and Goyal, 2008). Plates were incubated overnight at 37 ° C and all the plates were observed for the zone of inhibition, diameter of these zones were measured in millimeters. All the tests were performed under sterile conditions and repeated three times. The solvent control revealed no activity.

## DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

Minimum inhibitory concentration of active crude extract(s) was determined by broth microdilution method as recommended by National Committee for Clinical Laboratories Standards (NCCLS, 1997). The test was performed in 96 wells microtiter plates. Two fold serial dilutions of all active extracts were made in Cation-Adjusted Muller-Hinton Broth (CAMHB from Hi-Media) ranging from 1 to 512 µg/ml. Ciprofloxacin was used as standard antibiotic for the assay. Each inoculum was prepared in the same medium at a density adjusted to a 25% transmittance turbidity standard ($10^8$ cfu/ml) and diluted to 1:100 for the assay. The final volume in the wells was 200 µl. After 24 h of incubation at 37°C, the calculated amount of nitrogen dried cyanobacterial material present in the most diluted extract that produced a visible inhibition was defined as MIC.

## UV-VIS SPECTROSCOPIC ANALYSIS

The absorption spectrum of methanol extract of Nostoc commune was recorded in the UV-Vis spectrophotometer (Shimadzu; Model No. UV-1700 Pharmaspec) capable of producing monochromatic light in the range of 200-800 nm for measuring the absorbance.

## HPLC ANALYSIS

High performance liquid chromatography of methanol extract of Nostoc commune was performed on Shimadzu HPLC (Model No; 10 AVP) equipped with constant temperature column compartment, a sample injector capable of injecting 20 µl aliquots and a programmable variable photodiode array detector and an integrator. The C-18, 4.6 x 250 mm stainless steel column (waters) was used at room temperature. Extracts was eluted with mobile phase water:methanol (80:20) at the wavelength of 260 nm.

## RESULTS AND DISCUSSION

The antibacterial activity of different extracts of Nostoc commune is presented in table 1. The methanol extract exhibited significant activity against Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus pumilus and Escherichia coli, whereas no zone of inhibition was observed against Pseudomonas aeruginosa, Klebsiella pneumoniae and Salmonella typhi. Ethyl acetate extracts were found to inhibit the growth of Staphylococcus aureus, Bacillus subtilis, Bacillus cereus and Bacillus pumilus.

Aqueous extracts of the Nostoc commune exhibited antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus pumilus and Escherichia coli. Maximum zone of inhibition was observed against Gram-positive bacterium Staphylococcus aureus.

No antibacterial activity was detected in dichloromethane extracts and culture supernatant and none of the methanol extracts and aqueous extract showed antibacterial activity against Pseudomonas aeruginosa, Klebsiella pneumoniae and Salomonella typhi.

Minimum inhibitory concentration of methanol extract of Nostoc commune have been shown in Table 2. The results indicated that the MICs of methanol extract were 128 µg/ml against Staphylococcus aureus, 512 µg/ml against E. coli, 256 µg/ml against Bacillus subtilis and Bacillus cereus whereas it was more than 512 µg/ml against Bacillus pumilus.

UV-Vis spectrophotometric analysis of active methanol extract reveals the highest absorption spectra at 423 nm wave-length (Fig. 1-A). Several peaks were observed during HPLC analysis indicating the presence of active compounds in extract (Fig. 1-B). The antibacterial activity of these extracts might be due to the presence of one or more compounds. These extracts can further be subjected to various advanced techniques such as Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy to determine the exact chemical molecule responsible for their bioactivity. This would definitely be turning point for pharmaceutical sciences in determining a novel antibacterial compound.

The above findings indicate the discovery of novel chemicals that can lead to the development of pharmaceuticals by cyanobacteria. The current scenario of antibiotics is very threatening with significant emergence of resistance among bacterial pathogens against available antibiotics. The present investigation reveals that cyanobacterium Nostoc commune would be a major source in finding such metabolites with greater efficacy.

**Figure 1**

Table 1: Antibacterial activity of aqueous and organic extracts of

| Test Organisms | Zone of inhibition | | | | | |
|---|---|---|---|---|---|---|
| | Organic Extracts | | | | Aqueous extracts | Extracellular substances |
| | Hexane | Ethyl acetate | Dichloro methane | Methanol | | Culture Supernatant |
| *E. coli* MTCC-739 | - | - | - | +++ | + | - |
| *P. aeruginosa* MTCC-741 | - | - | - | - | - | - |
| *K. pneumoniae* MTCC-139 | - | - | - | - | - | - |
| *S. typhi* MTCC-531 | - | - | - | - | - | - |
| *S. aureus* MTCC-740 | - | ++ | - | +++ | ++ | - |
| *B. subtilis* MTCC-736 | + | ++ | - | ++ | ++ | - |
| *B. cereus* MTCC-430 | + | ++ | - | ++ | + | - |
| *B. pumilus* MTCC-1607 | + | + | - | ++ | + | - |

The diameter of the inhibition zone was scored as + (7to 10 mm), ++ (11to15mm), +++ (>15mm).(-) No activity.

**Figure 2**

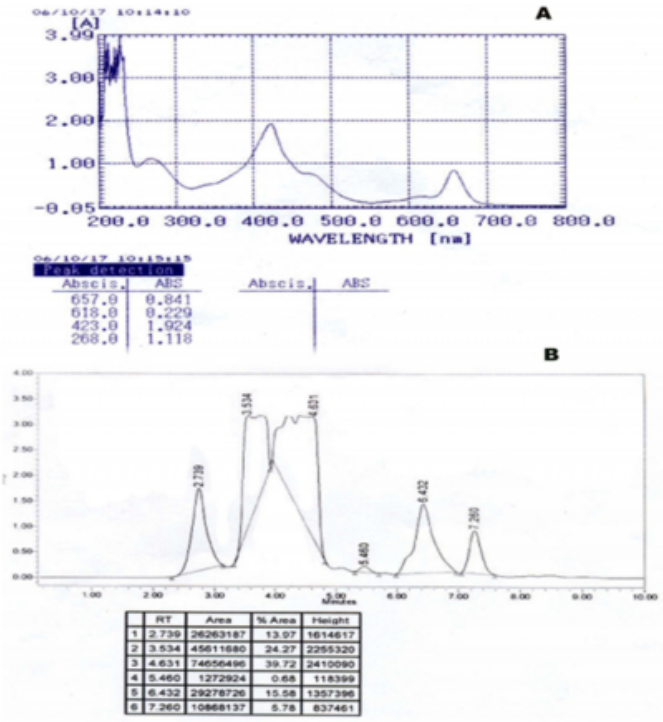
Table 2: Minimum Inhibitory Concentration (MIC) of crude methanol extract of

| Test organisms | Concentration of extracts in µg/ml | | | | | | | | | | MIC (µg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 512 | 256 | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | |
| *E. coli* | - | + | + | + | + | + | + | + | + | + | 512 |
| *S. aureus* | - | - | - | + | + | + | + | + | + | + | 128 |
| *B. subtilis* | - | - | + | + | + | + | + | + | + | + | 256 |
| *B. cereus* | - | - | + | + | + | + | + | + | + | + | 256 |
| *B. pumilus* | + | + | + | + | + | + | + | + | + | + | >512 |

( -) No growth observed, (+) Growth observed

**Figure 3**

Figure 1: UV-Vis spectrum (A) and HPLC chromatograph (B) of methanolic extract of




## References

1. Liu, X.J. and Chen, F. 2003. Cell differentiation and colony alteration of Nostoc flagelliforme, an edible terrestrial cyanobacterium, in different liquid suspension culture. Folia microbial, 48:619-626.
2. Falch, B.S., Konig, G.M., Wright, A.D., Sticher, O., Angerhofer, C.K., Pezzuto, J.M. and Bachmann, H. 1995. Biological activities of cyanobacteria: Evaluation of extracts and pure compounds. Planta Med, 61:321-328.
3. Mundt, S., Kreitlow, S., Nowotny, A. and Effmert, U. 2001. Biological and pharmacological investigation of selected cyanobacteria. Int J Hyg Environ Health, 203: 327-334.
4. Kaushik, P. and Chauhan, A. 2008. In vitro antibacterial activity of laboratory grown culture of Spirulina platensis. Indian Journal of Microbiology, 48.
5. Kaushik, P. and Goyal, P. 2008. In vitro evaluation of Datura innoxia (thorn-apple) for potential antibacterial activity. Indian Journal of Microbiology, DOI: 10.1007/s12088-008-0020-7.
6. MacMillan, J.B., Ernst-Russell, M.A., De Roop, J.S. and Molinski, T.F. 2002. Lobocyclamides A-C, lipopeptides from a cryptic cyanobacterial mat containing Lyngbya confervoides. J Org Chem, 67: 8210-8215.
7. Luesch, H., Yoshida, W.Y., Moore, R.E., Paul, V.J. and Mooberry, S.L. 2000. Isolation, structure determination and biological activity of Lyngbyabellin A from the marine cyanobacterium Lyngbya majuscula. J Nat Prod, 63: 611-615.
8. Papke, U., Gross, E.M. and Francke, W. 1997. Isolation, Identification and determination of the absolute configuration of fischerellin B. A new algicide from the freshwater cyanobacterium Fischerella musscicola. Tetrahedron Lett, 38: 379-382.
9. Koehn, F.E, Longley, R.E., and Reed, J.K. 1992. Microcolins A and B, new immunosuppressive peptide from the blue-green alga Lyngbya majuscula. J Nat Prod, 55: 613-619.
10. Hayashi, T. and Hayashi, K. 1996. Calcium spirulan, an inhibitor of enveloped virus replication, from a blue- green alga Spirulina platensis. J Nat Prod, 59: 83-87.
11. Stanier, R.Y., Kunisawa, R., Mandel, M. and Cohen, B.G. 1971. Purification and properties of unicellular blue-green algae (Order-chrococales). Bact Rev, 35: 171-205.
12. Perez, C., Pauli, M. and Bazerque, P.1990. An antibiotic assay by agar-well diffusion method. Acta Biologiae et Medecine Experimentaalis, 15: 113-115.
13. NCCLS, 1997. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard M7-A4, Wayne, Pa.

## Author Information

**P. Kaushik, M.Sc., Ph.D**
Department of Botany and Microbiology, Gurukul Kangri University

**Abhishek Chauhan, M.Sc., Ph.D (Submitted)**
Department of Botany and Microbiology, Gurukul Kangri University

**Garima Chauhan, M.Sc., Ph.D (Pursuing)**
Department of Botany and Microbiology, Gurukul Kangri University

**Pankaj Goyal, M.Sc., Ph.D (Submitted)**
Department of Botany and Microbiology, Gurukul Kangri University