*Research Article*
*J. Algal Biomass Utln*. 2013, 4 (1): 83–89
ISSN: 2229- 6905


Journal of Algal Biomass Utilization
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# Antibacterial Activity of Bloom forming Cyanobacteria against Clinically Isolated Human Pathogenic Microbes

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**Abstract**

Cyanobacteria are unique microbes and are potential sources of biologically active compounds with antiviral, antibacterial, antifungal and anticancer activities. We have isolated cyanobacteria *Anabaena variables* and *Synechococus elongates* from natural blooms and purified culture extracts were used for antibacterial efficacy against clinically isolated human pathogenic microbes- *E. coli*, *Enterococcus sp*., *Klebsiella sp*. Experimental results show that cyanobacterial extracts are capable of inhibiting the growth of pathogenic bacterial strains. Ethanol:acetic acid extract of *Anabaena variabilis* gave 17 ± 1.8 mm zone of inhibition against *E.coli* and methanol extract of *Synechococcus elongates* gave the highest antimicrobial activity against *E.coli* (17mm inhibition zone) and *Enterococcus sp*. (18mm inhibition zone). It was also concluded that ethanol was the best solvent for extracting the antibacterial agents from *Anabaena variabilis* while methanol was the best organic solvent for extracting antibacterial agents from *Synechococcus elongates*. In addition chloroform, acetone, water, acetic acid extracts of cyanobacterial strains also inhibit the growth of these pathogenic bacteria. Further exploration of antibacterial potential of cyanobacteria can open new horizons.

**Keywords**: Blooms; Antimicrobial activity; Cyanobacteria; Zone of inhibition.

## Introduction

Cyanobacteria or blue green algae are the most primitive form of the bacteria. They are the members of the plankton of marine, brackish and fresh waters throughout the world. They are also occurs on rocks & soils in symbiosis with plants, fungi & lichens. Harmful algal blooms have increased worldwide in fresh, estuarine and coastal marine waters [1-4]. Cyanobacteria are increasingly important in the biotechnology and resistant to the extreme environmental conditions. Rapid restorations of cyanobacterial activity under favorable conditions are characteristics of cyanobacteria [5]. The pharmacological activity of several classes of cyanobacteria has been identified and studied in last decades [6-8]. Cyanolichens (in symbiosis with lichens) have very potential applications in medicines [9] (Sunderaraman *et. al*, 1996), pharmaceuticals [10] (Gustafson *et. al*, 1989), enzymes, diagnostics, fuel and waste treatment, and recycling process [11]. Cyanobacteria are considered as the new therapeutic agents for a variety of diseases [12, 13]. Secondary metabolites from cyanobacteria are mainly associated with toxic, hormonal and antimicrobial effects [14, 15]. Antimicrobial effects of cyanobacterial extracts from aqueous and organic solvent are visiualized using selected microorganism as test microorganism [16-18].

Antibacterial activity is the antagonistic effect exerted by the microbes on other living species. Cyanobacteria have a tendency to produce secondary metabolites which prevent the growth of bacteria. Cyanobacteria inhibit the bacterial growth by interfering with the protein synthesis, retarding the metabolism and rupturing the cell wall.

In this study, we have explored the antibacterial potential of two bloom forming cyanobacteria *Anabaena variabilis* and *Synechococcus elongates* against clinically isolated human pathogenic gram negative bacteria *Escherichia coli*, *Enterococcus sp., Klebsiella sp.*

## Materials and Methods

### Cyanobacterial Cultures

*Anabaena variabilis* and *Synechococcus elongates* were isolated from natural blooms, purified in the laboratory and grown on BG-11 medium [19].

### Bacterial Strains

Gram negative bacteria *Escherichia coli, Enterococcus sp., Klebsiella sp.* obtained from Aadesh Medical College and Hospital (Bathinda). The overnight cultures of bacterial strains were used to check the antimicrobial activity were swabbed with sterile cotton on the surface of nutrient broth agar medium plates and incubated at $37^0$c for 24hrs. Sub culturing of bacteria was carried out every second day by taking the loop of colony from bacterial colonies grown on nutrient broth media in sterilized flask

containing peptone water (1.9g peptone in 125ml of water) incubated at $37^0$c and stored at $4^0$c.

**Preparation of Extracts**

Cyanobacterial cultures were harvested and dissolved in 3 ml extraction solvent Whattman paper discs (8mm) were saturated with 50µl of the test solution and dried under laminar air flow and placed on the nutrient agar plate, inoculated with bacteria. Plates were incubated at $37^0$c, for a period of 18-24h. Discs treated with 50µl of methanol, acetic acid were used as negative controls and streptomycin discs were used as positive controls which were dissolved in 50µl of streptomycin solution and air dried in laminar air flow. The extracts and supernatants containing antibacterial components produced distinct, clear, circular zones of inhibition of antibacterial activity. Antibacterial assay is carried out by disc and cupboard method.

**Disc method**

Discs of Whattman filter paper (8mm) in size were dissolved in the 50µl of solvents for about 2 to 3 times, air dried in laminar air flow and placed on the bacterial petriplates. After 24hr. zone of inhibition around the disc was observed.

**Cup board method/ Well method**

Petri plates containing solidified media before swabbing were punched and wells were developed of appropriate size. The overnight cultures of bacterial strains were swabbed with cotton on the plates and 50µl of solvent solution were added in the wells. After overnight incubation, the diameter of inhibition zone was observed.

**1. Methanol extract**

Methanol extract was prepared by taking 3 different concentrations of methanol: - 70%, 80%, 100%. The extracts containing methanol were prepared in the ratio 2:1 (2X methanol: 1X cells) and were stored at a temperature of 4 °C, except methanol 70% which was stored at 60- 80 °C (evaporation for 12 hours).

***2.* Ethanol extract**

100% ethanol extract was prepared in the ratio of 2:1, stored at 4 °C.

**3. Water extract**

Water extract was prepared with the crushed cells in the ratio of 1:1, stored both at 4 °C and at 60-80 °C.

**Extracts with different solvents**

**1. Chloroform: acetone extract**

A 2:1 chloroform acetone extract was prepared by adding two volumes of chloroform (cold) and one volume of acetone, stored at 4 °C.

**Methanol: acetone extract:** Extract was prepared in the ratio 1:1 methanol: acetone and store at 4° C, and 60° C.

**Butanol: methanol extract (1:1):** Prepared in the ratio of 1:1 and stored at 4° C

**Acetone: ethanol extract (1:2)** Prepared with equal volume of acetone and ethanol and stored at 4° C.

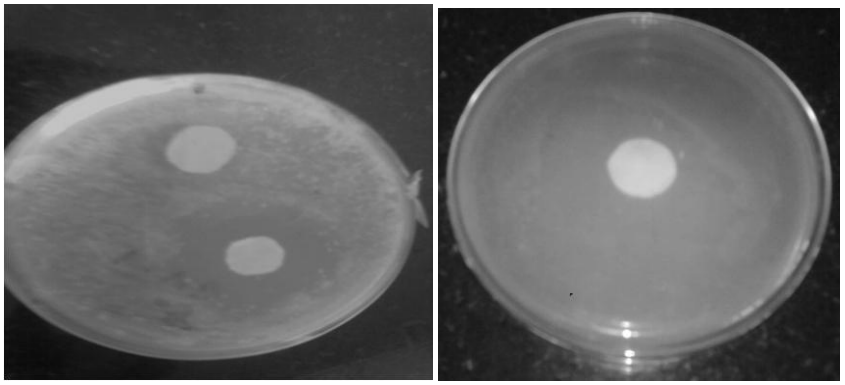
**Ethanol: acetic acid (2:1):** The extract prepared was stored at two different temperatures i.e. 4° C,and 60° C.

**Results and Discussion**

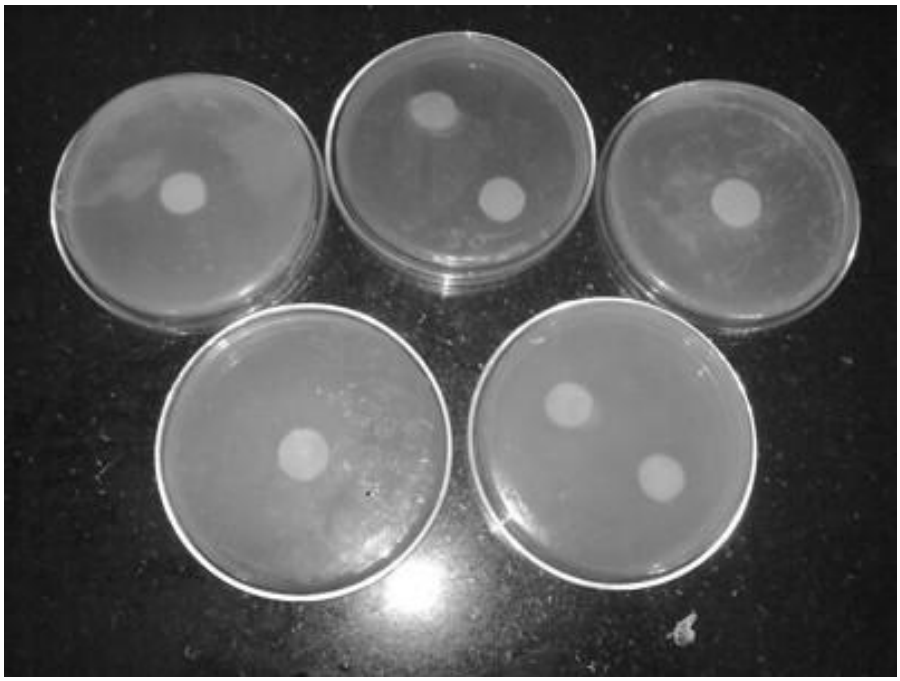
Bloom forming cyanobacterial strains were explored for their antibacterial activity. *Anabaena variabilis* and *Synechococcus elongates* were isolated from natural blooms. The strains were purified in the laboratory using serial dilution and solid liquid transfer technique. It was concluded from the study that the diameter of inhibition zone depends mainly on the type of the algal species, type of solvent used and the tested bacterial species.

The experimental analysis of antibacterial effects indicated that ethanol: acetic acid extract of (*Anabaena variabilis*) and methanol extract of (*Synechococcus elongates*) gave the highest antimicrobial activity against *E.coli* (17mm inhibition zone) and *Enterococcus sp.* (18mm inhibition zone). The results also indicated that ethanol: acetic acid had negative effect towards *Enterococcus sp.* and *Klebsiella sp.* whereas the methanol extract shows moderate activities with *Klebsiella* and *E.coli*. At the same time, ethanol, chloroform: acetone, methanol: acetone & acetone: ethanol extracts of *Anabaena variabilis* shows effects towards *Enterococcus* and *E.coli*. Ethanol extract of *Synechococcus elongates* shows moderate effects against *E.coli* and *Klebsiella sp.* and negative effect with *Enterococcus*. Thus, results proved that ethanol was the best solvent for extracting the antibacterial agents from *Anabaena variabilis* (Fig 1A, B) while methanol was the best organic solvent for extracting antibacterial agents from *Synechococcus elongates* (Fig 1C).

ISSN: 2229- 6905



**(A)** **(B)**



**(C)**

**Fig 1 – Antibacterial activity of some extracts (A)** Ethanol extract of *Anabena* **(B)** Ethanol: Acetic acid extract of *Anabena* **(C)** Methanol and Ethanol extracts of *Synechococcus* showing zone of inhibition against *E.coli*, *Enterococcus*, *Klebsiella* respectively.

The antimicrobial activity of the test microorganism against standard antibiotic was found that the effect of the standard antibiotic streptomycin was more than that of algal extracts on the *E.coli* and *Klebsiella sp*. On the other hand, the effect of standard antibiotic on *Enterococcus* was variable and was more or less similar to that of the investigated algal extracts (Table 1).

**Table** 1**: -** Antibacterial activity of the extracts of two cyanobacterial species such as methanol, ethanol, water, chloroform: acetone, methanol: acetone, acetone: methanol, ethanol: acetic acid against three bacterial species (*E.coli, Enterococcus*, *Klebsiella*) using agar plate diffusion method.

| **Antibiotic** | | | **Inhibition zone in diameter (mm)** | | |
|---|---|---|---|---|---|
| | | | ***Escherichia coli*** | ***Enterococcus*** | ***Klebsiella*** |
| Streptomycin 10ug/disc | | | 21 ± 2.7 | 19 ± 1.9 | 17 ± 3.1 |
| | *Anabaena variabilis* | Methanol (70%) | 8.5 ± 2.0 | _ | _ |
| | | Ethanol (100%) | 11 ± 0.6 | 8 ± 0.5 | 12.5 ± 1.6 |
| | | Water | _ | _ | _ |
| | | Chloroform: Acetone | 2.5 ± 0.8 | 3 ± 0.2 | _ |
| | | Methanol: Acetone | 4 ± 0.4 | 9 ± 1.1 | _ |
| | | Acetone: Ethanol | _ | _ | 5 ± 1.7 |
| | | Ethanol: Acetic acid | 17 ± 1.8 | _ | _ |
| | *Synechococcus elongates* | Methanol (70%) | 12 ± 3.0 | 18 ± 3.0 | 14 ± 2.1 |
| | | Ethanol (100%) | 6.5 ±1.1 | _ | 11 ± 0.9 |
| | | Methanol: Acetone | – | 4 ± 0.8 | _ |

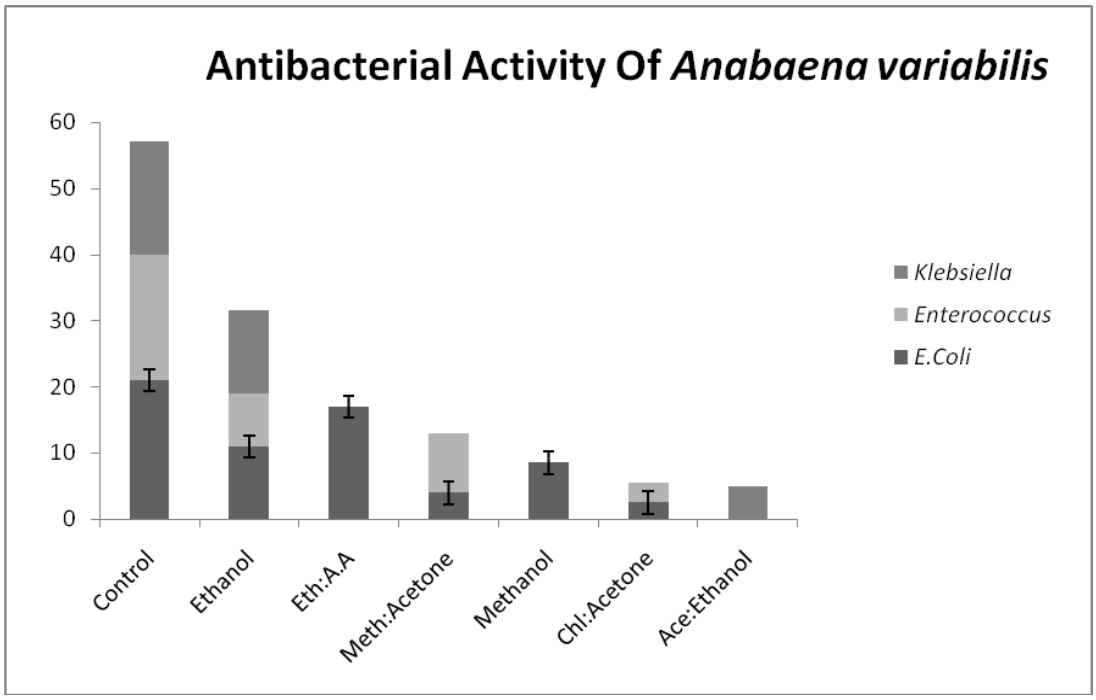



**Fig. 2: Antibacterial activity of *Anabaena variabilis*.**

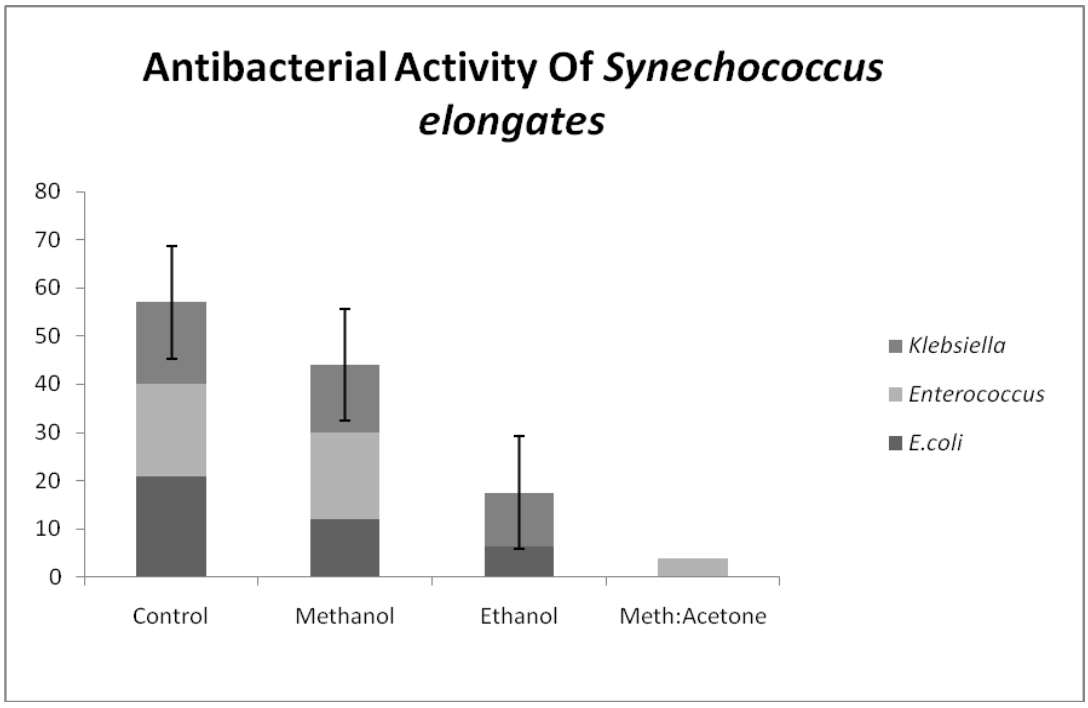



**Fig. 3: Antibacterial activity of *Synechococcus elongates*.**

Experimental results indicated that the cyanobacterial extracts contained different antibacterial substances and reflect the variety of environmental stress [15, 20]. It has been revealed that the maximum inhibition zone showed by the species affected by the chemical and physical properties of the growth medium and the size and ionic charge of the antibiotic molecule [21]. Campbell [22] reported that the toxic effects of cyanobacterial extracts on luminescent bacteria did not correlate with the concentration of microcystin-L-R,

but appeared to be due to other compounds present in the cyanobacteria. Acetone extract of *Nostoc* sp. (NTK28) showed minimal activity against S. *aureus* at higher (45 and 60µg) concentration [23]. Similarly, acetone extract of *Nostoc* sp. (NTK29) showed minimal activity against *Klebsiella* sp. at higher concentration. Unsaturated fatty acids extracted mixture of cyanobacteria *Oscillatoria redekei* has been reported to have antimicrobial activity against gram positive bacteria while no activity was found against gram negative bacteria [24, 25]. The reports showed that among 22 cyanobacterial samples isolated from freshwater and terrestrial environment, the extracts showed activity against gram positive bacteria but no activity on gram negative bacteria [26]. Three strains of cyanobacteria *Lyngbya majuscula*, *Microcystis aeruginosa* and *Plectonema boyamum* were tested for the antibacterial properties towards *Xanthomonas vesicatoria* [26, 27]. Cytotoxic activity exhibited by the cyanobacterial extracts could be of 38.6% [28].

Cyanobacteria produce different compounds include possible therapeutics, insecticides, and anti-fouling agents some have been lead-optimized and are in clinical trials. Cyanobacteria also produce mycosporines, UV-absorbing compounds useful for protecting biological samples or coating material surfaces. Cyanobacteria are unique prokaryotes with immense potentials. Exploring different cyanobacterial strains for antimicrobial efficacy and improvisation in methods to enhance their efficacy can prove to be a turning point.

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