Tropical Plant Research
An International Journal
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ISSN (E): 2349 – 1183
ISSN (P): 2349 – 9265
2(3): 288–291, 2015

**Research article**

# Phytoconstituents composition and *in vitro* antibacterial activity of a blue green alga *Anabaena variabilis* Kütz. ex Born. et Flah.

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[Accepted: 15 December 2015]

**Abstract:** Cyanobacterial species produce various types of secondary metabolites which are used in drug development as medicinal importance, dye and pigmentation and, food additives.The preliminary screening of phytoconstituents analyses in various solvent extracts (benzene, chloroform, acetone and methanol) of a microscopic blue green alga, *Anabaena variabilis* Kütz. ex Born. et Flah., collected from a pond at Diara in Hooghly district, West Bengal, India was done following standard methods and the results exhibited the presence of alkaloid, terpenoid, phenol, flavonoid, flavonol and phycocyanin phytoconstituents in those solvent extracts. The antibacterial activity of the said alga was detected using eight pathogenic bacterial strains out of which three are Gram positive (*Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*) and five are Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Shigella flexneri* and *Vibrio cholerae*) by agar well diffusion method with minor modifications. Maximum inhibition zones were observed in acetone and benzene extracts against the same Gram positive bacteria *Bacillus subtilis*. This study also indicated that acetone and benzene crude extracts were best active solvents against most of the studied bacterial strains followed by methanol and chloroform crude extracts. So, the present work suggested that this alga possessed several bioactive phytoconstituents which showed antibacterial potentiality on the tested pathogenic bacteria.

**Keywords:** *Anabaena variabilis* - Phytoconstituents screening - Antibacterial activity.

[**Cite as:** Halder N (2015) Phytoconstituents composition and *in vitro* antibacterial activity of a blue green alga *Anabaena variabilis* Kütz. ex Born. et Flah. *Tropical Plant Research* 2(3): 288–291]

## INTRODUCTION

Algae are one of the most important richest sources of novel bioactive compounds which may be used in the pharmaceutical industries for the development of pharmaceuticals. Cyanobacteria are well known incredible, primitive and prokaryotic algal group which produce various types of phytoconstituents with biological activities.

The search of cyanobacterial species with antimicrobial activities has gained much momentum in the recent times due to growing worldwide concerns about increases in the emergence of antibiotic resistance among the pathogenic bacteria (Chauhan *et al*. 2010). Cyanobacteria have been recognized as a good source of antibiotic with antimicrobial activities during the last two decades (Bhattacharyya *et al*. 2013). They have potentiality to produce an elaborate array of secondary metabolites with unusual structures and potent bioactivities. They also produce industrially important secondary compounds like antibiotic, algicide, cytotoxic, immune suppressive and enzyme inhibiting agents (Shaieb *et al*. 2014). The cyanobacterial algal group produces different biologically active compounds which may be used in drug development and some of the active components have potentially to inhibit anticancer, antimicrobial, antiviral, anti-inflammatory effects (Seal *et al*. 2014, 2015). The aims of the present study were preliminary screening of phytoconstituents and to investigate antibacterial potentiality of four organic solvent extracts of this alga against human pathogenic bacteria.

## MATERIALS AND METHODS

*Selected species*


Received: 14 September 2015 Published online: 31 December 2015

*Anabaena variabilis* Kütz. ex Born. et Flah. is a blue-green alga which belongs to the family Nostocaceae under the order Nostocales of class Cyanophyceae and it is grown in different types of aquatic bodies. The trichomes contain heterocystous stuctures and the alga acts a bio-fertilizer due to having the capability to fix atmospheric nitrogen in the soil.

*Collection of algal sample*

Algal material had been collected in plastic packets and sterilized glass containers from a pond of Diara (N 22° 79' E 88° 28') of Hooghly district (N 20° 30'–23° 1' E 87° 30'–80° 30'), West Bengal. Detailed study was made by examining the specimen under Olympus microscope (Model-CH20i). Identification of taxon was accomplished with the help of authentic literatures (Geitler 1932, Desikachary 1959).

*Preparation of algal extracts*

For extraction, the selected algal material, collected from pond was washed under running tap water to remove adhering soil particles, epiphytes and associated debris, if any, and dried up at room temperature. Benzene, chloroform, acetone and methanol were used for preparing the different solvent extracts. Extracts were prepared by grinding the algal material in a mortar and pestle at 20±1 °C. In a soxhlet extractor at 50–55 °C, extracts were concentrated under reduced pressure in a rotary evaporator and kept deep frozen until tests. Each time before extracting the powdered drug (marc) was dried up in a hot air oven. The concentrated extracts were obtained with each solvent were weighed. However, when the pH was out of range it was adjusted to 7.0 before assay of antibacterial activity.

*Preliminary qualitative phytoconstituent tests*

All the extracts were subjected to preliminary phytoconstituents screening as described by Trease & Evans (1989), Harborne (1998) and Silveira *et al*. (2007).

1. *Used Gram positive bacteria*

The tested bacterial strains were *Bacillus subtilis* MTCC441, *Micrococcus luteus* MTCC1538, *Staphylococcus aureus* MTCC3160. These strains were maintained on nutrient agar slant at 4°C and sub-cultured for 24 h before use.

2. *Gram negative bacteria*

The tested bacterial strains were *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC2581, *Shigella dysenteriae* (clinically isolated), *Shigella flexneri* MTCC1457, *Vibrio cholera* MTCC3904. The maintenance procedure of these bacteria was same as Gram positive bacteria.

*Antibacterial assay*

The antibacterial activity test of the above said alga was done using agar well diffusion method of Perez *et al*. (1990). 0.1 ml of diluted inoculum ($10^5$CFU $ml^{-1}$) of the bacterial strain was swabbed on the nutrient agar plates. Wells of 5.0 mm size diameter were made into the agar plates with the help of sterilized cork borer (5.0 mm). Using a micropipette, each of 100 µl of the algal extract was added to the wells made in plates. The plates were inoculated aerobically in an upright position at 37±2 °C for 24–48 h. Antibacterial activity was evaluated by measuring the zone of inhibitions (mm) against the bacterial strains. The tests were performed in triplicates with controls.

*Statistical analysis*

The results were presented as mean values ± standard errors (SE). The standard errors were calculated using statistical software package (SPSS v. 13, Inc.USA).

## RESULTS AND DISCUSSION

Qualitative screening of phytoconstituents of organic solvent extracts (benzene, chloroform, acetone and methanol) of *Anabaena variabilis* Kütz. ex Born. et Flah. showed the conformity of different types of bioactive compounds (Table 1). The result of the phytoconstituents screening revealed that this alga contained alkaloid, tannin, terpenoid, phenol, flavonoid, flavonol and phycocyanin compounds.

In table 2, the result of antibacterial activity of the said algal species against three Gram positive and five Gram negative bacterial strains was shown. The extracts of this alga confirmed antibacterial activities against only six tested pathogenic bacteria out of eight bacterial strains. Acetone and benzene extracts exhibited better antibacterial activities than other two organic solvent extracts. Regarding antibacterial activities, *Bacillus*

*subtilis* and *Escherichia coli* were more susceptible whereas, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed intermediate results. *Micrococcus luteus* and *Vibrio cholera* were comparatively less susceptible against all the four solvent extracts. Both acetone and benzene extracts executed the higher inhibition zones of 18.1 mm and 16.5 mm against the same bacteria *Bacillus subtilis*. Comparatively, somewhat lower antibacterial activities were recorded by methanol and chloroform extracts of *Anabaena variabilis.* The aqueous extract showed poor activities against the tested pathogenic bacteria (Table 2).

**Table 1.** Presence of phytoconstituents of *Anabaena variabilis* Kütz. ex Born. et Flah. in four solvents.

| | **Alkaloid** | **Tannin** | **Steroid** | **Saponin** | **Glycoside** | **Terpenoid** | **Phenol** | **Flavonoid** | **Flavonol** | **Phycocyaninn** |
|---|---|---|---|---|---|---|---|---|---|---|
| Benzene extract | + | - | - | - | - | + | + | + | + | + |
| Chloroform extract | + | - | - | - | - | + | + | + | + | + |
| Acetone extract | + | - | - | - | - | + | + | + | + | + |
| Methanol extract | + | + | + | - | - | + | + | + | + | + |

**Note:** + = Presence or positive reactions; – = Absence or negative reactions.

**Table 2.** Antibacterial activity of different solvent extracts of *Anabaena variabilis* Kütz. ex Born. et Flah.

| **Solvents** | **Zone of inhibition (mm) as (Mean ± SE ) in different bacteria** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Bs*** | ***Ml*** | ***Sa*** | ***Ec*** | ***Sd*** | ***Pa*** | ***Vc*** | ***Sf*** |
| Benzene | 16.5±0.07 | 10±0.03 | 15±0.05 | 16.1±0.06 | - | 14±0.05 | 11±0.03 | - |
| Chloroform | 14.4±0.06 | 8.6±0.02 | 12±0.04 | 14±0.05 | - | 12±0.04 | 10±0.03 | - |
| Acetone | 18.1±0.08 | 12±0.04 | 16.1±0.06 | 15.5±0.07 | | 14.2±0.06 | 13.8±0.04 | |
| Methanol | 14.6±0.05 | 9.0±0.02 | 13.2±0.04 | 14.8±0.04 | - | 13±0.04 | 8.0±0.03 | - |
| Water | 9.0±0.04 | - | 7.0±0.03 | 8.0±0.03 | - | 7.0±0.02 | - | - |

**Note:** *Ec= Escherichia coli, Sf= Shigellaflexneri, Pa= Pseudomonas aeruginosa, Sd= Shigelladysenteriae, Vc= Vibrio cholerae, Bs= Bacillus subtilis, Ml= Micrococcus luteus, Sa= Staphylococcus aureus*; "-"= Not detected.

Chauhan *et al*. (2011) carried out *in vitro* antibacterial evaluation of *Anabaena* sp. against several clinically significant pathogenic bacteria and HPTLC analysis of its crude extracts indicated that different solvents possessed significant antibacterial effects on both Gram positive and Gram negative bacteria. In the current scenario, similar observation was noticed.

Ethyl acetate solvent among three solvents (chloroform, ethyl acetate and n-butanol) was proved as a most effective organic solvent for the extraction of the antibacterial compounds in five species of *Anabaena* namely *Anabaena solitaria*, *Anabaena variabilis*, *Anabaena cylindrical*, *Anabaena spiroides* and *Anabaena circinalis* (Abdel-Raouf & Ibraheem 2008). Among various solvent extracts (acetone, methanol, ethanol. diethyl ether, chloroform and hexane) methanol extract of *Anabaena circinalis* appeared to be the most effective solvent by showing maximum antibacterial activity against the selected bacterial pathogens *viz. Escherichia coli, Salmonella typhi, Proteus vulgaris, Streptococcus pyogens, Pseudomonas solanocearum* (Sivakami *et al*. 2013). In this study, it was noticed that acetone and benzene extracts were more effective over the other two solvent extracts for extraction of antibacterial compounds.

Malathi *et al*. (2014) while working on the screening of three cyanobacterial strains observed significant antibacterial activities of *Anabaena variabilis* against the bacteria *Bacillus subtilis* (ATCC-11774) and *Pseudomonas aeruginosa* (ATCC-15442) in chloroform and methanol crude extracts respectively. In the present work, *in vitro* antibacterial activities of this alga showed the similar result. Maximum size of inhibition zone (9.67±0.57) of *Anabaena* BT2 was observed in methanol extract against *Pseudomonas* sb1 (Yadav *et al*. 2012) but in this study, higher inhibition zone (18.1 mm) was observed in acetone extract against a Gram positive bacteria *Bacillus subtilis*.

## CONCLUSION

It is quite evident from the discussion that the above said alga possessed several bioactive compounds of pharmaceutical interests and the formation of inhibition zones (mm.) depended on various factors like types of

algal species, solvents used and the kind of tested pathogenic bacterial stains. Therefore, this study could be used for future research and to produce antibacterial drugs of Cyanobacterial origin.

**ACKNOWLEDGEMENTS**

The author is indebted to Dr. S. N. Sinha, University of Kalyani, Nadia, West Bengal for his guidance and supports. The author is also grateful to Dr. T. Seal and K. Chaudhuri, Plant Chemistry Department, BSI, Howrah for their kind co-operations and suggestions.

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