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EVALUATION OF ANTIMICROBIAL PROFILE OF CYANOBACTERIAL SPECIES ISOLATED FROM BILASPUR TOWN OF CHHATTISGARH STATE

Biological Science

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ABSTRACT

Cyanobacteria produces certain bioactive compounds as their secondary metabolites that have antimicrobial nature against pathogenic microbial strains. Under present research work we have evaluated cyanobacteria which isolated from Bilaspur Town of Chhattisgarh State, for their antimicrobial profile against pathogenic microbial strains. Out of eighteen cyanobacterial isolated Anabeana iyengerii showed maximum antimicrobial activity. Result exhibited that Methanol extract (100%) of Anabeana iyengrii shown maximum clear zone of 14.4 ±0.59 mm (Mean ±SD) against Staphylococcus aureus and 18.7 ±0.46 mm (Mean ±SD) against Bacillus cereus. Further study could be done to characterize bioactive compounds produced by Anabeana iyengerii to find which bioactive compound are responsible for antimicrobial activity and after purification, toxicity and efficiency of purified bioactive compound could be evaluated against pathogenic microbial strains.

KEYWORDS

Cyanobacteria, Antimicrobial Profile, Bioactive compounds, Bilaspur Town

1. INTRODUCTION

Cyanobacteria are photosynthetically active bacteria and well known for the oxygenation of Earth's atmosphere (Kasting & Siefert, 2002) and nitrogen fixation and biocidal (release exotoxin) (Dittmann et al., 2013). Cyanobacteria referred as blue-green algae but classified under prokaryotes (Whitton, 1992) and comprising more than 2000 species. Cyanobacteria has been largely studied for their bioindicators to identify environmental quality (Mateo et al., 2015; Monteguardo, 2016), exotoxins production and some other secondary metabolites (Abed et al., 2009; Ducat et al., 2011; Vijajacumar & Mengakha, 2015). Cyanobacterial species are good sources of bioactive compounds having antimicrobial and toxic properties (Namikoshi & Rinehart, 1996). Antimicrobial effects of Cyanobacterial extracts are visualized by various bioassays against selected test organisms (Frankmoll et.al, 1992).

Cyanobacteria are the rich source of organic acids, peptides, growth substances, antibiotics, enzymes and toxic compounds (Codd, 1997 and Carmichael, 2001) and biofertilizer (Bano and Siddiqui, 2004). Plenty of cyanobacterial strains are known to produce intracellular and extracellular secondary metabolites with antimicrobial properties (Sultan et al., 2016). These secondary metabolites consisted of phenolics, terpenoids, N-glycosides and isonitrile-containing indole alkaloids (Mundt et al., 2003; Neuhof et al., 2005). Presently more than 75% of drugs used for the treatment of infectious diseases are derived from natural sources (El Gendy et al., 2016).

Cyanobacterial secondary metabolites (or bioactive compounds) have been widely studied in last few decades. Researchers communities have focused towards the cyanobacterial derived bioactive compounds and their biological effects on other life forms (Namikoshi and Rinehart, 1996; Sivonen, 1996; Welker and von Dohren, 2006) because bioactive toxins play an important role in biological control (Jeong-Dong Kim, 2006). Many of cyanobacterial species have been evaluated towards biomedical applications (especially as antibiotics against microbial infection) and around 100 species known for their significant bioactive secondary metabolites (e.g. peptide, alkaloides, macrolactones and heterocyclic compounds) and each of them exhibits specific toxicity (Codd & Bell, 1985). Main toxin producers are *Microcystis, Oscillatoria, Anabaena, Scytonema, Gleotrichia, Aphanizomenon, Nostoc, Synechocystis, Cylindrospermum, Schizotrix, Nodularia, Cylindrospermopsis* and *Hapalosiphon*. Lots of literature available on biological control of plant pathogens (Adams, 1990; Stirling, 1991; Tjamos et al., 1992; Dwivedi, 1956; Mehrotra et al., 1997) and they investigated bioactive compounds as bacterial and fungal control agents because they are causing various kind of diseases over agricultural crops and lead to large scale spoilage and are also injurious to humans and environment (R. J. Cook, 1983). To minimize such issues and damages caused by microbes could potentially be used as bio-control agents. Thus the present work has been done to evaluate the cyanobacterial species for their efficiency as antimicrobial agent.

2. MATERIALS AND METHODS

Isolation and identification of Cyanobacteria flora of Bilaspur Town of Chhattisgarh state of India has been done and their antimicrobial activities against pathogenic microbes (Bacteria) have been analyzed. Active cultures of pathogenic bacterial strain were procured from Chhattisgarh Institute of Medical Sciences (CIMS), Bilaspur and Barrister Thakur Chhedilal College of Agriculture & Research Station, Bilaspur. Active cultures were brought in the laboratory and maintained by regular sub culturing and as agar slant. Three procured bacterial pathogen *Escherichia coli* (for screening), *Staphylococcus aureus* and *Bacillus cereus* were maintained in Luria-Bertani (LB) broth media.

2.1. SAMPLE COLLECTION

Sample was collected from Bilaspur Town of Chhattisgarh State. To do so Bilaspur Town was divided into four different sectors and each sector has three sampling sites viz., Agriculture field, Pasture field and Pond water (Table - 1). Samples were collected on clean and sterilized plastic bags in triplicates and were brought to laboratory. Plastic bags were labeled with sampling site, sampling date, temperature and pH of the sample. The samples were kept in refrigerator until used.

2.2 Isolation And Identification of Cyanobacteria

Cyanobacteria were isolated from samples using standard protocol. BG-11 growth media was used for the isolation of cyanobacteria as suggested by Shrivastava (2000) & Shrivastava et al. (2005).

2.2.1 Isolation

The samples were spreaded on growth media and incubated for overnight inside at 25°C. Colonies observed were aseptically transferred in sterile glass capillary tubes consisted of 10 ml of sterile BG-11 growth medium (composition mentioned in Table - 2) and incubated inside a growth chamber having 25°C temperature, supplied with 5% CO_2 and illuminated with fluorescent lamps with irradiance of 3.0 to 5.0 k lux.

2.2.2. Identification

The cyanobacterial isolates were observed under compound light microscope (magnification of 100X x 15X). cyanobacterial isolates were characterized and identified by key suggested by Desikarchary (1959, 1972), and Anand (1989).

2.3 Screening of Exo-Toxin Releasing Cyanobacteria

Crude extract of each pure culture of cyanobacterial isolates were

prepared with hot water. Cyanobacterial isolates were screened against *E. coli* for their antimicrobial activity by disc diffusion protocol. Bacterial culture broth (12 h old) were prepared and 0.5 ml from that broth, spreaded on the surface of the Nutrient agar Media (for bacteria) by using cotton swab aseptically. Then, filter paper discs soaked in 0.5 ml cyanobacterial hot water crude extracts was placed over the media surface. Positive control (Gentamycin) and negative control (hot water) were placed on inoculated agar plates. The plates were incubated at 37°C for 2 days. The antibacterial activity was observed as clear zone size in millimeter occurred surrounding the disks.

2.4. Assessment of Antimicrobial Nature of Cyanobacteria

Crude extracts of cyanobacteria in Ethanol and Methanol and hot water, were assessed against pathogenic bacterial strains. Antimicrobial assay done as per described in section 2.3. Larger clear zone diameter means the greater is the antimicrobial activity.

2.5. Statistical Analysis

All the experiments were done in three replicates to minimize error. The quantitative data obtained through appropriate statistical analysis. Mean and Standard Deviation were calculated in Microsoft Excel software.

3. RESULT AND DISCUSSION

Present study has been focused on the assessment of antimicrobial activity of isolated cyanobacterial strains. Total 18 species were observed to have an antimicrobial activity against selected pathogens.

4.1 Servey and Sampling

Total of 36 samples were collected from 12 sites of Bilaspur districts. From each site 3 samples were collected. pH of the sampling site were ranges from 5.7 to 6.8 in soil samples and from 6.1 to 7.5 in water samples. Temperature of the sampling sites was ranges from 19 ^{0}C to 33 ^{0}C in soil sample and 16 ^{0}C to 30 ^{0}C in water sample.

4.2 Isolation and Identification of Cyanobacteria

From 36 samples, 18 Cyanobacterial isolates were isolated and these cyanobacterial isolates were further go through screening against pathogenic bacteria mentioned under section 4.3. Cyanobacterial isolates were identified by Microscope at a magnification of 100X x 15X as per metioned by Desikarchary (1959, 1972), and Anand (1989).

4.3 Screening of Exo-Toxin Releasing Cyanobacteria

Morphologically identified cyanobacterial strains were screened against *Escherichia coli.* Cyanobacterial strains exhibiting maximum antimicrobial activity against *E. coli* were selected and evaluated for further assessment of antimicrobial property against bacterial strains. Hot water extract of each cyanobacterial strain was prepared and assessed antimicrobial activity against selected pathogens. Results exhibited that *Anabeana iyengerii* exhibited maximum activity of 13.9 ±1.6 mm (Mean ±SD) *E. coli.*

4.3 Assessment of Antimicrobial Nature of Cyanobacteria

As per the observation in 4.2 section *Anabeana iyengerii* was selected due to their maximum activity against *E. coli.* Hot water extract, ethanol extract and methanol extract of *Anabeana iyengerii* were prepared. and evaluated for antimicrobial activity. Each cyanobacterial crude extracts were prepared four different concentration (0%, 25%, 75% and 100%). Antimicrobial activity of *Anabeana iyengerii* was evaluated against S*taphylococcus aureus* and *Bacillus cereus*. Standard Antibiotic Gentamycin were used. Result exhibited that Methanol extract (100%) of *Anabeana iyengrii* has been shown maximum clear zone of 14.4 ±0.59 mm (Mean ±SD) against *Staphylococcus aureus* (Table - 3) and Ethanol extract (100%) of *Anabeana iyengrii* 18.7 ±0.46 mm (Mean ±SD) against *Bacillus cereus* (Table - 4). Bhattacharyya et al. (2013) also reported that Methanolic extract of fresh water algae *Anabaena* species (*Anabaena fertilisimia*) have been shown maximum antimicrobial activity (23.66 mm) against gram *Staphylococcus aureus* (positive bacteria). It has been claimed by Ghosh et al. (2008) that organic extracts has more potential than that of aqueous extract in terms of bioactivity. Cyanobacteira have been reported to produce bioactive compounds viz., lipopeptides, amino acids, fatty acids, macrolides and amides (Singh et al., 2005) and most of these compounds have been reported to have antibacterial activity (Bloor and england, 1989). *Anabaena* species have been most widely studied. Further, Seddek et al., (2019) has been evaluated the antimicrobial activity of organic solvent extracts of *Anabaena oryzae* against human pathogenic bacterial strains as well as for antioxidant and cytotoxic activity against human breast adenocarcinoma (MCF-7) and found that acetone extract of *Anabaena oryzae* to be the most potential against bacterial strains while acetone and methanol extracts of *Anabaena oryzae* exhibited high toxicity against MCF-7 cell line. They also assessed acetone extracts by Gas Chromatography Mass Spectrophotometer (GC–MS) and found diacetone alcohol, acetic acid butyl ester, mesityl oxide and heptadecane as the major compound.

4. CONCLUSION

Present study has been done to evaluate antimicrobial profile of cyanobacterial species isolated from Bilaspur Town of Chhattisgarh State. Samples were collected for the isolation of cyanobacteria and evaluated them for assessment of antimicrobial profile. *Anabeana iyengrii* was found to be potential antimicrobial property against *Staphylococcus aureus* and *Bacillus cereus.* Every cyanobacterial species that has antimicrobial activity possessing certain kinds of bioactive compounds which are most often secondary metabolites of the cell. It may be extracellular or intracellular. Bioactive compounds are the mixture of different biologically active compounds but only few of them are actually responsible for antimicrobial activity and such compound need to be isolated and identified to make it more effective and efficient.

Future Scope

Further study could be done to assessment of physiological property, chemical stability, kinetic and dynamics of methanol and ethanol extract of *Anabeana iyengrii* spectrum of activity. Anther dimension of research could be done to identify, isolation and characterization the most potential bioactive compound present in methanol and ethanol extract of *Anabeana iyengrii.*

Table 1 - Sample

Sample Zone	Sample Sector	Sample Site	Sample size	Sample Processing
Bilaspur Town (C.G.)	4 Sector-I Sector-II Sector-III Sector-IV	3 Agriculture field, Pasture field and Pond water	36	C.M.D. College, Bilaspur (C.G.)

Table 2 - Media Composition:BG-11

Ingredients	Concentration (mg/l)
$CaCl_2$	36.00
Citric Acid	6.00
$Co(NO)_3,6H_2O$	0.05
$CuSO_4 5H_2O$	0.079
De ionized Water (DD)	1000 ml
H_3BO_3	2.86
Iron (III)ammonium citrate	6.00
K_2HPO_4, $3H_2O$	40.00
$MgSO_4$. $7H_2O$	75.00
$MnCl_2$. $4H_2O$	1.81
NaCl	18,000.00
Na_2EDTA	1.00
Na_2MoO_4. $2H_2O$	0.39
*Na_2NO_3	150.00**
$ZnSO_4$. $7H_2O$	0.222
pH	7.1 ±0.2
Distilled water	1000 (ml)

Table 3 - Antimicrobial activity of *Anabeana iyengrii* against S*taphylococcus aureus*

Cyanobacteria	Extract	(Concentration %)	*Staphylococcus aureus* mm (Mean ±SD)
Anabeana iyengrii	Methanol	100 %	14.4 ±0.59
	Methanol	75 %	12.7 ±0.42
	Methanol	50 %	9.5 ±0.28
	Methanol	25 %	6.2 ±0.20

Table 4 - Antimicrobial activity of *Anabeana iyengrii* against *Bacillus cereus*

Cyanobacteria	Extract	(Concentration %)	*Bacillus cereus* mm (Mean ±SD)
Anabeana iyengrii	Ethanol	100 %	18.7 ±0.46
	Ethanol	75 %	15.2 ±0.31
	Ethanol	50 %	11.5 ±0.27
	Ethanol	25 %	8.2 ±0.19

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