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Antibacterial potentialities of exotoxin releasing cyanobacterial isolates of local pond

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Abstract
Cyanobacteria produce variety of secondary metabolites that are not essential for primary metabolism of organisms and include compounds that can operate as allelo-chemicals, antibiotics, hormones, and toxins. Secondary metabolites of so many strains of cyanobacteria have been studied extensively and their antimicrobial properties such as antibacterial, antifungal, antialgal, & anticyanobacterial have been observed. To focus on the antibacterial behavior of the exogenous cyanotoxins, in-vitro study was carried out. Cyanobacterial strains, *Anabaena variabilis*, *Microcystis robusta,* and *Oscillatoria splendida* has been isolated from local ponds. Actions of methanol extract of *isolated Cyanobacterial strains* against clinically important standard pathogenic bacterial strains i.e. *E. coli* (ATCC-25922) and *Staphylococcus aureus*-(ATCC 25923) were investigated. Zone of inhibition was (ZOI) formed by all the antibiotics and extracts, were measured. The result showed that there is gradual suppression of growth exhibited by the pathogen with increasing concentration of extract over control. The outcome indicated that all the *Mirocystis robusta and Oscillatoria splendida.* posses a potential broad spectrum of antibacterial activity then *Anabaena variabilis* and that may lead in the development of new pharmaceuticals that address unmet therapeutic needs.

Keywords: Cyanobacteria, cyanotoxins, methanolic extracts, pathogenic bacterial strains, antibacterial behavior

Introduction
Cyanobacteria are the Gram negative photosynthetic prokaryotes found in almost all the ecological habitats. They are morphologically, physiologically and metabolically very diverse group and have been the intrested area for investigation since long. However, increasing trend of identifying of biological resources for the production of bioactive molecules prompted scientific community to screen cyanobacteria in recent past.
Several bioactive substances with antibacterial, antiviral, fungicidal, enzyme inhibiting activity have been isolated from cyanobacteria, in their spent culture media, or the water containing the cyanobacterial blooms in the field (Nowotny *et al*., 1997; Jaki *et al*., 1999; Pulz & Gross., 2004; Singh *et al*., 1999) [10, 8, 12, 13]. Several cyanobacterial species can produce toxic secondary metabolites, the cyanotoxins (Carmichael., 1997; Codd *et al*., 1999.) [2, 3]. At present, only a few records on the occurrence of cyanotoxins have been documented for the African continent, principally from South Africa, where associated animal poisonings have been reported for over 50 years (Wicks & Thiel., 1990; Scott., 1991; Harding & Paxton., 2001.) [7]. Cyanotoxins have been reported more recently from Morocco (Oudra *et al*., 2002) [11].

Material and Methods
Water Samples were collected in sterilized glass bottles. Cyanobacterial strains were identified after microscopic observation with the help of key given by Desikachari, 1959 & Anand, 1989 [1]. They were then cloned as laboratory culture intensively under aseptic condition at proper light & aeration using synthetic media i.e. BG-11 (Rippka *et al*., 1979) by proper laboratory methods. Dense populated pure cultures of Cyanobacteria were taken for extraction and for toxicity assessment, Cyanobacterial cells were concentrated by using continuous centrifugation. A portion of the concentrated samples were filtered through a glass fiber filter (What men-41) and air dried in an oven at 600 ^{0}C. Dried cell mass-100mg/100ml (w/v) were extracted with methanol, for 3 to 4 hours; then centrifuged at 5000 rpm for 7min. The supernatant was separated in fresh glass vials and filtered with 45 µm pore size. Different Dilutions (25%, 50%, 100%) were prepared for assessment of toxic behavior of crude extracts

against pathogenic bacterial strains. For assessment of toxicity of three Cyanobacterial strains releasing exotoxins against two bacterial strains disc diffusion method was applied and zone of inhibition in different concentrations (25%, 50% & 100%), were measured properly that was compared with the zone of inhibition in control set supplemented with antibiotics (Commercially standard and available in the market).

Result and Discussion

Table 1: Toxic behavior of crude extracts of Cyanobacterial isolates against pathogenic bacterial strains and its comparison with standard antibiotics.

Supplementation of Crude Extracts and Standard Antibiotic		Conc. of Extracts	Zone of inhibition in mm (Mean ±SD)	
			Staphylococcus aureus (ATCC 25923)	*E.coli* (ATCC-25922)
Control (Without supplementation)		0%	00	00
Crude Extracts	*Anabaena variabilis*	25%	00	00
		50%	2.52 ± 0.12	3.42 ±0.16
		100%	5.10 ±0.13	6.83 ±0.12
	Microcystis robusta	25%	7.52 ±0.13	8.92 ±0.11
		50%	10.7 ±0.22	13.8 ±0.13
		100%	18.8 ±0.12	20.7 ±0.18
	Oscillatoria splendid	25%	5.70 ±0.17	6.10 ±0.12
		50%	8.20 ±0.16	10.30 ±0.19
		100%	12.10 ±0.16	15.30 ±0.15
Antibiotics	Gentamycin	1mg/ml	18.0 ±0.21	22.0 ±0.35
	Ciprofloxacin	1mg/ml	20.3 ±0.22	25.4 ±0.12

During present investigation fifteen Cyanobacterial samples were collected and identified out of which three cyanobacterial strains (*Anabaena variabilis*, *Microcystis robusta*, *Oscillatoria splendida*) have been used for toxicity assessment against two laboratory bacterial strains *(E. coli and Staphylococcus aureous*). toxic behavior of three cyanobacterial strains against two bacterial isolates have been observed on culture plates and zone of inhibition was measured in triplicate set, which mean value with ± SD have been tabulated (Table -1), (Fig-1).

Experimental result of present investigation clarifies the toxicity of each Cyanobacteria against two bacterial strains in both methods i. e. zone of inhibition and growth pattern observation.

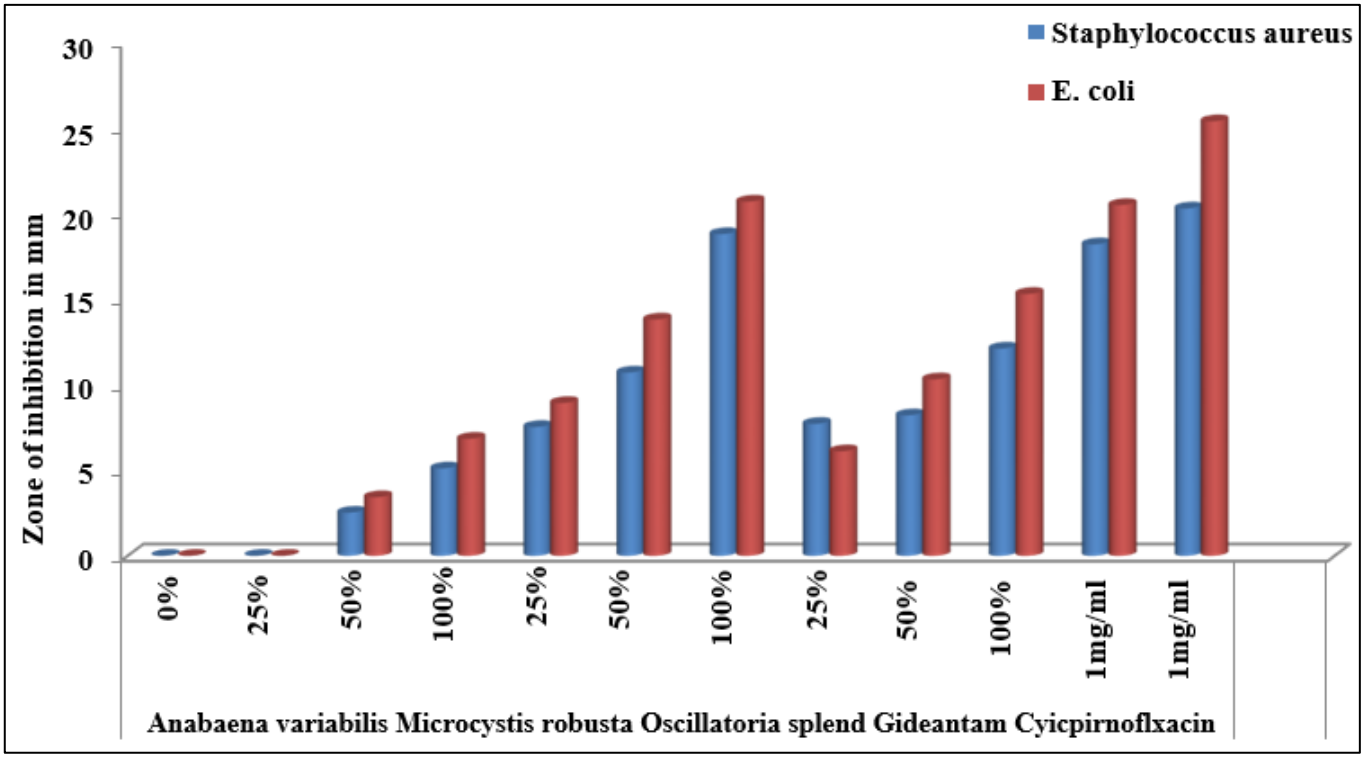

Fig 1: Toxic behavior of crude extracts of Cyanobacterial isolates

Zone of inhibition was found maximum in *E. coli* that are more or less closer to standard antibiotics and minimum in *Staphylococcus aureous* that is lesser than standard antibiotics.where as crude extract of *Microcystis robusta* and *Oscillatoria splendida* observed more toxic rather than *Anabaena variabilis* correspondingly.

Conclusion

Present investigation reveals that secondary metabolites of Cyanobacteria can be used as biological agent with potential application in pharmaceuticals. The result obtained in the present investigation was based on crude extracts, however suitable bacterial bioassays have been established to recognize and quantify antibacterial effect of Cyanobacterial extracts. During present course of investigation it was noticed that non heterocystous *Microcystis robusta* and *Oscillatoria splendid* were more toxic than that of *Anabaena variabilis* that was nitrogen fixing heterocystous strains. Cyanotoxins have the capacity to alter pathogenic bacterial strains these findings open new horizons for further research and developments on Cyanobacterial bioactive compounds.

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