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EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE DIFFERENT CULTURE EXTRACTS OF CYANOBACTERIAL SPECIES

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ABSTRACT

The main aim of the present investigation was to assess the efficacy of the antimicrobial activity of *Anabaena circinalis, Nostoc muscorum, Stigonema ocellatum* and *Hapalosiphon welwitschii*. The antimicrobial activity of cyanobacterial species were screened by using Agar disc diffusion method against pathogenic bacteria and fungi. Chloroform extract of *A. circinalis* was resulted the highest antibacterial activity 21.00 ± 0.57 mm against *E. coli* followed by *K. pneumonia.* Similarly, highest antifungal activity 15.00 ± 1.15 mm was recorded with *N. muscorum* followed by *S. ocellatum* and *A. circinalis* against *Trichophyton mentagrophytes*, *Aspergillus fumigatus* and *Aspergillus niger* respectively. Chloroform extract was showed highest antimicrobial activity among all the solvents tested; ethyl acetate and methanol were the next preferred solvents. Statistical analysis of the data showed a significant ($P \leq 0.05$) difference between the different solvents, cyanobacterial species against bacteria and fungi tried. In conclusion the present investigation suggested that cyanobacteria used as a potential natural drug sources owing to the antimicrobial efficiency.

KEYWORDS: ***Anabaena circinalis, Nostoc muscorum, Stigonema ocellatum, Hapalosiphon welwitschii*, Agar disc diffusion technique, Antimicrobial activity**

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INTRODUCTION

Cyanobacteria (blue green algae) are Gram negative autotrophic bacteria had a variety of metabolic capabilities and the ability to form symbiotic associations with plants, fungi and protists[1]. Cyanobacteria are a group of extraordinarily diverse, photosynthetic, oxygen-evolving, prokaryotes. Cyanobacteria occur in varied habitats ranging from marine to fresh water, from desert sand to hot springs and from snow field to ice caps and moist soil habitats [2]. Blue green algae also contain rich source of nutrients, including vitamin B, vitamin E, beta-carotene, manganese, zinc, copper, iron, selenium and fatty acid [3]. Most species of cyanobacteria are known to produce bioactive compounds with diverse biological activities in two ways either within the cell biomass i.e. intracellular and extracellular metabolites towards the environments. Generally, these activities may include cytotoxic, anticancer, antimalarial, antihepatoxic, immunosuppressive, hypo cholesterolemic, anticardiotoxic, antialgal, antibacterial, antifungal and antiviral activities [4,5,6,7]. *Fischerella* species had the ability to produce broad-spectrum of antimicrobial substance [8]. Cyanobacteria are morphologically, physiologically and metabolically very diverse group, which makes them as a promising group of organisms for research in drugs discovery. Besides diversity and their role in improving soil fertility, cyanobacteria, as a source of pharmacologically active compounds, have attracted immense interest in the field of microbiology [9, 10, 11]. Cyanobacteria with rich source of structurally novel and biologically active secondary and primary metabolites which are potential bioactive compounds [12]. Microalgae and cyanobacteria offer numerous advantages for antimicrobial investigations because of their enormous biodiversity and fast growth rate. The antimicrobial activity of cyanobacterial extracts are generally assayed using various organic solvents which always provide a higher efficiency in extracting compounds for antimicrobial activity [13, 14, 15]. Many marine and fresh water cyanobacterial species have good antimicrobial activity [16]. In the present study very few reports are available on antimicrobial properties of *A. circinalis, N. muscorum, S. ocellatum, H. welwitschii*. Hence the present study on evaluation of antimicrobial activity of cyanobacterial species against four pathogenic bacteria (*B. subtilis*, *S. aureus*, *E. coli* and *K. pneumoniae*) and (*A. fumigatus*, *A. niger*, *Mucor* sp. and *T. mentagrophytes*).

MATERIALS AND METHODS

Chemicals and Reagents

Chloroform, Ethyl acetate, Hexane, Methanol, DMSO, BG-11 media composition, Standard antibiotics Ciprofloxacin (10 µg/disc) and Nystatin (50 µg/disc) were used throughout the present study procured from Hi-Media Laboratories, Mumbai, India.

Sampling Area

Fresh water samples were collected from different locations of Warangal, Telangana State, India. The Warangal situated between North Latitude 17^0 19' and 18^0 36' and East Longitude 78^0 49' and 80^0 43'. All the samples were brought to laboratory in plastic vials and washed with distilled water to prevent potential contaminants.

Microorganisms used for the present study

To study the biological activity four bacterial strains of *Bacillus subtilis* (MTCC-1427), *Staphylococcus aureus* (MTCC-1430), *Escherichia coli* (MTCC-1302) and *Klebsiella pneumoniae* (MTCC-4030) and four fungal strains of *Aspergillus fumigatus* (MTCC-4163), *Aspergillus niger* (MTCC-4325), *Mucor* sp. (MTCC-3340) and *Trichophyton mentagrophytes* (MTCC-8476) were obtained from Microbial Type Culture Collection, Chandigarh, India.

Isolation and identification of cyanobacterial species

All the samples were kept in plastic vials to transfer to the lab. Streaking plate method was used for the isolation of cyanobacterial strains. Purified cyanobacteria cultures were transferred to 100 ml inorganic BG-11 medium. The inoculated conical flasks were incubated for 28 days at 26 ± 2^0C and 4000 lux light intensity. The identification of cyanobacterial species was made based on the morphological observations using standard monographs and protocols [17, 18].

Preparation of cyanobacterial extracts

At the end of the incubation period, each cyanobacterial cultures were harvested to obtain the biomass. The resulted biomass (5.0 g) was air dried under room temperature, powdered was used for the extraction by different solvents. Five hundred (500 mg) mg of dried powder of four cyanobacterial species were extracted in each of 20 ml of Aqueous, Chloroform, Ethyl acetate, Hexane and Methanol and all the solvents were refluxed until it gets to saturation at 24 hrs as outlined by [19]. The resultant crude extract 1 mg was weighed and dissolved in 1 ml of Dimethyl sulfoxide (DMSO) as stock solution and it was preserved at 4ºC until it use for further studies. For the bioassay study 50 µg/ml concentration of crude extract was used for the antimicrobial activities.

Assessment of biological activity

Antimicrobial activity was carried out by using agar disc diffusion method. The 20 ml of sterilized Muller-Hinton Agar medium for bacteria and Sabouraud Dextrose Agar medium (Hi Media- Mumbai, India) for fungi were used and the plates were seeded with 100 µl of selected test organisms [20]. Then 50 µl/ml of different cyanobacterial species crude extract was saturated on 6 mm filter paper disc and allowed to dry for 5 minutes. Then loaded discs were placed on the surface of the agar containing media. Finally the plates were incubated for 24 hrs at 37^0 C for bacteria and 48-72 hrs at 28^0 C for fungi. Ciprofloxacin 10 µg/disc (bacteria) and Nystatin 50 µg/disc (fungi) were

used as standard control. The zone of inhibition was recorded in millimeters (mm) by using Hi Antibiotic Zones Scale-C™ Hi Media (Mumbai, India). Antimicrobial activity was evaluated by measuring the zone of inhibitions against the tested microorganisms and their mean and standard errors were calculated.

Statistical analysis

The results of the present data obtained were statistically analysed and the results were expressed as the mean ($\bar{x}$) and standard error (SE) of three experiments (n=3) by using Graph Pad Prism version 5.03 (Graph Pad Software, Inc.,).

RESULTS

Isolation and identification of cyanobacterial species

Samples collected were analysed for the presence of cyanobacterial species. The obtained species were identified as *Anabaena circinalis, Nostoc muscorum, Stigonema ocellatum* and *Hapalosiphon welwitschii* shown in (Figure-1).

Antibacterial activity

The formation of zone of inhibition in the chloroform extract of *A. circinalis* exhibited maximum (21.00 ± 0.57 mm) antibacterial activity against *E. coli* and ethyl acetate extract has exhibited minimum (7.33 ± 0.33 mm) inhibition zone against *B.subtilis.* Whereas, the aqueous, chloroform and ethyl acetate extracts were did not expressed antibacterial activity against the *B. subtilis, S. aureus, E. coli* respectively. Similarly the hexane and methanol culture crude extracts were also not showed the activity against *S. aureus* (Figure-2: A). The observation of the extracts of *N. muscorum* exhibited with the widest spectrum activities, that inhibited the growth of chosen pathogenic bacteria with maximum (17.33 ± 1.20 mm) in the solvent extract of hexane against *K.pneumoniae* and minimum inhibition zone (8.33 ± 0.66 mm) in the solvent extracts of methanol against *E.coli* respectively. However, chloroform, ethyl acetate and methanol extract was did not showed any activities against *K.pneumoniae*, *E.coli* and *S. aureus* (Figure-2: B). The *S. ocellatum* culture extract was also expressed good antibacterial activity with chloroform extract and resulted (19.33 ± 0.88 mm) zone of inhibition against *K. pneumoniae* and hexane extract had shown with lowest inhibition zone (10.33 ± 0.33 mm) against *E.coli* respectively. While, the hexane extract of *S. ocellatum* had failed to show inhibition zone against *B. subtilis.* Similarly, the aqueous extract against *B.subtilis* and *S. aureus* and the methanol extract against *E. coli* were not shown the zone of inhibition **(**Figure-2: C). The chloroform crude extract of *H. welwitschii* was shown with the maximum zone of inhibition (19.00 ± 0.57 mm) against the *E. coli* and aqueous extract was exhibited with the minimum (7.66 ± 0.66 mm) zone of inhibition against the *S. aureus*. Whereas, the aqueous, chloroform and hexane extracts were not expressed any inhibition zone against *K. pneumoniae* and the same results were found in the methanol extract against *B. subtilis* and *E. coli* (Table-1 & Figure-2: D).

Antifungal activity

The antifungal activity of culture extracts of *A. circinalis* was shown in Table-2. In *A. circinalis* the highest zone of inhibition (12.33 ± 0.33 mm) in the chloroform extract against *A .niger* and lowest inhibition zone (7.00 ± 0.00 mm) was found in the methanol extract against *T. mentagrophytes*. The fungal pathogens, *A. fumigatus, A. niger* and *Mucor* sp. did not respond to *A. circinalis* in ethyl acetate extract and same results were found with the strains of *A. niger* and *Mucor* sp. in methanol extract. Similarly, the chloroform extract also did not exhibit the activity against *A. fumigatus* and *Mucor* sp. and aqueous extract against *A. niger* and *Mucor* sp. **(**Figure-3: A). The different culture crude extracts of *N. muscorum* were studied against *A. fumigatus, A. niger, Mucor* sp. and *T. mentagrophytes.* The hexane extract of *N. muscorum* expressed with maximum zone of inhibition (15.00 ± 1.15 mm) against *T. mentagrophytes* and aqueous extract exhibited with less zone of inhibition (7.66 ± 0.66 mm) against *Mucor* sp. The pathogenic strains, *A. fumigatus* did not respond to the methanol, ethyl acetate and chloroform extracts. The fungal strain, *A. niger* was also not expressed zone of inhibition against the extracts of hexane, methanol and aqueous. The culture extracts of ethyl acetate and chloroform were also not exhibited antifungal activity against *T. mentagrophytes.* Methanol extract not exhibited zone of inhibition against *Mucor* sp. **(**Figure-3: B). The screening of extracts of *S. ocellatum* in five different solvents were studied in which the resulted maximum (13.00 ± 1.00 mm) zone of inhibition in Ethyl acetate extract against *A. fumigatus* and lowest inhibition zone (7.33 ± 0.33 mm) in the aqueous extract against *A.niger.* The chloroform extract had failed to show the activity against *A. fumigatus* and *A. niger* (Figure-3: C). The *H. welwitschii* culture extracts were expressed with varied zone of inhibition against four fungal pathogens. The highest antifungal activity (12.00 ± 1.15 mm) was found in ethyl acetate extract against *A. niger* and lowest antifungal activity (7.33 ± 0.33 mm) was found in methanol extract against *A. niger.* The ethyl acetate extract failed to express zone of inhibition against *T. mentagrophytes* and same results were found in the chloroform extract against two fungal pathogens (*A. fumigatus* and *Mucor* sp.) and also methanol extract against *A. fumigatus* and *T. mentagrophytes.* No antifungal activity was detected in the aqueous extracts against *A. fumigatus, A. niger*, *Mucor* sp. and *T. mentagrophytes* (Table-2 & Figure-3: D). When compared with the standard Ciprofloxacin (10 µg/disc, Figure-2: E) and Nystatin (50 µg/disc, Figure-3: E) all the culture crude extracts of cyanobacterial species were exhibited with less inhibition zones. The results obtained from the present study indicates that the development of antibacterial and antifungal substances from different selected genera of cyanobacterial extracts with chloroform and hexane extract solvents are potential sources of bioactive compounds and it warrants further study to identify these bioactive compounds.

DISCUSSION

According to the preliminary reports, cyanobacteria are rich sources of antibacterial and antifungal bioactive compounds. The different solvents extracts of *S. platensis* exhibited different zone of inhibitions of antimicrobial activity on both Gram-positive and Gram-negative bacteria [21]. The results obtained from the present investigations are in agreement with the reports of that the methanol extract of cyanobacterial species showed highest activity against *A. niger* and *R. stolonifer* [22]. Two cyanobacterial species *A. variabilis* and *S. elongatus* have shown potential antibacterial activity against *E.coli, Enterococcus* sp. and *Klebsiella* [23]. In the present investigations, acetone and methanol extracts of *S. platensis* showed more or less similar inhibition zones against *S. aureus* and *S. typhimurium* were positively correlated [24]. The methanolic extract of *Fischerella* sp. was noticed with antibacterial activity [25]. *P. corium*, *L. martensiana* and *M. aeruginosa* were responsible for maximum antimicrobial activity in acetone, methanol and diethyl ether extracts in a descending order which are also similar to the reports published [26]. The cyanobacterial extracts of *P. boryanum* and *A. variabilis* were also reported with inhibition zones (17 & 12 mm) against *S. epidermidis* [27].

Table -1

Antibacterial activity of Cyanobacterial species against different culture crude extracts.

Cyanobacteria species	Culture crude extracts (50 µl)	Zone of inhibition (diameter in mm)			
		Bacillus subtilis **(MTCC-1427)**	***Staphylococcus aureus*** **(MTCC-1430)**	***Escherichia coli*** **(MTCC-1302)**	***Klebsiella pneumoniae*** **(MTCC-4030)**
Anabaena circinalis	Aqueous	--	--	--	9.00 ± 1.00
	Chloroform	8.00 ± 0.57	--	21.00 ± 0.57	11.66 ± 0.33
	Ethyl acetate	7.33 ± 0.33	14.33 ± 0.88	--	13.00 ±0.57
	Hexane	7.66 ± 0.33	--	11.00 ± 0.57	12.66 ± 0.88
	Methanol	17.00 ± 0.57	--	12.66 ± 0.88	14.66 ± 0.33
Nostoc muscorum	Aqueous	11.66 ± 1.20	8.66 ± 0.33	8.33 ± 0.88	16.00 ± 0.57
	Chloroform	11.00 ± 0.57	11.66 ± 0.88	14.66 ± 0.88	--
	Ethyl acetate	10.33 ± 0.33	13.66 ± 0.33	--	13.66 ± 0.88
	Hexane	13.33 ± 0.88	15.00 ± 0.57	9.00 ± 0.57	17.33 ± 1.20
	Methanol	12.66 ± 0.66	--	8.33 ± 0.66	12.00 ± 1.15
Stigonema ocellatum	Aqueous	--	--	13.66 ± 1.20	12.00 ± 0.57
	Chloroform	14.33 ± 0.33	11.33 ± 0.33	15.00 ± 1.15	19.33 ±0.88
	Ethyl acetate	14.00 ± 1.15	13.33 ± 0.33	15.66 ± 0.88	10.33 ± 1.45
	Hexane	--	11.66 ± 0.88	10.33 ± 0.33	12.00 ± 1.15
	Methanol	13.33 ± 0.88	13.00 ± 0.57	--	14.66 ± 0.88
Hapalosiphon welwitschii	Aqueous	9.33 ± 0.66	7.66 ± 0.66	13.33 ± 1.45	--
	Chloroform	12.66 ± 1.20	14.66 ± 0.33	19.00 ± 0.57	--
	Ethyl acetate	8.33 ± 0.88	11.33 ± 1.20	17.66 ± 0.88	13.00 ± 0.00
	Hexane	10.33 ± 0.33	13.00 ± 1.15	16.00 ± 1.15	--
	Methanol	--	8.33 ± 0.88	--	15.66 ± 0.66
Standard Control (Ciprofloxacin 10 µg/disc)		25.33 ± 0.33	24.66 ± 0.66	28.33 ± 0.33	29.66 ± 0.33

"--" No inhibition zone

Diameter of the inhibition zone including disc diameter (6 mm)

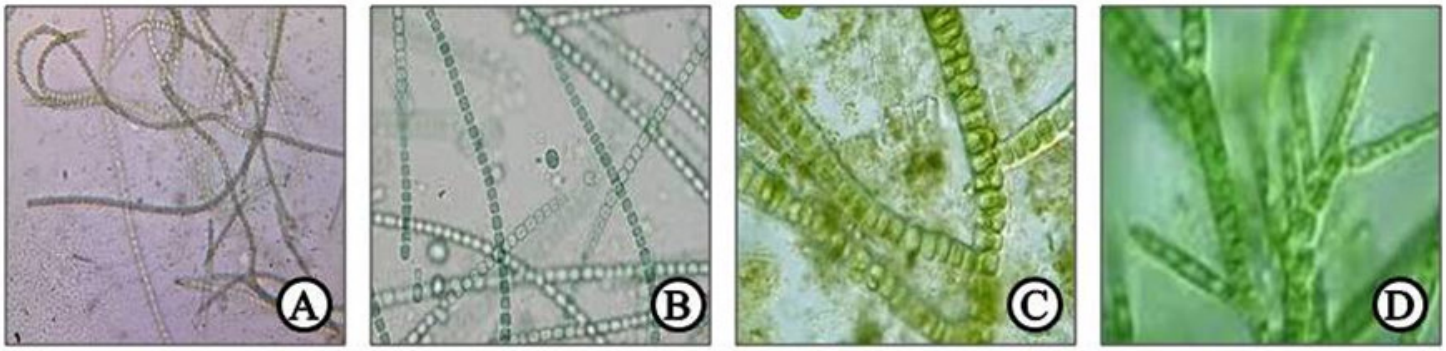
Values were with mean ± SE of three separate experiments (n=3)

Table -2
Antifungal activity of Cyanobacterial species against different culture crude extracts.

Cyanobacteria species	Culture crude extracts (50 µl)	Zone of inhibition (diameter in mm)			
		Aspergillus fumigatus (MTCC-4163)	*Aspergillus niger* (MTCC-4325)	*Mucor* sp. (MTCC-3340)	*Trichophyton mentagrophytes* (MTCC-8476)
Anabaena circinalis	Aqueous	9.00 ± 0.57	--	--	10.00 ± 0.57
	Chloroform	--	12.33 ± 0.33	--	12.33 ± 0.88
	Ethyl acetate	--	--	--	8.00 ± 0.57
	Hexane	11.33 ± 0.33	10.66 ± 0.33	8.00 ± 0.57	9.33 ± 0.33
	Methanol	10.33 ± 0.88	--	--	7.00 ± 0.00
Nostoc muscorum	Aqueous	12.33 ± 0.88	--	7.66 ± 0.66	8.00 ± 0.57
	Chloroform	--	8.33 ± 0.33	10.33 ± 1.20	--
	Ethyl acetate	--	12.00 ± 0.57	8.66 ± 0.33	--
	Hexane	13.00 ± 0.57	--	9.00 ± 0.57	15.00 ± 1.15
	Methanol	--	--	--	10.33 ± 0.88
Stigonema ocellatum	Aqueous	8.00 ± 0.57	7.33 ± 0.33	8.00 ± 0.57	9.00 ± 1.15
	Chloroform	--	--	11.33 ± 0.33	8.66 ± 1.20
	Ethyl acetate	13.00 ± 1.00	9.33 ± 0.33	10.66 ± 0.33	9.66 ± 0.66
	Hexane	8.00 ± 0.57	10.00 ± 1.15	7.66 ± 0.66	8.33 ± 0.33
	Methanol	10.33 ± 0.88	8.00 ± 0.57	8.00 ± 0.57	8.00 ± 0.57
Hapalosiphon welwitschii	Aqueous	--	--	--	--
	Chloroform	--	10.66 ± 0.66	--	9.33 ± 0.88
	Ethyl acetate	11.00 ± 0.57	12.00 ± 1.15	9.66 ± 1.20	--
	Hexane	9.66 ± 0.88	8.33 ± 0.88	8.00 ± 0.57	11.66 ± 0.66
	Methanol	--	7.33 ± 0.33	7.66 ± 0.33	--
Standard Control (Nystatin 50 µg/disc)		23.33 ± 0.33	22.00 ± 0.57	21.33 ± 0.88	21.66 ± 0.66

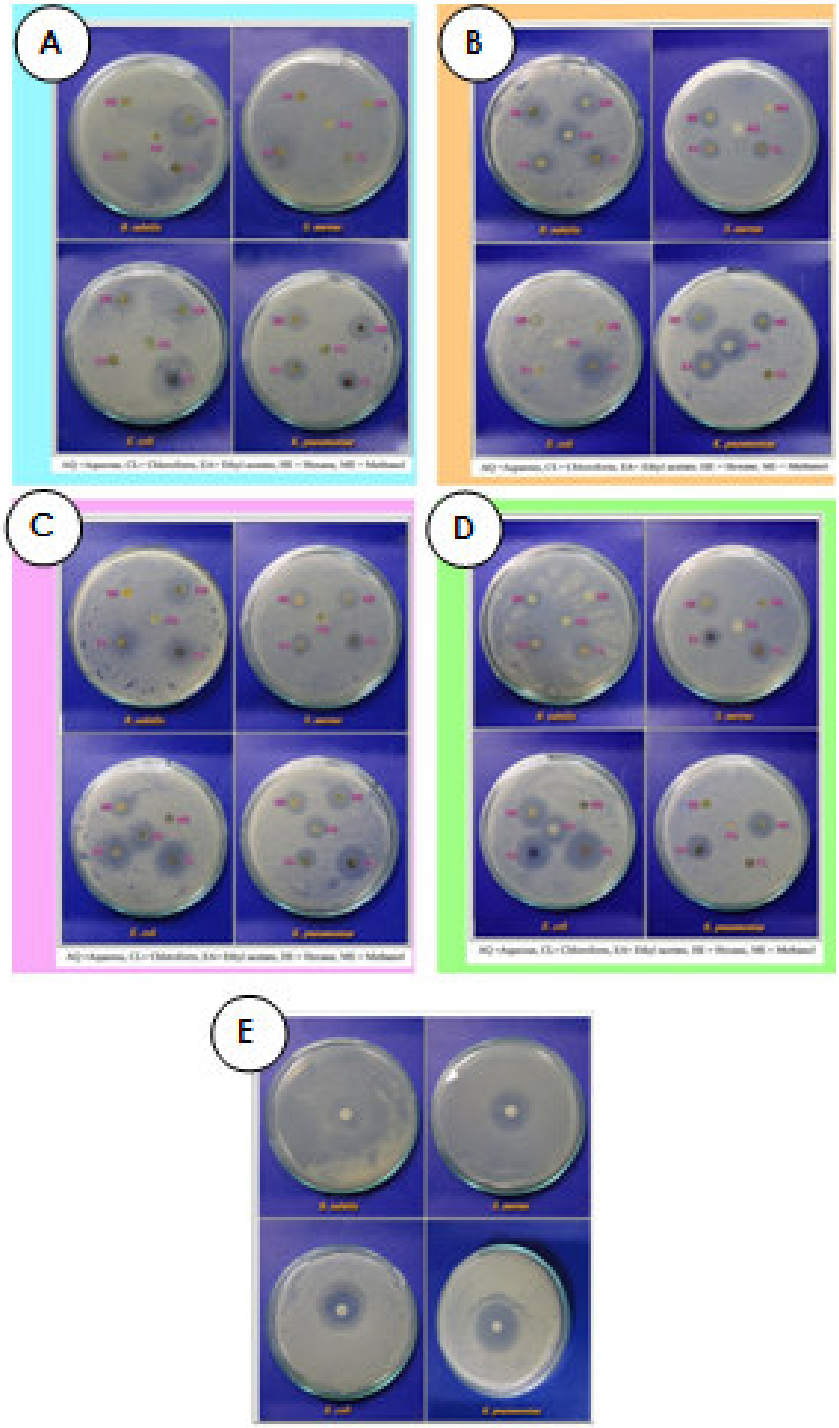
"--" No inhibition zone
Diameter of the inhibition zone including disc diameter (6 mm)
Values were with mean ± SE of three separate experiments (n=3)

Figure-1
Cyanobacterial species



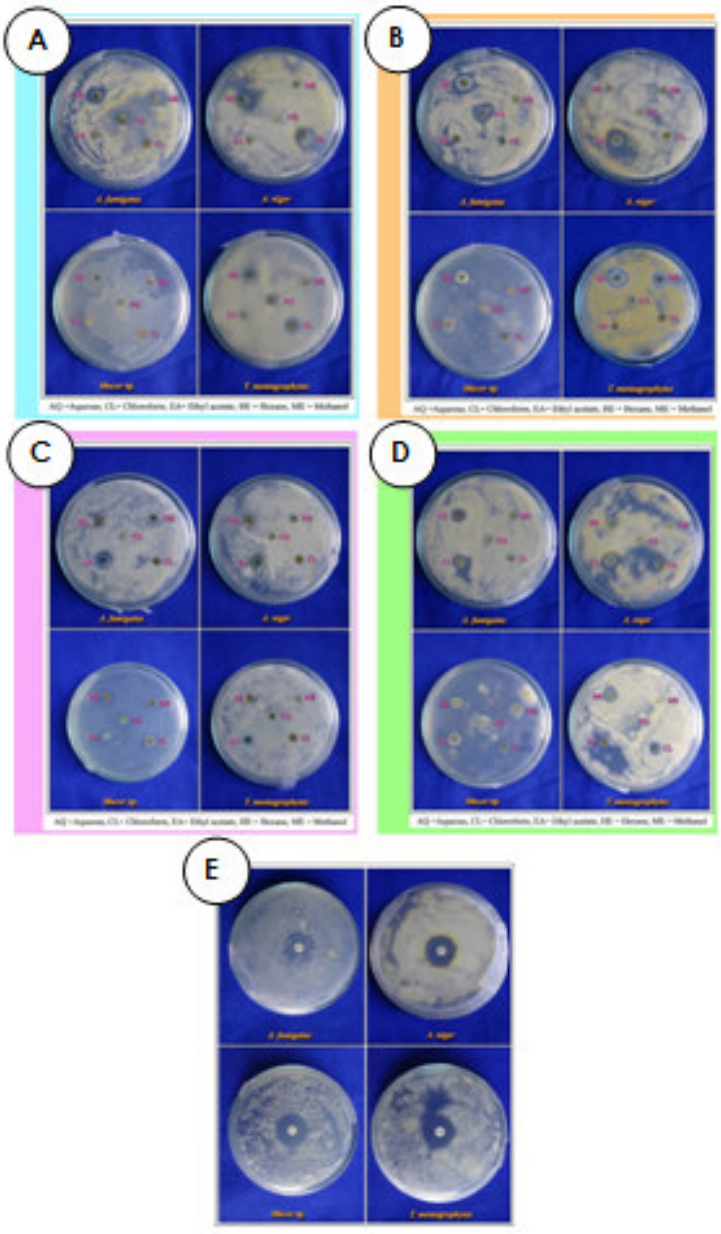
A. *Anabaena circinalis* B. *Nostoc muscorum* C. *Stigonema ocellatum* D. *Hapalosiphon welwitschii*

Figure-2
Antibacterial activity of Cyanobacterial species against different culture crude extracts



A). *Anabaena circinalis* B). *Nostoc muscorum* C). *Stigonema ocellatum* D). *Hapalosiphon welwitschii*
E). Standard Control, Ciprofloxacin (10 µg/disc)

Figure-3
Antifungal activity of Cyanobacterial species against different culture crude extracts



A). *Anabaena circinalis* B). *Nostoc muscorum* C). *Stigonema ocellatum* D). *Hapalosiphon welwitschii*
E). Standard Control, Nystatin (50 µg/disc)

CONCLUSION

The present investigation revealed that the chloroform and hexane extracts were proved to be potential antimicrobial activity.. Fresh water cyanobacteria are the substantial resources for natural bioactive substances with potential use in the pharmacological industry. Antimicrobial activities of culture extracts of cyanobacterial species were showed broad spectrum activities against bacterial and fungal pathogens. Hence, further study is required for the isolation, identification and chemical characterization of crude extracts in order to develop future pharmaceuticals for the benefit of the society.

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