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MARINE CYANOBACTERIA: ASSESSMENT OF TOXICITY AND ANTIMICROBIAL POTENTIAL OF CRUDE SAMPLES AND ISOLATED STRAINS FROM COASTAL WATERS OF KARACHI, PAKISTAN

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Abstract

Microalgal communities, such as, cyanobacteria, diatoms and dianoflagellates, etc., generally bloom under eutrophic conditions in both fresh and marine water bodies. Blooms of harmful algae have been reported from various parts of the world that inflicts losses to the economy and impact environmental/human health. Here we present on antimicrobial activity and lethal/toxic effects in a rat model of some cyanobacteria collected from the coastal waters (Manora Channel, Karachi, Pakistan). Antibacterial activity was targeted against nine species of gram +ve and gram -ve bacteria, namely, *Bacillus subtilus*, *Staphlococcus aureus*, *Staphlococcus albus, Streptococcus pyogens* and *Streptococcus fecalis, Pseudomonas aeruginosa*, *Klebsiella* sp., *Eschierichia coli*, *Salmonala typhi*. The cyanobacterial extracts showed promising antibacterial activity against Gram +ve bacteria. The ethanol and chloroform extracts showed high antibacterial activity compared to water extracts. *Bacillus subtilus*was the most susceptible to cyanobacterial extract. Acute lethal toxicity was caused by one marine cyanobacterial species, *Leptolyngbya angustissima* and a mixed crude sample for which LD50 values were recorded. All extracts generally showed typical signs of hepatotoxicity, though some of the symptoms resembled neurotoxicity. The data suggested the presence of toxic microalgae for which essentially requires regular monitoring in the coastal and near-shore waters.

Key words: Cyanobacteria, Mixed culture, Toxicity, LD_{50}, Marine environment.

Introduction

Phototrophic microbial communities, including cyanobacteria, diatom, dianoflagellates, etc., generally bloom under eutrophic conditions in both fresh and marine water bodies. Blooms of harmful algae have been reported from various parts of the world from fresh, brakish and marine water (Shaika *et al*., 2023). Microalgal toxin poisonings are also reported from these waters (Lee *et al*., 2023). The toxins produced by microalgae pose lethal, acute and chronic toxicity to human (Louzao *et al*., 2022) and other wild and domestic animals including aquatic species of mammals (Louzao *et al*., 2022; Aklakur *et al*., 2023) birds, fish (Aklakur *et al*., 2023) and molluscs (Zgouridou *et al*., 2022). Toxic marine cyanobacteria have also been reported from freshwater and marine environments (Zhang *et al*., 2022). Increasing cyanobacterial toxic blooms of several species, such as, *Oscillatoria, Trichodesmium erythraeum* and *Nodularia,* etc. are being reported from various parts of the world (Shaika *et al*., 2023). Such toxic cyanobacterial blooms not been reported from Pakistani waters. Only the diversity and distribution of cyanobacteria Pakistan have been reported (Siddiqui *et al*., 2000; Bano & Siddiqui, 2004; Ahmed *et al*., 2016; Bano & Siddiqui, 2017; Munawar & Aisha, 2017; Mansoor *et al*., 2023). Similarly, information on potential bloom forming species and blooms of other microalgal species including dinoflagellate (Munir *et al*., 2012, 2016; Burhan *et al*., 2018; Khokhar *et al*., 2018, 2021; Hamid *et al*., 2023) and diatom (Naz *et al*., 2010, 2011, 2012; Hamid *et al*., 2023) in the coastal water of Pakistan is available but data on bloom forming and toxic cyanobacteria is scares. Cyanobacteria (blue green algae) are photosynthetic prokaryotes and are known to produce a multitude of biologically active compounds (Ahmed *et al*., 2020; Abdi *et al*., 2023; Shahbaz *et al*., 2023).

For the management of environmental health and water quality as well as potential toxic microalgae/cyanobacterial species, it is inevitable to understand the frequency of occurrence, properties and exposure routes of these toxins. Cyanobacterial toxins and their toxicity has been the subject of many studies and it has been suggested that these toxins enter in mammalian system through skin contact, inhalation, hemodialysis and ingestion (Lad *et al*., 2022). Studies of cyanobacterial toxins and other bioactive compound have led to the conclusion that these organisms possess various natural products which, on one hand, are toxic in human, animals and other aquatic life, and on the other hand, they may provide a range of new and unique therapeutic drugs, antibiotics and fine chemicals (Shahbaz *et al*., 2023). Cyanobacterial species and their biological activities have not been reported in Pakistan's waters. Therefore, the aim of this study was to isolate and identify cyanobacterial species in the coastal waters, assess the lethal and toxic effects of certain cyanobacterial isolates using rat models, and evaluate their potential to produce antibiotic molecules.

Material and Methods

Collection of samples and identification of cyanobacteria: Water sample were collected from Manora Channel (24° 51.300'N, 66° 55.533'E) Karachi (Fig. 1) and brought to the laboratory for further analysis. In the laboratory water samples were inoculated in ASN III medium (Rippka *et al*., 1979). Any visible growth was aseptically transferred into other tube containing fresh media and final isolation was achieved using serial dilution technique or by streaking onto solidified (agarized) ASN-III medium. Isolates were kept under 12h/12h light/dark cycle with a light intensity of ca. 2000 Lux at room temperature (ca. 30±2°C). The original mixed culture was also

maintained for scaling up. Taxonomic identification of cyanobacteria was carried out according to botanical mode of classification provided by Komarek & Anagnostidis (1986, 1989), Anagnostidis & Komarek (1985), Anagnostidis, (1988) and Desikachary (1959). Nomenclature for all given taxa were validated according to currently used names on Algae Base (Guiry & Guiry, 2023).

Scaling up of cultures and extraction: The isolated cyanobacteria and the mixed cyanobacterial culture were sub-cultured in large volumes of media and incubated under optimum light and temperature conditions. Dense growth of each species was harvested through centrifugation and the pallets were dried briefly, weighed and extracted with ethanol, chloroform and water for 5 to 6 days and the extracts obtained were evaporated to dryness at 55°C (Jiang *et al*., 2019).

Antibacterial activity: The antibacterial activity of cyanobacterial extracts was assessed by the disk diffusion method (Gheda & Ismail, 2020). Positive and negative control discs were prepared in the same way by impregnating 20 µl streptomycin (2mg/ml) and DMSO, respectively, to the discs for antibacterial assay.

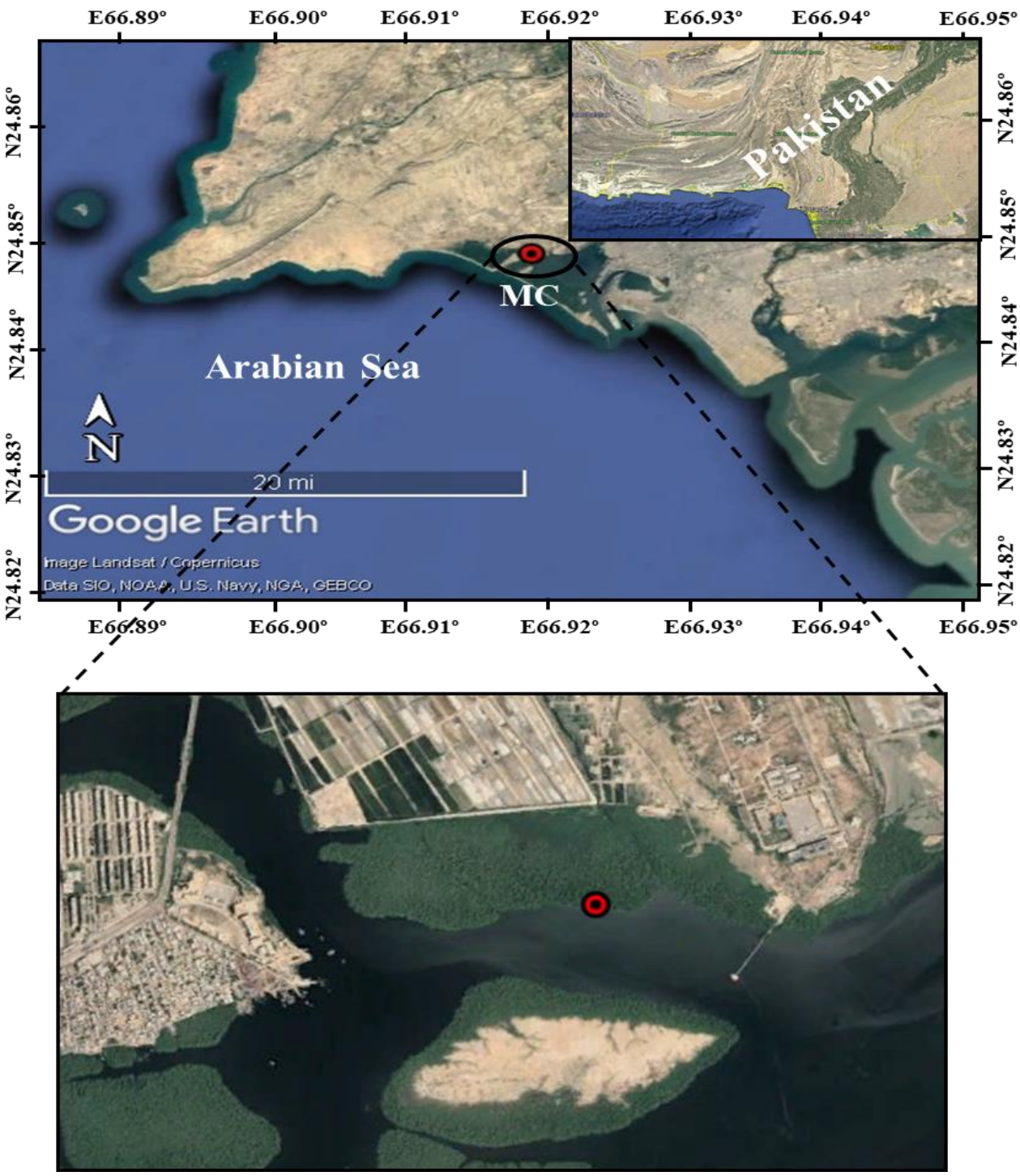

Fig. 1. Map showing study site located at Manora channel (MC) along Karachi coast.

Table 1. Antibacterial assay was performed using disc diffusion method against nine bacterial strains. The positive results are shown as width (mm) of inhibition zone around the test disc.

Cyanobacterial isolates	Bacterial strains*								
	1	2	3	4	5	6	7	8	9
Ethanolic extract									
Leptolyngbya angustissima	-	7	8	-	12	-	-	-	-
Oscillatoria limosa	-	-	-	-	8	-	-	-	-
Phormidesmis mollis	-	-	12	-	7	-	-	-	-
Pseudanabaena lonchoides	-	-	10	-	-	-	-	-	-
Synechocystis aquatilis	-	-	-	-	8	-	-	-	-
Mixed crude sample	-	8	-	-	11	-	-	-	-
Chloroform extract									
Leptolyngbya angustissima	7	8	10	12	8	-	-	-	-
Oscillatoria limosa	-	-	-	-	-	-	-	-	-
Phormidesmis mollis	8	13	15	15	12	-	-	-	-
Pseudanabaena lonchoides	-	-	10	-	-	-	-	-	-
Synechocystis aquatilis	-	-	-	-	7	-	-	-	-
Mixed crude sample	-	-	-	-	-	-	-	-	-
Water extract									
Leptolyngbya angustissima	-	-	-	-	-	-	-	-	-
Oscillatoria limosa	-	-	-	-	-	-	-	-	-
Phormidesmis mollis	-	-	-	-	-	-	-	-	-
Pseudanabaena lonchoides	-	-	-	-	-	-	-	-	-
Synechocystis aquatilis	-	-	-	-	-	-	-	-	-
Mixed crude sample	-	7	-	-	8	-	-	-	-

*1: *Staphlococcus aureus*, 2: *Staphlococcus albus*, 3: *Streptococcus pyogenes*, 4: *Streptococcus faecalis*, 5: *Bacillus subtilus*, 6: *Pseudomonas aeruginosa*, 7: *Escherichia coli*, 8: *Salmonella typhi*, 9: *Klebsiella* sp.

Table 2. Antibacterial capacity of cyanobacterial extract. Values are total number of bacterial species inhibited by each extract.

Cyanobacteria	Water	Ethanol	Chloroform	Total
Laptolayngbya angustissima	-	3	5	8
Oscillatoria limosa	-	1	-	1
Phormidesmis mollis	-	2	5	7
Pseudanabaena lonchoides	-	1	1	2
Synechocystis aquatilis	-	1	1	2
Mixed crude sample	2	2	-	4
Total	**2**	**10**	**12**	**24**

Rat bioassay: Albino rats of either sex (100-150 gm) were injected intra-peritoneally (i.p.) with 1 ml of the diluted alcohol extracts (30% dilution v/v in DMSO). The animals were monitored for any abnormal behavioral signs at frequent intervals. Blood was collected in centrifuge tube immediately after they expired due to toxic effects of extracts. Blood from the other rats that survived i.p. dose was drawn after 48 hrs of injection. Blood samples were centrifuged and sera were separated and frozen immediately for chemical component analysis by enzymatic methods on Hitachi 912. General condition of some vital organs (heart, kidneys, liver, spleen, and lungs) were noted after the animal died or after 48 hrs to study any gross morphological changes.

For toxicity assessment, rat bioassay (Carmichael, 1992) was applied using crude extract of *Leptolyngbya angustissima* and mixed culture of cyanobacteria. Rats of 100-150 gm of body weight were used in the experiment. Cyanobacterial crude extract was dissolved in DMSO and injected i.p. to rats (8 replicates). Toxicity was quantified as LD_{50} (lethal dose for 50% mortality of tested rats) within four hours of the injection were determined following the previously established procedure (Finney, 1985).

Results

Antimicrobial Activity: The antimicrobial assay of five isolated cyanobacterial starins and one crude mixed sample, extracted in ethanol, chloroform and water, were tested against 9 species of gram +ve (*Staphlococcus aureus*, *Staphlococcus albus*, *Bacillus subtilus*, *Streptococcus pyogens* and *Streptococcus fecalis*) and gram -ve bacteria (*Pseudomonas aeruginosa*, *Eschierichia coli*, *Klebsiella sp*., and *Salmonala typhi*). Results of antibacterial assay are set out in (Tables 1 & 2). Clear zone of inhibition around disc was taken as positive result (Fig. 2). The negative control showed no clear zone around the disc. Fifteen percent (24 tests) of the total 162 tests showed positive results against bacteria including, 10 ethanolic (42%), 12 chloroform (50%) and 2 water (8%) extracts (Table 1 and Fig. 3). Only five gram +ve bacteria appeared to be sensitive to cyanobacterial extracts tested. On the other hand, none of the other four gram -ve bacteria depicted sensitivity to cyanobacterial extracts. Most cyanobacterial extracts showed antagonistic activity against *Bacillus subtilus* (9 +ve results) followed in descending order by *Streptococcus pyogens* (6), *Staphlococcus albus* (5), whereas *Staphlococcus aureus* and *Streptococcus fecalis*had lowest sensitivity (2 each) to cyanobacterial extracts tested (Fig. 4).

Toxicity / Lethality: Clinical signs of the rats injected i. p. with ethanolic extracts of cyanobacteria are given in Table 3. Ataxia, restlessness, hurdling behavior and lethargic condition were common among test rats. However, difficult breathing and partial paralysis of extremities was observed in rats injected with some cyanobacterial extracts. Most of these symptoms were either mild or absent in the DMSO injected rats, where ataxia and crawling movement were observed initially and the rats recovered by the end of 24hrs.

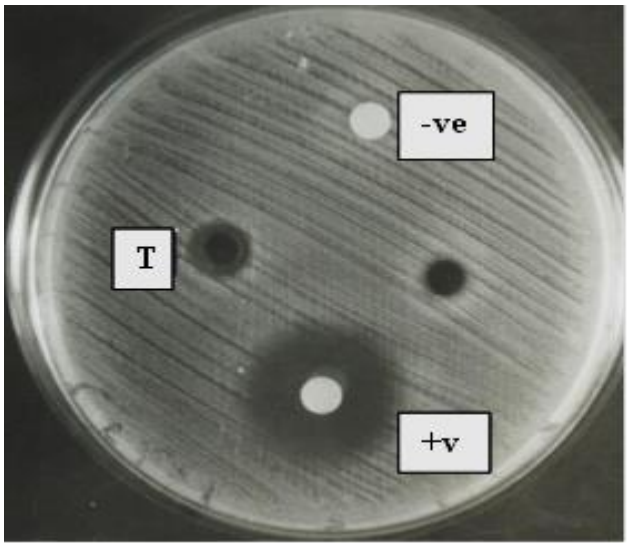

Fig. 2. Zone of inhibitions around +ve control and test discs were taken as positive result. No clear zone was seen around –ve control disc.

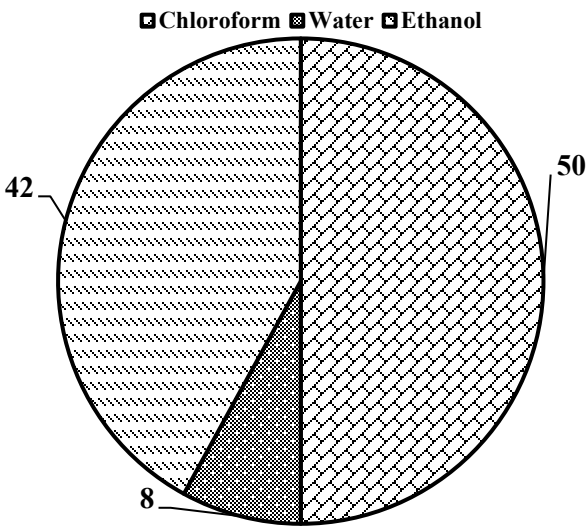

Fig. 3. Percentage of total positive antibacterial test exhibited by cyanobacterial extracts against nine bacterial species.

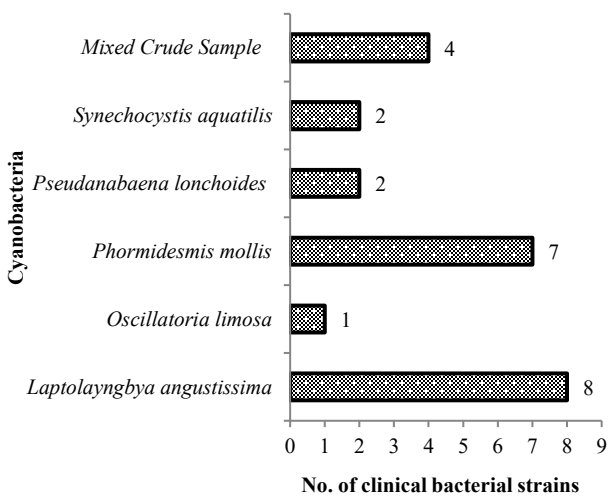

Fig. 4. Susceptibility of five cyanobacterial isolates and one mixed crude sample of cyanobacteria against 9 clinical bacterial strains.

Table 3. Behavioral response of rats injected (i.p) with ethanolic extracts of cyanobacteria. Clinical signs manifested by rats were noted for 24 hrs.

Cyanobacteria extracts administered (mg/ml)	Behavioral response of rats during experiment						
	1st hrs.	2nd hrs.	3rd hrs.	4th hrs.	5th hrs.	6th hrs.	24th hrs.
Leptolyngbya angustissima (82.4)	Ataxia, marked restlessness	Lethargy and withdrawal symptoms appear	Lethargy and withdrawal continues	Loss of some mobility, coma like condition with difficult respiration	Coma like condition continues with occasional respiratory gasps. Died within 5 hrs.	-	-
Oscillatoria limosa (60)	Ataxia, hurdling. Hard breathing, occasional toxic convulsion showing marked neurotoxicity	Ataxia disappears, lethargy and withdrawal	Slow mobility, drink water	Slow mobility, toxicity signs start disappears	Almost normal	Almost normal	Normalcy regained
Phormidesmis mollis (75.5)	Ataxia and hurdling	Ataxia disappears, lethargy and withdrawal	lethargy and withdrawal	lethargy and withdrawal	lethargy and withdrawal	lethargy and withdrawal	Normalcy regained
Pseudanabaena lonchoides (90)	Ataxia, hurdling, Restlessness, mild sign of paralysis in limbs	Ataxia disappears, sign of paralysis continue, lethargy and withdrawal	Lethargy and withdrawal sign of paralysis continue	Lethargy and sign of paralysis continue, drinks water	Lethargy and sign of paralysis disappears, drinks water	Lethargy, drinks water	Normalcy regained
Synechocystis aquatilis (63)	Ataxia, hurdling, sign of paralysis in hind limbs	Ataxia disappears, paralysis disappears, lethargy, drink water	Lethargy, drinks water	Lethargy, drinks water	Lethargy, drinks water	Lethargy, drinks water	Normalcy regained
Mixed crude sample (66.4)	Ataxia, hard breathing, marked sign of paralysis	Lethargy and withdrawal, paralysis continues	Lethargy and withdrawal, paralysis continues, difficult breathing	Complete loss of mobility. Died during 4th hr.	-	-	-
DMSO	Ataxia, regular breathing, crawling movement	Ataxia and crawling movement disappear, gets normal	Normal	Normal	Normal	Normal	Normal

Table 6. Changes in the levels of serum enzymes and other components of test rats injected with ethanolic extracts of cyanobacteria and DMSO in comparison with normal rats.

Serum enzymes & Other components	Ethanol extracts of cyanobacteria							
	Leptolyngbya angustissima	*Oscillatoria limosa*	*Phormidesmis mollis*	*Pseudanabaena lonchoides*	*Synechocystis aquatilis*	Mixed crude sample	DMSO	Normal rat
Creatine kinase (CK)	$787.23^{a} \pm 24.32$	$611.70^{a} \pm 33.62$	$500.4^{a} \pm 17.99$	$810.2^{a} \pm 18.62$	$174.79^{a} \pm 7.79$	$16.17a \pm 0.15$	$61.45^{b} \pm 4.21$	35.89 ± 5.99
Lactate dehydrogenase (LDH)	$898.87^{a} \pm 15.36$	$891.6^{a} \pm 23.45$	$922.3^{a} \pm 18.8$	$1063.0^{a} \pm 157.9$	$835.3^{a} \pm 27.28$	$90.97a \pm 6.25$	$181.55^{b} \pm 19.02$	162.25 ± 0.87
Glutamate oxaloacetate transaminase (SGOT)	$151.24^{a} \pm 1.20$	$153.7^{a} \pm 4.99$	$185.7^{a} \pm 4.5$	$231.3^{a} \pm 4.99$	$112.7^{a} \pm 9.98$	$13.67a \pm 2.49$	$49.98^{b} \pm 3.96$	47.23 ± 4.25
Glutamate pyruvate transminase (SGPT)	$151.11^{a} \pm 6.93$	$148.00^{a} \pm 6.53$	$178.0^{b} \pm 7.79$	$201.0^{a} \pm 11.86$	$102.3^{a} \pm 6.34$	$14.33a \pm 2.62$	$46.88^{b} \pm 5.13$	36.53 ± 7.88
Alkaline phosphate (ALP)	$461.22^{a} \pm 7.02$	$488.8^{a} \pm 6.86$	$501.1^{b} \pm 7.11$	$480.4^{a} \pm 7.23$	$200.7^{a} \pm 13.07$	$30.83a \pm 1.6$	$79.98^{b} \pm 5.45$	66.12 ± 13.24
β-amylase (AMY)	$198.99^{a} \pm 4.17$	$361.5^{a} \pm 4.82$	$306.3^{b} \pm 19.7$	$488.3^{a} \pm 7.27$	$749.7^{a} \pm 41.65$	$78.91a \pm 3.83$	$120^{b} \pm 10.12$	108.46 ± 15.87
Glucose	$201.21^{a} \pm 3.84$	$199.9^{a} \pm 13.19$	$210.03^{a} \pm 6.60$	$239.83^{a} \pm 3.29$	$89.33^{b} \pm 1.70$	53.33 ± 7.41	$113.7^{b} \pm 6.21$	110.2 ± 3.01
Creatinine	$3.01^{b} \pm 0.12$	$3.36^{a} \pm 0.04$	$3.56^{a} \pm 0.43$	$3.82^{a} \pm 0.16$	$1.67^{a} \pm 0.12$	2.28 ± 0.02	$0.66^{b} \pm 0.08$	0.60 ± 0.05
Urea	$113.23^{a} \pm 3.25$	$135.27^{a} \pm 6.21$	$135.27^{a} \pm 6.21$	$120.95^{a} \pm 6.62$	$78.37^{a} \pm 5.69$	116.7 ± 3.53	$17.89^{b} \pm 2.45$	14.05 ± 3.20
Protein	$15.56^{a} \pm 2.13$	$3.05^{a} \pm 0.36$	$3.08^{a} \pm 0.20$	$15.5^{a} \pm 3.02$	$4.9^{a} \pm 0.37$	7.90 ± 0.08	$7.05^{b} \pm 0.89$	5.98 ± 1.03
Lipid	$901.20^{a} \pm 9.88$	$757.2^{a} \pm 47.32$	$889.96^{a} \pm 14.22$	$889^{a} \pm 8.09$	$488^{a} \pm 10.23$	287.0 ± 9.42	$610.21^{b} \pm 11.58$	628.31 ± 18.59
Cholesterol	$612.21^{a} \pm 10.23$	$830.33^{a} \pm 7.92$	$499.77^{a} \pm 7.74$	$481.93^{a} \pm 14.40$	$61.0^{a} \pm 2.16$	98.33 ± 2.87	$9.52^{b} \pm 4.11$	7.26 ± 2.17
Triglycerides	$267.66^{a} \pm 3.12$	$227.83^{a} \pm 5.68$	$246.83^{a} \pm 3.97$	$291.13^{a} \pm 6.66$	$101.33^{a} \pm 10.66$	50.67 ± 6.55	$178^{b} \pm 12.01$	180.12 ± 11.20

Values represent mean ± S.D. Superscript letters a = Values significantly different from normal value ($p<0.05$; Student T-test) and b = Values not significantly difference from normal value ($p<0.05$; Student T-Test)

Table 4. LD_{50} values of ethanolic extracts of a cyanobacterium (*Leptolyngbya angustissima*) and mixed crude sample of cyanobacteria (collected from Manora Channel) in rat model.

Cyanobacterial extract	LD_{50} (mg/kg)
Leptolyngbya angustissima	274.8
Mixed crude sample	221.5

Some of the test rats injected with ethanolic extracts of one cyanobacteria (*L. angustissima*) and the mixed collected material from Manora channel (Mixed crude sample) exhibited severe distressing effects starting from 3rd hr. Rats received *L. angustissima* extract did not show sign of paralysis in limbs but lost mobility and fall in a comma like condition which gained severity during 3rd and 4th hour and the rats died during the 5th hour. In another group of rats that received i.p. dose of ethanol extract of mixed crude sample the lethal signs appeared from the beginning exhibiting signs of paralysis in limbs and loss of complete mobility during 4th hour and died. LD_{50} values are given in (Table 4).

The gross morphological appearances of vital organs of the rats injected with cyanobacterial extracts were generally normal and not different from the control rats (Table 5). However, livers of rats charged with *L. angustissima* and mixed crude sample showed discoloration, and were dark red with mottled appearance. Lungs in rats challenged with *L. angustissima* and *Pseudanabaena lonchoides* extracts had turned whitish in appearance with red patches.

Serum component analysis: In most of the rats injected (i.p.) with cyanobacterial extracts caused either a significant increase or decrease in the levels of blood serum enzymes and other components. However, DMSO did not produce any significant effect on the serum levels of enzymes and other components when compared to the normal levels. Although the extract of mixed crude sample did not elevate the serum levels of any of the enzymes significantly, but a decline in levels of certain enzymes were observed. On the other hand, all other extracts, in general, have caused the enzyme levels to increase compared to their levels in the normal rats. The altered pattern of serum enzymes and components (see Table 6) in experimental rats administered with ethanol extracts clearly indicates chemical toxicity to their normal physiological system (Table 6). The levels of various serum components were compared with those from normal and DMSO-injected rats, and the results of the Student's t-test are presented in (Table 6).

Table 5. Gross morphological appearance of the vital organs of rats injected with cyanobacterial extracts, mixed crude sample from collected from Manora Channel and DMSO.

Extracts	Liver	Heart	Kidney	Lungs	Spleen
Leptolyngbya angustissima	Discolored and mottled in appearance	Normal	Normal	Dark red patches	Normal
Oscillatoria limosa	Normal	Normal	Normal	Normal	Normal
Phormidesmis mollis	Normal	Normal	Normal	Normal	Normal
Pseudanabaena lonchoides	Normal	Normal	Normal	Whitish/ pale with dark red patches	Normal
Synechocystis aquatilis	Normal	Normal	Normal	Normal	Normal
Mix crude sample	Dark red in color	Normal	Normal	Normal	Normal
DMSO	Normal	Normal	Normal	Normal	Normal

Discussion

The present study evaluated the lethal/toxic effects of certain cyanobacterial isolate from coastal waters using a rat model and antimicrobial assay. These cyanobacteria were collected from mangrove environment and nearby water channels at Manora backwaters. The Manora channel is significantly impacted by pollution from domestic and industrial sources, leading to high levels of organic pollution. This pollution is likely to promote microalgal blooms, including cyanobacteria, in the area, which could pose potential health risks. The exact triggering factors for the coastal algal blooms is not known (Weber *et al*., 2020), the level of nutrients, water temperature, light and wind conditions are generally considered to be the potential controlling factors (Ibelings *et al*., 2021). Although diatoms and dinoflagellate populations have been studied from this area (Naz *et al*., 2010, 2012; Munir *et al*., 2012, 2016; Khokhar *et al*., 2016, 2018, 2021), but the cyanobacteria that may produce toxins and/or harmful to human and environmental health have not been registered. This study also gathers some information on the possible role of cyanobacteria as the producers of therapeutic agents. Marine cyanobacterial species have been reported as a promising source of antimicrobial compounds (Abdi *et al*., 2023). All cyanobacteria tested here showed antagonistic activity against Gram positive bacteria, which is in agreement with similar results obtained in some previous studies (Dussault *et al*., 2016; Behzadnia *et al*., 2024). It may however be noted that some studies have also demonstrated activity of marine alga against Gram -ve bacteria (Alsenani *et al*., 2020). Filamentous cyanobacteria tested in the present study had significant antibacterial activity which is line with the previous studies where most of the antimicrobial compounds have been isolated from filamentous cyanobacteria (Swain *et al*., 2023). Overall, our results indicate that the ethanol and chloroform extracts exhibited strong antibacterial activity against the human pathogens tested in this study, suggesting their potential as promising therapeutic agents in the future. Lethal/toxic effects of marine cyanobacteria have been tested using rat bioassay, another screening procedure. One marine cyanobacterial species, *Leptolynbya angustissima*, caused acute lethal toxicity (Lee *et al*., 2020). There are only few species have been confirmed for toxic potential and further screening of cyanobacterial species for such activity is required. Previous literature depicts that most toxin producers commonly belong to genera, for example, *Anabaena*, *Aphanizomenon*, *Calothrix*, *Croccocus*, *Cylindrospermopsis, Gloeotrichia*, *Lyngyba*, *Microsystis*, *Nodularia*, *lanktothrix*, *Tychonema* and *Xenococcus* (Van Hassel *et al*., 2022). This is a first report on toxic cyanobacterium, *Leptolyngbya angustissima,* from northern Arabian Sea bordering Pakistan. This species has not been investigated and reported for the secondary metabolite production. However, a reports is available that confirms the presence of microcystin (hepatotoxin) *mcyE* gene in *Leptolyngbya* sp. (Usman *et al*., 2022). Similarly, the mixed crude sample was also lethally toxic to rats causing death within four hours of extract injection. This indicates that there may be other cyanobacteria present in the study area which produce toxins and thus further pose threat to the human and environmental health. The general signs and symptoms caused by the lethally toxic samples in this study were indicative of hepatotoxicosis, including ataxia, lethargy, labored respiration, and the development of a coma-like state before death. Paralysis of the hind limbs, an unusual manifestation of hepatotoxicity, was also observed following the administration of the mixed crude sample extract from Manora Channel. These findings correspond well with result reported earlier for hepatotoxic blue-green algae (Abdi *et al*., 2023). Necropsy findings of the lethal extract revealed livers with dark red colour and mottled appearance, an indication of hepatotoxicity (Beasley *et al*., 2023). This pathologic characteristic is caused by destruction of the hepatic sinusoidal endothelial lining, resulting in massive hemorrhage and pooling of blood (Raghuvanshi *et al*., 2022). This intestinal hemorrhage into the liver leads to hypovolemic shock, which is the actual cause of death in rats due to hepatotoxins of cyanobacteria (Svirčev *et al*., 2022). Cyanobacterial hepatotoxin including tissue damage and injuries have been confirmed using advanced magnetic resonance techniques in conjunction with histo-pathological and serum enzyme function tests (Svirčev *et al*., 2022).

The survival time of the rats injected with lethally toxic extracts was about 3-4 hours, consistent with previous reports (Anon., 2020; Casas-Rodriguez *et al*., 2022) for hepatotoxins derived from cyanobacteria. Additionally, some rats exhibited mild to moderate symptoms indicative of neurotoxicity, which resolved completely within 24 hours. Neurotoxic substances have been isolated from various cyanobacterial genera, including *Anabaena*, *Aphanizomenon*, *Microcystis*, *Oscillatoria*, and *Planktothrix* (Nowruzi & Lorenzi, 2021; Aranda *et al*., 2023). In some cases, rats injected with other cyanobacterial extracts displayed atypical signs, but all recovered normal function within a few hours. Previous studies on cyanotoxins in animals have suggested that the observed symptoms are due to chemosensory stimulation rather than neuromotor inhibition, with full recovery occurring over time (Pauluhn, 2018). Additionally, previous research indicated that mice injected with sub-lethal doses of neurotoxins showed rapid and complete recovery following exposure (Pellett *et al*., 2019). Consequently, it is plausible that certain cyanobacteria, which induced neurotoxic symptoms in the present study but did not cause permanent damage, could be lethally toxic if administered at higher intraperitoneal doses. The limited availability of extracts constrained further investigations involving higher doses, a consideration that may warrant future exploration.

Earlier reports indicated that planktonic cyanobacteria are mostly toxic (Paerl, 2018). This is concomitant with our findings that the samples which appeared to be lethally toxic were collected from the water channel. According to the reports hepatotoxins have been the pre-dominant toxins in cyanobacteria, involved in cases of fatal animal poisonings (Napiórkowska-Krzebietke *et al*., 2023). Thus it can be said that hepatotoxic blooms are more common in world than neurotoxic bloom. These facts are in agreement with our findings which showed typical signs of hepatotoxicosis by rats injected with the lethally toxic extracts as well as other extracts.

High LD_{50} values recorded in the present study are in concurrence with the previous studies. A lower range of LD_{50} has been observed in case of purified cyanobacterial toxins (32-122 µg/Kg body weight) (Dawson, 1998). However, in studies where whole cell extracts were injected in rats, higher LD_{50} values ranging from 31 to 400 mg/Kg (Xu *et al*., 2020) were observed. Different LD_{50} values for crude ethanol extracts of different samples indicate variation in the concentration of toxins present and the species of constituting cyanobacteria biomass.

Conclusion

In conclusion, the cyanobacterial extracts, particularly those obtained with ethanol and chloroform, exhibited antibacterial activity, predominantly against Gram-positive bacteria such as *Bacillus subtilis*. However, no sensitivity was observed in the Gram-negative bacterial strains tested. These results suggest that cyanobacteria may possess selective antibacterial properties, varying in efficacy against different bacterial species. Similarly, the ethanolic extracts of cyanobacteria administered intraperitoneally in rats showed signs of toxicity, ranging from mild distress to severe effects, including paralysis and death, particularly with extracts from *Leptolyngbya angustissima* and a mixed crude sample. Moreover, alterations in blood serum enzyme levels indicated significant physiological disruption. While no major structural changes were noted in the gross morphological examination of vital organs, some discoloration and abnormal appearances were observed in the liver and lungs. The overall results of this study highlights the potential of cyanobacterial extracts as antimicrobial agents but emphasize the importance of evaluating their toxicity to ensure safety for clinical or therapeutic applications. Further studies are necessary to explore the safety profile and therapeutic potential of these extracts.

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