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ISOLATION AND CHARACTERIZATION OF A NOVEL ANTIMICROBIAL COMPOUND FROM SUB-AERIAL CYANOBACTERIUM *Fischerella* sp., ISOLATED FROM BUILDING FACADES

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ABSTRACT

This work was aimed at to extract a novel antimicrobial compound, especially against certain human pathogens, from a sub-aerial cyanobacterium *Fischerella* sp., isolated from the building facades of Bhubaneswar, Odisha (India). The antimicrobial activity was determined in different solvents and aqueous extracts using agar well diffusion method which were purified using column chromatography and TLC (twice), followed by direct bioautography, MIC, cytotoxicity and was characterized using UV-Vis, FTIR and LC-MS spectroscopic methods. The lowest MIC value (0.312 and 0.625 mg mL^{-1}) was depicted by the acetone extracted single compound against *Escherichia coli* and *Candida albicans.* The bioautography and spectroscopic analysis revealed the compound to be an alkaloid which was identified as pentaphenyl ferrocene carboxamide and found to be non-toxic against *Catla* thymus macrophage and *Mus musculus* up to 72 h. The cyanobacterium was identified as *Fischerella* sp. (accession No. MN593556) through 16S rRNA technique. The compound has potential for use as a therapeutic drug.

Keywords: Antimicrobial, cytotoxicity, *Fischerella* sp., natural bioactive compound, spectroscopic analysis

INTRODUCTION

Infectious diseases are one of the root causes of high morbidity and mortality in human all over the world, especially in the developing countries. Most of the bioactive microbial products lead to the induction of resistance to antibiotics. This has stimulated the researchers to explore and screen the less exploited microbial groups endowed with a more versatile secondary metabolite having drug therapeutic values. Sub-aerial cyanobacteria are believed to produce diverse biologically active compounds in the form of secondary metabolites due to their dwelling in extreme environments such as rock surfaces, walls and roofs of building facades and monuments, etc. (Keshari and Adhikary, 2014; Vasiliki *et al*., 2015). Some of these metabolites have the potential to be exploited for the development of new pharmaceutical compounds having biotechnological applications (Dobretsov *et al.,* 2011). *Fischerella* sp. is one of the members of cyanobacteria belonging to the order Stigeonematales which is identified by thallus bluish-green, spongy, branched, heterocystous and cell quadrant to rectangular in shape. This microbe is a rich source of bioactive compounds having antibacterial and antifungal activities (Tyagi *et al*., 2014; Singh *et al*., 2016; Safari *et al*., 2019).

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Very few studies have reported the antimicrobial potential of sub-aerial cyanobacteria isolates collected from the building facades (Panigrahi *et al*., 2015). In India, the state of Odisha, especially Bhubaneswar known as temple city, has rich cultural heritages including old/modern building which are inhabited by diverse cyanobacteria (Adhikary *et al*., 2015). This induced us to carry out a study on sub-aerial cyanobacterium *Fischerella* strain (isolated from cement wall, building facades of Bhubaneswar) and evaluate their antimicrobial activity against some selected pathogens by characterizing and identifying the pure bioactive compound and elucidate its chemical structure. Further, the toxic effects of pure compound were assessed against two different cell lines.

MATERIALS AND METHODS

Isolation and identification of cyanobacteria

The biofilms, collected from the cement wall surfaces of building facades of Bhubaneswar (India), were soaked in sterile distilled water, then incubated under a fluorescent light for 72 h and observed microscopically. Since morphological features needed for the identification were not distinct even after the prolonged soaking (up to 7 to 14 days), a small amount of sample was transferred with the help of an inoculation needle to the Petri-plates containing BG-11 agar medium with and without nitrogen (Rippka *et al.*, 1979). The cultures were incubated at 28 ± 1°C under continuous fluorescent light at an intensity of 2000 lux. After 10 to 14 days incubation, the cyanobacteria appearing in culture medium were isolated by sub-culturing methods (Ferris and Hirsch, 1991). The axenic cultures were cultured in a 500 mL BG-11 medium for biomass production, and maintained under controlled conditions at 28 ± 1°C and continuous illumination of 2000 lux with 16:8 h light and dark regime in laboratory. The cultures were identified as *Fischerella* sp. through morphological variation studies and compared with standard monographs (Desikachary, 1959; Komárek and Hauer, 2015).

Biochemical analysis

Cells grown on 10 mL BG-11 medium at 28 ± 1°C for 40 days were harvested by centrifugation technique at 4 days interval. By using standard protocols, the estimations were made for growth rate (Sfriso *et al*., 2014), total protein (Lowry *et al*., 1951), total carotenoids (Jensen, 1978), total carbohydrates (Herbert *et al*., 1971) and phycobiliproteins (Bennet and Bogard, 1973). All the experiments were conducted in triplicates and the values represented as mean ± SD.

Preparation of crude extracts

After 32nd days of incubation, the isolates grown on BG-11 broth were harvested using Whatman filter paper No. 1, dried in shade for 30 min and grinded to fine powder with a help of glass homogenizer. One gram of above powder was extracted separately in a Soxhlet apparatus using 200 mL each of water and different solvents like hexane, benzene, chloroform, ethyl acetate, acetone and methanol at 45^{o}C for 48 h. The extracts were collected in pre-sterilized air tight containers and preserved at 4^{o}C for further characterizations.

Antimicrobial efficacy and screening of extracts

The aqueous and organic (acetone, hexane, benzene, chloroform, ethyl acetate and methanol) crude extracts of microbial culture were screened for antimicrobial activity using agar well diffusion technique (Perez *et al*., 1990) against the six human pathogens which included four bacteria [*Bacillus subtilis* MTCC121, *Pseudomonas aeruginosa* MTCC741, *Staphylococcus aureus* MTCC902 and *Escherichia coli* MTCC723] and two fungi [*Candida albicans* MTCC4748 and *Epidermaphyton flocossum* MTCC7880]. These cultures were procured from the Microbial Type Culture Collection (IMTECH), India. All the experiments were conducted in triplicates. The bacterial and fungal pathogens were grown in nutrient broth and potato dextrose broth media and

incubated for 24 and 72 h at 37 and 28ºC, respectively. Then the bacterial and fungal pathogens were lawn cultured in Petri-plates containing Mueller-Hinton agar and Sabouraud dextrose agar, respectively, using sterile swab. A well of 6 mm dia. was made in the centre of plate and the bottom of well was sealed with soft agar. Cyanobacterial extracts (100 µL) were poured into the well and incubated at 37ºC for 24 h and 28ºC for 72 h, respectively, for bacterial and fungal pathogens to study the antimicrobial effect. The zone of inhibition was measured in mm and the break point for efficacy was taken as zone of inhibition ≥10 mm. Ciprofloxacin (10 $mgmL^{-1}$) and carbazine (10 mg mL^{-1}) were used as positive controls and pure solvents as negative controls.

Column chromatography and TLC purification

The most effective cyanobacterial crude extract showing higher antimicrobial efficacy during screening was fractionated by column chromatography (Rao *et al*., 2007). Silica gel (20 g) was dissolved in 500 mL of pure solvent (acetone) and loaded on the top of column and allowed to dry for 24 h. Then acetone cyanobacterial extracts (50 mL) were poured into the column. The solvent mixture composition (10% solvent) was used in column chromatography. The elutes were collected in fractions (30 mL each). Each fraction was further purified by preparatory TLC using ethyl acetate and hexane (1:1) as the mobile phase. The fraction was visualized under UV and the bands were marked and their R_f values determined. Additional tests were carried out on obtained TLC band by spraying of phytochemical screening reagents (Raaman, 2006) for phytochemical constituents for confirmation of purity and the identity of isolated compound. The purified active fraction was scrapped out separately, concentrated *in vacuo* to isolate the pure compound, dissolved in 3 mL of respective solvent and the antimicrobial activity against test pathogens was done by using agar well diffusion method as described earlier. The MIC (Wayne, 2012) and their toxicity assay were also conducted using different cell lines like *Catla* thymus macrophage (Challouf *et al.*, 2011) and mouse (*Mus musculus*) [Martins *et al*., 2008]. The antimicrobial activity index (AI) of purified compound was calculated using the formula of Welling (1983):

$$\text{Antimicrobial activity index (AI)} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of the standard}} \times 100$$

The bioactivities of compound were confirmed by secondary screening technique (bioautography method) following the method of Dewanjee *et al*. (2015).

Minimum inhibitory concentration (MIC)

The purified compound was serially diluted with acetone solvents to obtain a concentration in the range of 0.312 to 10.0 mg mL^{-1} in Mueller-Hinton broth and Sabouraud dextrose broth for bacteria and fungi, respectively, in 96 well micro-titre plates by micro-dilution method as per National Committee for Clinical Laboratory Standards guidelines (NCCLS) (Wayne, 2012). Overnight freshly grown pathogens (20 µL) were inoculated to each well. The wells without compound served as negative control (acetone) and the antibacterial (ciprofloxacin, 10 mg mL^{-1}) and antifungal (carbazine, 10 mg mL^{-1}) agents were included in the assays as positive controls. The test plates were incubated at 37 and 28°C for bacteria and fungi, respectively. After incubation, one loopful of sample was sub-cultured onto nutrient agar and Sabouraud dextrose agar plates for bacteria and fungi, respectively. A dilution where no growth of pathogens was recorded the MIC of purified compound.

Identification of bioactive compounds

The purified compound obtained in powdered form was further subjected to the following analysis in order to elucidate its structure as much as possible.

1. UV/Vis spectroscopy (Model: ƛ365, Perkin Elmer) [Rastogi and Incharoensakdi, 2013]
2. FT-IR spectroscopy (Nicolet 6700, USA spectrophotometer) [Bakir *et al.*, 2018]
3. LC-MS analysis (1260 Infinity Binary LC coupled with 6530B accurate mass Q-TOF, Agilent Technologies, USA) [Draisci *et al*., 2001]

The purified compound (2.5 mg) was extracted using 5 mL acetone. Then 1.5 µL extract was injected inside the inlet of LC-MS chamber through monolithic column (4 mm dia.) by direct injection method. Formic acid (0.1%) and isopropyl alcohol was used (Merck, Germany). The sample was subjected to LC-MS analysis for 20 min to identify the structure of compound. The peak of purified compound obtained was analyzed by the software TopPIC.

Cytotoxicity studies of purified compound

The cytotoxicity of bioactive compound, dissolved in acetone in various concentrations from 0.875 to 4.00 mg mL^{-1}, were assessed with the help of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) assay and WST1 against *Catla* thymus macrophage and *Mus musculus* (mouse) (Martins *et al*., 2008; Challouf *et al*., 2011), respectively. The absorbance was measured at 595 and 460 nm (DR 6000, HACH spectrophotometer). The experiments were conducted in 4 sets. The percentage of cytotoxicity was calculated by following formula:

$$\% \text{ cytotoxicity} = 100 - \text{cell viability } \%$$

$$\text{Cell viability} = \{(At-Ab)/(Ac-Ab)\} \times 100$$

Where, At - absorbance of test compound, Ab - absorbance of blank and Ac - absorbance of control

16S rRNA gene-based identification

The 16S rRNA gene-based identification was out-sourced and the sample was sent to SRM-DBT Platform for Advanced Life Science Technologies, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu (India). As per the details provided, the molecular identification was carried out through 16S rRNA by following the standard protocol of Gracia-Pichel *et al*. (2001). The experimental cyanobacterial sample (3 g) was grinded with the help of liquid nitrogen in a pestle mortar. Then 10 mL TESC buffer [100 mM tris-HCl, pH 8, 100 mM EDTA, 1.5M NaCl, 1% hexadecylmethyl ammonium bromide) was added followed by 550 mL 10% (w/v) sodium dodecyl sulfate and 30 mL proteinase K and incubated at 50°C for 20 min. Chromosomal DNAs were extracted in 10 mL phenol-chloroform-isoamyl alcohol (25:24:1) at 65°C and allowed to precipitate by adding isopropyl alcohol for 30 min at room temperature. PCR amplifications were done by using Cyclogene thermal cycler. Primers CYA 359F and CYA 781R were applied for amplifying the DNA fragment. Then 10 mL PCR buffer (100 mM tris-HCl [pH 9.0], 15 mM MgCl, 500 mM KCl, 1% triton X-100, 0.1% gelatin) was added and 10 mg DNA sample dissolved in 100 mL water. Thirty-five incubation cycles were performed. The consensus sequence was obtained from forward and reverse sequences using Bio-edit software and phylogenetic tree constructed using Mega5 software. The BLAST sequence was obtained from the National Centre for Biotechnology Information (NCBI) (*www.ncbi.nlm.nih.gov*) and the organism thus identified was deposited at NCBI Gene Bank, USA to obtain the accession number.

Statistical analysis

The data obtained from the experiments were expressed as mean ± SD of three replicates using one-way analysis of variance (ANOVA, SPSS version 17.0 statistical software program) and the treatments compared at 5% significance level.

RESULTS AND DISCUSSION

Fischerella sp., a sub-aerial cyanobacterium isolated from the building facades (cement wall surfaces) of Bhubaneswar, Odisha (India), was examined for specific biochemical contents as well as for the characterization and identification of antimicrobial potential compound against some pathogenic bacterial and fungal strains. The biochemical studies revealed that during mid-stationary phase, the specific growth rate was 6.78 µg mL^{-1}, chlorophyll-*a* 6.98 µg mL^{-1}, soluble cell-protein 48 µg mL^{-1}, carotenoids 18.98 µg mL^{-1}, cellular and extracellular carbohydrate content 56.36 µg mL^{-1} and 55.97 µg mL^{-1} on 28th days of incubation. The phycobiliproteins analyses revealed phyco-

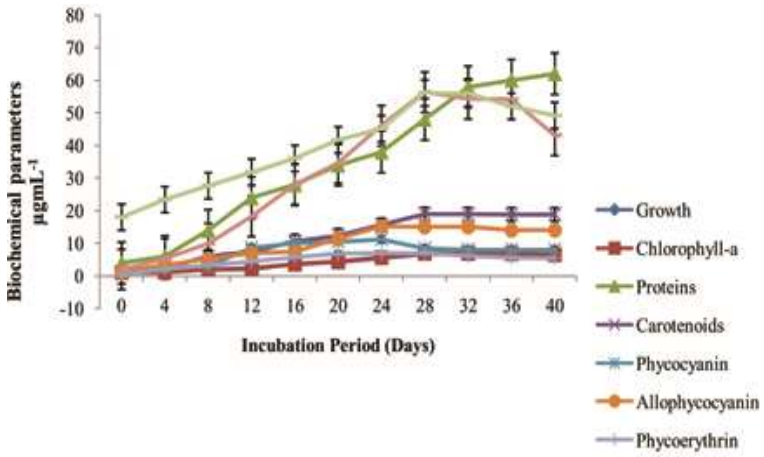

Fig. 1: Temporal variations of in growth rate (absorbance at 750 nm), pigment composition, soluble cell-protein, cellular and extracellular carbohydrates of *Fischerella*

cyanin content of 11.45 µg mL^{-1}, allophycocyanin 15.19 µg mL^{-1} and phycoerythrin 6.98 µg mL^{-1} on 28th day of growth (Fig. 1). The overall biochemical component contents in *Fischerella* sp. increased with increase in incubation time up to 28th day and decreased thereafter. This finding is comparable with the reported studies on *Fischerella muscicola*, isolated from freshwater sources (Paul and Rout, 2017).

The study revealed that the isolate has pharmaceutical potential. Swain *et al.* (2017) reported that the cyanobacteria releasing diverse secondary metabolites could be used in the development of anti-microbial compounds against resistant bacteria and fungi as base molecules which can be exploited in drug development. Bioactive metabolite analyses have mostly been studied in marine (Shaieb *et al.*, 2014) and freshwater algae (Ramya and Muralitharan, 2019). Very little is known about the algae isolated from terrestrial and sub-aerial habitats (Panigrahi *et al.*, 2015; Sahu *et al.*, 2017). The sub-aerial cyanobacteria isolated from building facades, known as extremophiles, are capable of producing secondary biologically active metabolites and are usually abundant in stationary phase (Drobac-Cik *et al*., 2007).

The isolate was screened for its antimicrobial potential using aqueous and crude organic extracts *viz.*, hexane, benzene, chloroform, ethyl acetate, acetone and methanol against certain human pathogens such as *Bacillus subtilis* MTCC121, *Pseudomonas aeruginosa* MTCC741, *Staphylococcus aureus* MTCC902, *Escherichia coli* MTCC723, *Candida albicans* MTCC4748 and *Epidermaphyton flocossum* MTCC7880. The cyanobacterial extract showed effective antimicrobial efficacy in polar solvents rather than in non-polar solvents against *E. coli*, *S. aureus*, *C. albicans* and *E. flocossum.* On the other hand, these extracts showed no activity against *B. subtilis* and *P. aeruginosa*. The aqueous, ethyl acetate and chloroform extracts showed no zone of inhibition; while hexane and benzene mimicked very little antibacterial effect and methanol extract showed moderate antimicrobial activity towards *S. aureus* and *E. flocossum* which might be attributed to less solubility of active components in these solvents (data not shown). The antimicrobial activity of aqueous, ethanol, diethyl ether, hexane and chloroform extracts of *Fischerella* have previously been reported (Asthana *et al*., 2006; Ravesh and Carmeli, 2007; Panigrahi *et al*., 2015). The variation observed in present study may be attributed to the inherent potential of strain used or the degree solubility of bioactive compounds in different solvent systems (Philip *et al*., 2009).

Based on the screening tests, the examination of antimicrobial activity of various extracts of *Fischerella* sp. revealed acetone to be the best solvent for the extraction of active antagonistic material and was found more effective against two pathogens, *E. coli* and *C. albicans*. The compounds were further purified by column and thin layer chromatography (twice). The chromatographic studies of 2 g biomass dissolved in 50 mL acetone extract of *Fischerella* sp. resulted in 15 elutes (fractions). Of these, elute No. 2 showed activity during screening. Further purification by TLC using ethyl acetate and hexane (1:1) revealed four pure fractions which were labelled as A_1, A_2, A_3 and A_4. One single fraction (A_2), out of four fractions on TLC plate, depicted antimicrobial activity against *E. coli* and *C. albicans* while others were ineffective. The confirmation

Table 1: Antimicrobial activity and antimicrobial index (in parentheses) of the compounds derived from *Fischerella* sp.

Sources	Compounds (R_f value)	Inhibition zone (mm)/Antimicrobial index (AI)					
		B. subtilis MTCC 121	*E. coli* MTCC 723	*P. aeruginosa* MTCC 741	*S. aureus* MTCC 902	*C. albicans* MTCC 4748	*E. flocossum* MTCC 7880
Fischerella sp.	A_1	-	-	-	-	-	-
	A_2 (0.96)	-	Complete inhibition	-	-	16.3 ± 6.0 (AI = 68)	-
	A_3	-	-	-	-	-	-
	A_4	-	-	-	-	-	-
Ciprofloxacin (10 mg mL^{-1})	IZ (mm)	-	28	18	26	-	-
Carbazine (10 mg mL^{-1})	IZ (mm)	-	-	-	-	24	30

[a]R_f = Retention factor; IZ = Inhibition zone; A = Acetone; numerals with the compounds indicate the number of spots on TLC plate

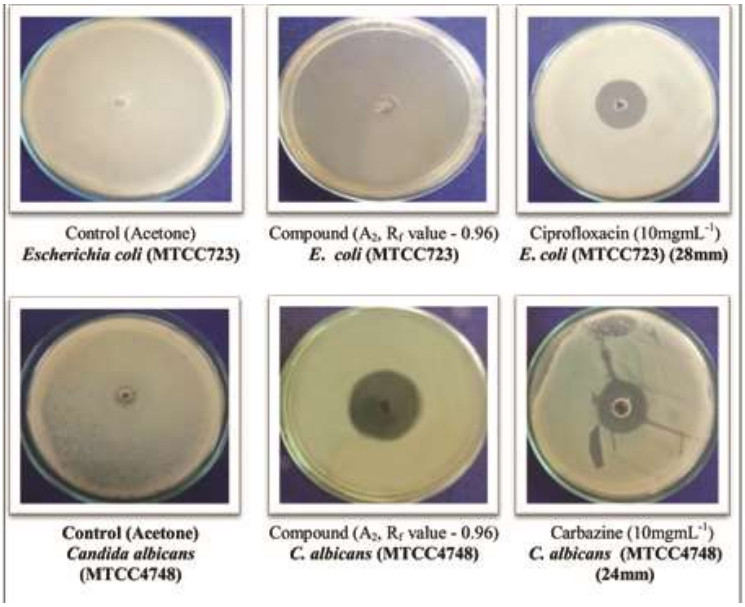

Fig. 2: Antimicrobial activity of the purified compound (A_2, R_f value - 0.96) extracted from acetone of *Fischerella* sp.

of bioactivity of this fraction (A_2) was carried out after second time purification on TLC plate which recorded a single major spot depicting the same response. The spot (A_2) was carefully scrapped out and dissolved in acetone solvent as it was found soluble in acetone (3 mL) only. This extract was centrifuged to remove the silica gel powder. The supernatant was concentrated *in vacuo* to isolate the white powdered compound and stored in 4°C till use. The compound A_2 showed complete inhibition against *E. coli* (Table 1, Fig. 2). The zone of inhibition obtained against *C. albicans* was 16.3 ± 6.0 mm (antimicrobial index, 68%). In comparison to the positive control ciprofloxacin (10 mg mL^{-1}) and carbazine (10 mg mL^{-1}), the compound A_2 showed better activities against *E. coli* and completely inhibited its growth. Through direct bioautography on TLC plates and phytochemical spraying, the pure compound A_2 with R_f value of 0.96 was found to be an alkaloid (Fig. 2). Singh *et al*. (2017) and Safari *et al*. (2019) reported

Table 2: Minimum inhibitory concentrations (MIC) of compound (A_2) from *Fischerella* sp.

Sources	Compound (R_f value)	MIC values (mg mL^{-1})					
		B. subtilis MTCC 121	*E. coli* MTCC 723	*P. aeruginosa* MTCC 741	*S. aureus* MTCC 902	*C. albicans* MTCC 4748	*E. flocossum* MTCC 7880
Fischerella sp.	A_2 (0.96)	-	0.312	-	-	*0.625*	-
Ciprofloxacin (10 mg mL^{-1})	MIC (mg mL^{-1})	2.5	0.625	0.625	2.5	-	-
Carbazine (10 mg mL^{-1})	MIC (mg mL^{-1})	-	-	-	-	0.312	0.625

[a]R_f = Retention factor; A = Acetone; The numerals with compound indicate the number of spots on TLC plate

Table 3: IR- spectrum of the purified active compound from *Fischerella* sp.

Functional groups	Types of vibrations	Characteristics absorption (cm^{-1}) of A_2 compound (R_f value 0.96)
Hydroxyl	O-H (stretch)	3534.7
Amide A	N-H (stretch)	
Alkanes	$-CH_2$ (stretch)	-
	$-CH_3$ (bending)	-
Alkenes	=C-H (stretch)	3003.8
	=C-H (bending)	-
Alkynes	-C=C- (stretch)	-
	C-H (bend)	
Amine II	C-N (stretch)	1220.5
	N-H (bending)	903.0
Aromatic rings	C-C (stretch)	1420.3
Alcohol, carboxylic	C= O (stretch)	1707.8
acids, esters	C- O (stretch)	1092.1
Nitro compound	N-O (stretch)	1358.8
Alkyl halides	C-Br (stretch)	529
Phosphodiester	>P=O	-
Iron containing	Fe	420

reported that antimicrobial effects of cyanobacterial extracts against pathogens may include fatty acids, alkaloids, flavonoids, phenolic compound, quinones and steroids. In present study, direct bioautography showed the presence of alkaloids in the active purified compound A_2 of *Fischerella* sp. (Walton and Berry, 2016). In contrast, many studies have reported the presence of terpenoids, tannins and saponin in the extracts of cyanobacteria (Pattanaik and Lindberg, 2015). Further, the minimum inhibitory concentration (MIC) of compound was determined to assess the effectiveness of compound. The lowest and moderate MIC of A_2 was 0.312 and 0.625 mg mL^{-1} against *E. coli* and *C. albicans*, respectively, which was co-related with MIC values (Table 2) indicating that acetone extract contained

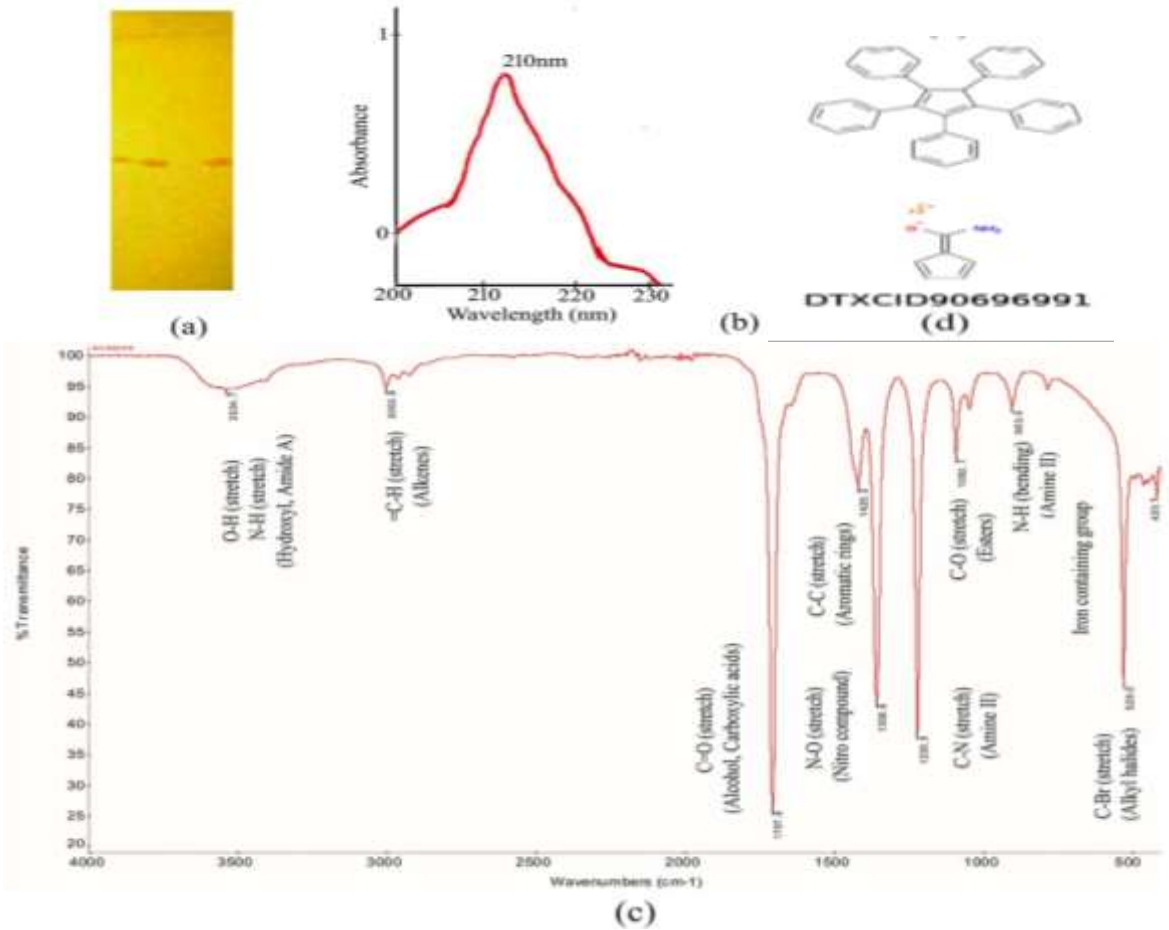

Fig. 3: Spectroscopic analysis of the purified compound (A_2, R_f value - 0.96) derived from acetone extract of *Fischerella* sp. (a) Single spot/compound on TLC plates; (b) UV absorption spectrum; (c) FT-IR spectrum, and (d) LC-MS

better quality bioactive compound in concomitant to previous reports (Panigrahi *et al.*, 2015; Singh *et al.*, 2016).

The elucidation of chemical structure of the isolated pure A_2 compound (R_f value 0.96) from *Fischerella* sp. was determined by UV-Vis, FTIR and LC-MS analysis (Fig. 2). UV spectrum of bioactive compound showed a single peak at 210nm and the compound was found to be an UV compound which revealed the presence of functional groups of absorption bands at 3534 cm^{-1} due to to NH_2, OH and NH groups. The peak at 3003.8 cm^{-1} indicated the presence of C-H aliphatic compounds. The absorption band at 1707.8 cm^{-1} was due to carboxylic acids, at 1420.3 cm^{-1} due to aromatic ring, at 1358.8 cm^{-1} due to amide group and at 1220.5 cm^{-1} due to phosphodiester groups.

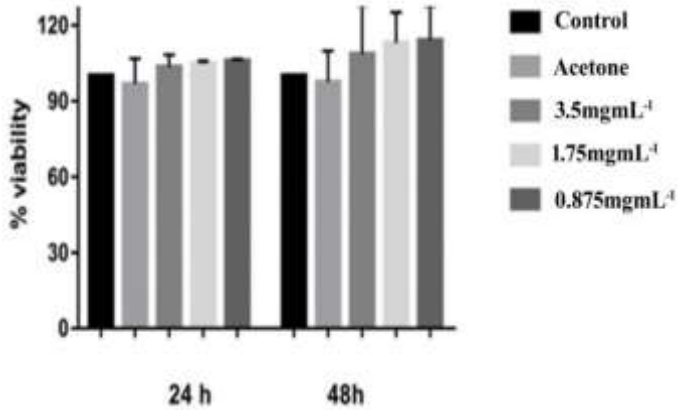

Fig. 4: Cytotoxicity activity (%) of the compound (A_2, R_f value 0.96) of *Fischerella* sp. against *Catla* thymus macrophage

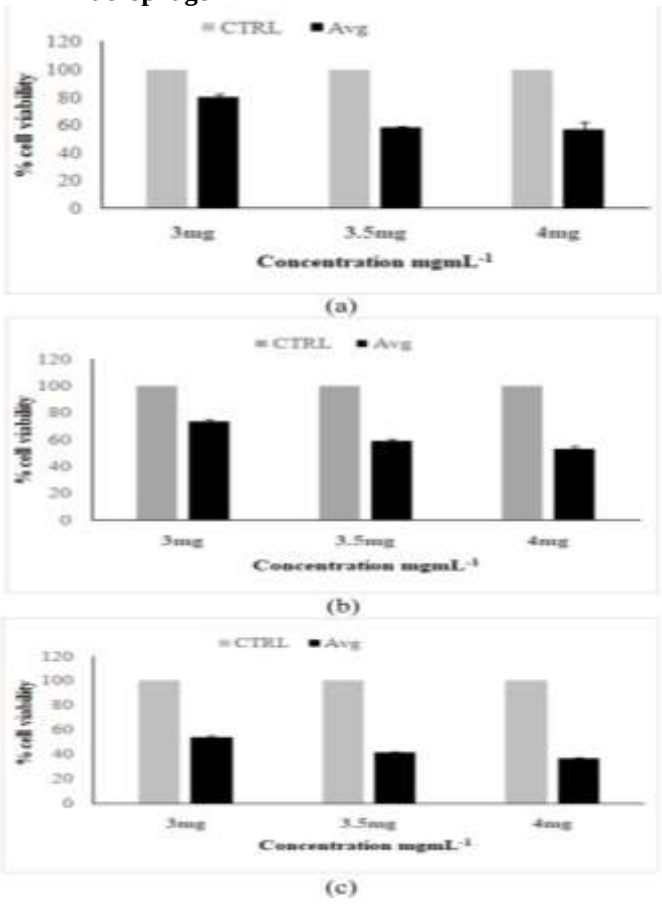

Fig. 5: Cytotoxicity activity (%) of the compound (A_2, R_f value 0.96) of *Fischerella* sp. against *Mus musculus* cell line at different time interval (a) 24 h, (b) 48 h, (c) 72 h

The peak at 1092.1 cm^{-1} revealed the presence of ester groups (C-O-C stretching), at 903 cm^{-1} for C-H, at 529 cm^{-1} for alkyl halides and at 420 cm^{-1} for iron group after FT-IR analysis (Table 3). The LC-MS analysis revealed the single pure compound A_2 to be iron (2+) amino (cyclopenta 2,4 diene-1-ylidine) methanolate 1,2,3,4,5-pentapheny-cyclopenta-2,4 dien-1-ide (pentaphenyl-ferrocene-carboxamide) $C_{41}H_{31}FeNO$:MW, 610 g mol^{-1} (Fig. 2). The resultant compound was surveyed with the available data base of PubChem, provided by NCBI but we found no biological activities.

The spectroscopic studies suggested that the antimicrobial activity of the extracts may be due to the presence of these secondary metabolites within it (Özer *et al.*, 2016). In earlier studies, the compounds such as ambiguines, a hapalindole type alkaloid, parsiguine, fischerllin A, a allelochemicals and hapalindole from *Fischerella* sp., *F. ambigua* and *F. muscicola* (Singh *et al.*, 2016) exhibiting antibacterial and antifungal activities against *Mycobacterium tuberculosis* H37Rv, *Bacillus anthracis*, *Staphylococcus aureus* ATCC25923, *Salmonella typhi* MTCC3216, *Pseudomonas aeruginosa* ATCC27853, *E. coli* ATCC25992 and *Enterobacter aerogene* MTCC2822 were reported where in pentaphenyl ferrocene carboxamide was reported

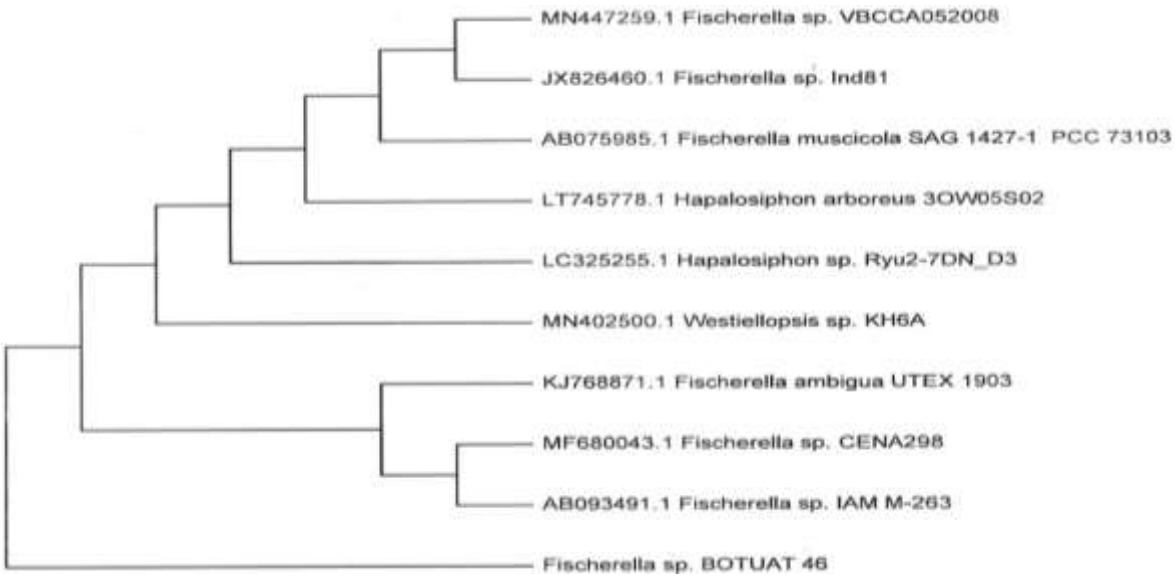

Fig. 6: Phylogenetics tree of sub-aerial cyanobacterium *Fischerella* sp. (Accession No. MN593556)

from *Fischerella* sp. collected from sub-aerial habitat (cement walls), building of Bhubaneswar, Odisha. The suggested compound A_2 was assayed for *in vitro* cytotoxicity test by comparing two different cell lines i.e. *Catla* thymus macrophage and osteoblast precursor of *Mus musculus* with different concentrations 0.875-4.0 mg mL^{-1}, respectively (Fig. 4-5). Further, there was no pronounced cytotoxic effect in cell lines derived from the healthy tissue of *Catla* thymus macrophage and *Mus musculus* up to 48 h with a concentration range of 0.875-4.0 mg mL^{-1}, but little lethal effect was noticed at 3.5 mg mL^{-1} (IC_{50}) after 72 h. Previous studies have reported hapalindole from *F. muscicola* UTEX strain number LB1829 investigated with HT-29, MCF-7, NCI-H460, SF268 and IMR90 cells was non-toxic (Kim *et al*., 2012) which are in agreement with present finding.

The species was identified by molecular characterization using 16S rRNA sequencing where maximum likelihood tree was obtained from the analysis of 16S rRNA sequences (Fig. 6). It was identified as *Fischerella* sp. which is the most potential species with accession No. MN593556.

Conclusion: The sub-aerial cyanobacterium *Fischerella* sp. (accession No. MN593556) revealed promising antimicrobial activities. In addition, the compound identified in acetone extract of *Fischerella* sp. was found non-toxic in nature which can be potentially explored further leading to the development of natural antimicrobial agents. This is the firsthand information regarding the isolation of an extremophilic sub-aerial cyanobacterium from building facades of Bhubaneswar, Odisha (India) with ability to produce antimicrobial compound. Further scientific studies are required to find its use in pharmaceutical industries against resistant antibiotics and chemotherapeutics.

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REFERENCES

Adhikary, S.P., Keshari, N., Urzi, C. and De Phillipis, R. 2015. Cyanobacteria in biofilms on stone temples of Bhubaneswar, Eastern India. *Algological Studies*, **147**: 67-93.

Asthana, R.K., Srivastava, A., Kayastha, A.M., Nath, G. and Singh, S.P. 2006. Antibacterial potential of γ-linolenic acid from *Fischerella* sp. colonizing neem tree bark. *World Journal of Microbiology and Biotechnology*, **22**: 443-448.

Bakir, E.M., Younis, N., Mohammad, M.E. and El-Semary, N.A. 2018. Cyanobacteria as nanogold factories: Chemical and anti-myocardial infarction properties of gold nanoparticles synthesized by *Lyngbya majuscule*. *Marine Drugs*, **6**(6): 217. [*https://doi.org/10.3390/md16060217*].

Bennett, A. and Bogorad, L. 1973. Complimentary chromatic adaptation in a filamentous blue green alga. *Journal of Cell Biology*, **58**(2): 419. [*https://doi.org/10.1083/jcb.58.2.419*].

Challouf, R., Trabelsi, L., Dhieb, R.B., Abed, O.E., Yahia Ghozzi, K., Ammer, J.B., Omran, H. and Ouada, H.B. 2011. Evaluation of cytotoxicity and biological activities in extracellular polysaccharides released by cyanobacterium *Arthrospira platensis*. *Brazilian Archives of Biology and Technology*, **54**(4): 831-838.

Desikachary, T.V. 1959. *Cyanophyta*. Indian Council of Agricultural Research, New Delhi, India.

Dewanjee, S., Gangopadhyay, M., Bhattabharya, N. and Khanra, R. 2015. Bioautography and its scope in the field of natural product chemistry. *Journal of Pharmaceutical Analysis*, **5**: 75-84.

Draisci, R., Ferretti, E., Palleschi, L. and Marchiafava, C. 2001. Identification of anatoxins in blue-green algae food supplements using liquid chromatography-tandem mass spectrometry. *Food Additives and Contaminants*, **18(**6): 525-531.

Dobretsov, S. and Thomason, J.C. 2011. The development of marine biofilms on two commercial non biocidal coatings: A comparison between silicone and fluoropolymer technologies. *Biofouling*, **27**(8): 869-880.

Drobac-Čik, A.V., Dubic, T.I., Stojanović, D.B. and Svirčev, Z.B. 2007. The importance of extremophiles cyanobacteria in the production of biologically active compounds. *Natural Product Sciences*, **112**: 57-66.

Ferris, M.J. and Hirsch, C.F. 1991. Method for isolation and purification of cyanobacteria. *Applied and Environmental Microbiology*, **57**(5): 1448-1452.

Garcia-Pichel, F., López-Cortés, A. and Nübel, U. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology*, **67**(4): 1902-1910.

Herbert, D., Phipps, P.J. and Strange, R.E. 1971. Chemical analysis of microbial cells. pp. 209-234. **In***: Methods of Microbiology* (eds. J.R. Norris and D.W. Ribbons). Academic Press, New York, USA.

Jensen, A. 1978. Chlorophylls and carotenoids. pp. 59-70. **In**: *Hand Book of Phycological Methods. Physiological and Biochemical Methods.* (eds. J.A. Hellebust, and J.S. Craigie). Cambridge University Press, Cambridge, UK.

Keshari, N. and Adhikary, S.P. 2014. Diversity of cyanobacteria on stone monuments and building facades of India and their phylogenetic analysis. *International Biodeterioration and Biodegradation*, **90**: 45-51.

Kim, H., Lantvit, D., Hwang, C., Kroll, D., Swanson, S., Franzblau, S. and Orjala, J. 2012. Indole alkaloids from two cultured cyanobacteria, *Westiellopsis* sp. and *Fischerella muscicola. Bioorganic and Medicinal Chemistry*, **20**(17): 5290-5295.

Komárek, J. and Hauer, T. 2015. *CyanoDB.cz-On-line Database of Cyanobacterial Genera*. World-Wide Electronic, University of South Bohemia and Institute of Botany. Trebon, Czech Republic.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**: 265-275.

Martins, R., Ramos, M.F., Herfindal, L., Sousa, J., Skarven, K. and Vasconcelos, V.M. 2008. Antimicrobial and cytotoxic assessment of marine cyanobacteria - *Synechocystis* and *Synechococcus*. *Marine Drugs*, **6**(1): 1-11.

Mckinney, G. 1941. Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry,* **221**: 315-322.

Ozer, T., Yalcin, D., Acikgoz Eekaya. I. and Udoh, A.U. 2016. Identification and characterization of some species of cyanobacteria, chlorophyta and bacillariophyta using Fourier-transform infrared (FTIR) spectroscopy. *Journal of Pharmacy and Biological Sciences*, **11**(6): 20-27.

Panigrahi, S., Sethi, A.K. and Samad, L.K. 2015. Antibacterial activities of *Scytonema hofman* extracts against human pathogenic bacteria. *International Journal of Pharmacy and Pharmaceutical Science*, **2017**(5): 123-126. [*doi https://index.php/ijpps/article/view/ 3978*].

Pattanaik, B. and Lindberg, P. 2015. Terpenoids and their biosynthesis in cyanobacteria. *Marine Drugs*, **5**: 269-293.

Paul, A. and Rout, J. 2017. Biochemical evaluation of some cyanobacterial strains isolated from the lime sludge wastes of a paper mill in Southern Assam (India). *Phykos,* **47**(1): 8-15.

Perez, C., Paul, M. and Bazerque, P. 1990. An antibiotic assayed by the agar well diffusion method. *Acta Biologiae et Medicinae Experimentalis,* **15**: 113-135.

Philip, K., Malek, S.N.A., Sani, W., Shin, S.K., Kumar, S., Lai, H.S., Sem, L.G. and Rahman S.N.S.A. 2009. Antimicrobial activity of some medicinal plants from Malaysia. *American Journal of Applied Sciences,* **6**: 1047-1058.

Raaman, N. 2006. *Phytochemical Techniques*. New India Publishing Agency, New Delhi, India.

Ramya, V.P. and Muralitharan, G. 2019. Evaluation of antibacterial activity of cyanobacteria isolated from fresh water ecosystem of Tiruchirappalli district, Tamil Nadu, India. *Research Journal of Life Science, Bioinformatics, Pharmaceutical and Chemical Sciences*, **5**(2): 331-344.

Rao, M., Malhotra, S., Fatma, T. and Rattan, A. 2007. Antimycobacterial activity from cyanobacterial extracts and phytochemical screening of methanol extract of *Hapalosiphon. Pharmaceutical Biology*, **45**(2): 88-93.

Rastogi, R.P. and Incharoensakdi, A. 2013. Characterization of UV-screening compounds, mycosporine-like amino acids, and Scytonemin in the cyanobacterium *Lyngbya* sp. CU2555. *FEMS Microbiology Ecology*, **8**(1): 254-256.

Ravesh, A. and Carmeli, S. 2007. Antimicrobial ambiguines from the cyanobacterium *Fischerella* sp. collected in Israel. *Journal of Natural Products*, **70**(2): 196-201.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. 1979. Generic assignments, strain histories and properties of pure culture of cyanobacteria. *Journal of General Microbiology*, **111**: 1-61.

Safari, M., Nowruzi, B., Estalaki, S. and Shokri, M. 2019. Biological activity of methanol extract from *Nostoc* sp. N42 and *Fischerella* sp. S29 isolated from aquatic and terrestrial ecosystems. *International Journal of Algae*, **21**: 373-391.

Sahu, E., Giri, D., Bhakta, S., Panda, S.K. and Bastia, A.K. 2017. Phytochemical screening of a corticolous cyanobacterium *Hassalia byssoidea* Hass. Ex Born. Et Flah. for antibacterial and antioxidant activity. *World Journal of Pharmacy and Pharmaceutical Sciences,* **6**(3): 1161-1172.

Samad, L.K. and Adhikary, S.P. 2008. Diversity of micro-algae and cyanobacteria on building facades and monuments in India. *Algae,* **23**(2): 91-114.

Sfriso, A.A., Marchetto, D., Gallo, M. and Baldi, F. 2014. Biochemical characterization of some cyanobacterial strains from salt marshes of the Venice lagoon. *Journal of Applied Phycology*, **26**: 273-278.

Shaieb, F.A., Issa, A.A.S. and Meragaa, A. 2014. Antimicrobial activity of crude extracts of cyanobacteria *Nostoc commune* and *Spirulina platensis*. *Archives of Biomedical Sciences,* **2**: 34-41.

Singh, N.K. 2016. Role of cyanobacteria in crop protection. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **86**(1): 1-8.

Singh, J.S., Kumar, A., Rai, A.N. and Singh, D.P. 2016. Cyanobacteria: A precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Frontiers in Microbiology*, **7**: 529. [*https://doi.org/10.3389/fmicb.2016.00529*]

Singh, R., Parihar, P, Singh, M., Bajguz, A., Kumar, J., Singh, S., Singh, V.P. and Prasad, S.M. 2017. Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: Current status and future prospects. *Frontiers in Microbiol*ogy, **8**: 515. [*https://doi.org/ 10.3389/fmicb.2017.00515*].

Swain, S.S., Paidesetty, S.K. and Padhy, R.N. 2017. Antibacterial, antifungal and antimycobacterial compounds from cyanobacteria. *Biomedicine and Pharmacotherapy*, **90**: 760-776.

Tyagi, R., Kaushik, D.B. and Kumar, J. 2014. Antimicrobial activity of some cyanobacteria. pp. 463-470. **In**: *Microbial Diversity and Biotechnology in Food Security* (eds. R.N. Kharwar, R. Upadhyay**,** N. Dubey and R. Raghuwanshi). Springer, New Delhi, India.

Vasiliki, L., Kyriaki, T., Emmanuel, N.V., Alkiviadis, V., Smaragdi, A., Elizabeth, F., Adriani, P. and Athena, E.A. 2015. Cave cyanobacteria showing antibacterial activity. *International Journal of Speleology*, **4**(3)**:** 281-238.

Walton, K. and Berry, J.P. 2016. Indole alkaloids of the stigonematales (Cyanophyta): Chemical diversity, biosynthesis and biological activity. *Marine Drugs*, **14**(73): 1-28.

Wayne, P.A. 2012. *Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard* (11^{th} edn.). Clinical and Laboratory Standards Institute, CLSI Publication M02-A11, Pennsylvania, USA.

Welling, P.G. 1983. Oral controlled drug administration. *Drug Development and Industrial Pharmacy,* **9:** 1185-1225.