In vitro screening of, antibacterial antifungal and cytotoxicity activities in crude extract of freshwater cyanobacterium *Oscillatoria* sp.

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Abstract

Cyanobacteria, highly diverse group of prokaryotes are recognized as a potent source of biologically active compounds with antiviral, antibacterial, antifungal, and anticancer properties.The aim of the present study was to screen antibacterial, antifungal and cytotoxic activities of intracellular secondary metabolites of freshwater cyanobacterium *Oscillataria* sp. Cyanobacterium *Oscillatoria* sp. was isolated from Senanayaka Samudraya reservoir (7^0 11' 37.37^0N 81^0 31' 47.13^0E), Sri Lanka.In vitro antibacterial and antifungal activity of *Oscillatoria* sp. was screened against Gram-positive Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 25923, *Bacillus anthracis* and Gram-negative *Pseudomonas aeruginosa* (ATCC 25853), *Salmonella typhi* and *Escherichia coli* (ATCC 25922) and fungi, unicellular *Candida albicans* (ATCC 60192) and *Candida tropicalis* using agar disc diffusion method.The Minimum Inhibitory Concentrations (MIC), Minimum Bacteriocidal Concentration (MBC), Minimum Fungicidal Concentrations (MFC) and cytotoxic effects (brine shrimp bioassay) of *Oscillatoria* crude extract were determined. 10% and 60% of biomass was extracted with hexane and methanol respectively.Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify compounds in the crude extract. The highest antibacterial and antifungal activity of crude extract were detectedin methanol extract against *S.aureus* (19±2 mm)and *C.albicans* (10±1 mm) within 24 hours wherein the hexane extract, antibacterial activity was detected only for *S. aureus* and mean diameter of inhibition zone was 11±1mm within 24 hours.The lowest MIC of methanol extract against *S. aureus* wasfound as 156.25 µg/ml. The lowest MBC and MFC of methanol extract against*S. aureus* and *C.albicans* were 0.63 mg/ml and 1.25mg/ml respectively. Lethal concentration, 50% of the crude extract against brine shrimp was recorded at 2.50 g/l, 1.25 g/l and 0.625 g/l for 6, 12, 24 hrs intervals respectively. GC-MS analysis revealed that the methanol crude extract of *Oscillatoria* sp. contains important fatty acid namely hexadecanoic acid methyl ester, methyl tetradecane and 13-tetradecanoic acid and n-hexane extract contains Bis (2-ethylhexyl hydroxypyridine oxide, 1 2-benzenedicarboxylic acid mono (2-ethylhexyl) ester, Phthalic acid 6-ethyl-3-Octyl heptyl ester and Phthalic acid dodecyl nonyl ester which may possess antibacterial and antifungal properties.

Keywords:Oscillatoria sp., MFC, MIC, MBC, Cytotoxicity

1. Introduction

Over the past decades, an alarming number of clinically efficacious antibiotics have become less effective due to the direct implications in human morbidity and mortality, leading to the development of microorganisms that are resistant to antibiotics (Liyanage and Manage, 2016).

Consequently, an increase in the failure of chemotherapeutic and antibiotic resistance by pathogenic microorganisms has led to the screening of several other alternative sources for potential antimicrobial activity (Osman et al., 2011). Eventually, researchers came up with a rational alternative for synthetic drugs; bioactive compounds derived from cyanobacteria and algal extracts and it has always been recognized that humans, animals, and plants benefit from cyanobacteria and algae and their extracts (Chojnacka et al., 2012).

Cyanobacteria are morphological, physiological, and metabolically a diverse group which originated 3.5 billion years ago (Kaushik and Chauhan, 2008;Sethunga and Manage, 2009). They are not only widespread in freshwater, marine and terrestrial ecosystems but also found in extreme habitats such as hot water springs, hypersaline localities, freezing environments and arid deserts (Wijesekara and Manage, 2016; Manage et al., 2009a). To survive in a highly competitive environment, often exhibiting widely fluctuating chemical and physical parameters, they have developed defensive and adaptive strategies, including the synthesis of a tremendous diversity of compounds from different metabolic pathways (Yadav et al., 2010). Recent scientific studies have documented that various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal, cytotoxic, algaecide, immunosuppressive and antiviral chemicals (Ghasemi et al., 2003; Jaki et al., 1999; Manage et al., 2009b).The majority of them were originated from the filamentous genera *Lyngbya*, *Oscillatoria*, and *Symploca* (Williams et al., 2001; Osswald et al., 2007; Grindberg et al., 2008,) and a high degree of diversity in the bioactivities due to the presence ofsecondary metabolites.

Recent studies on biologically active secondary metabolites from cyanobacteria led to the identification of a wide range of antibiotic compounds (Singh et al., 2005). Ethyl acetate extract of *Spirulina platensis* consisted of heptadecane and tetradecane which can inhibit some Gram +ve and Gram-ve bacteria and *Candida albicans*(Ozdemir et al., 2004). El-Sheekh et al. (2005) showed that phenolic compounds from *Nostoc muscorum* exhibited antagonistic activity against Gram +ve and Gram-ve bacteria. Ghasemi et al. (2003) isolated substances belonging to groups of peptides, polypeptides, amides and alkaloids from *Fischerella ambigua*, *Anabaena* spp. which produce a number of bioactive compounds, mostly lipopeptides that have antibiotic, antialgal, anticancer, anti-inflammatory, cytotoxic and enzyme-inhibiting effects (Burja et al., 2001; Fujii et al., 1997).

Thus the present study was aimed to screen the antibacterial and antifungal activity of the isolated *Oscillatoria* sp. against both Gram positive and Gram negative human pathogenic bacteria and fungal species and evaluate the cytotoxicity effect against *Artemia*. Finally, to identify the individual components of their methanol and hexane extract by gas chromatography (GC) coupled with a mass spectrometer (MS).

2. Material and Methods

2.1 Isolation of cyanobacteria

Cyanobacteria used in this study was isolated fromfreshwater sample collected from the Senanayake Samudraya reservoir (7^0 11’ 37.370 N 81^0 31’ 47.130 E), Sri Lanka. Water samples were brought to the laboratory in an aseptic condition and the BG11 medium was used for isolation and maintenance of the cyanobacterium (Manage et al., 2001; Manage et al., 2009). Unifilamentous *Oscillatoria* sp. was isolated using a pasture pipette following the spread plate method (Manage et al., 1999). Isolated *Oscillatoria* sp. in BG-11culture was maintained without aeration at 25±1°C, at a light intensity of 2,000-3,000 Lux (10 μmol $m^{-2}s^{-1}$) provided by cool white tubes and with a light/dark cycle of 18 h /6 h. Bacterial contamination states were confirmed by standard TYG (Tryptone, Yeast extract and Glucose) protocol (Vaara et al., 1979). The fernbach and mass cultures of *Oscillatoria* sp.

was prepared and mass culture was grown under laboratory condition for a period of 30 days in 10 litres of BG 11 medium. The exponential growth phase of the culture was determined by chlorophyll-*a* content and the culture was harvested within 10- 15 days by centrifugation at 10°C, 6,500 rpm for 10 minutes.

2.2 Preparation of Oscillatoria crude extract

One gram of dried biomass was ground to fine powder and extracted using two different extraction solvent; hexane and methanol. The powder form of biomass was dissolved in 100 ml of n-hexane and the cells were broken in an ultrasonic water bath for 7 min at 35 KHz. Then, the biomass was extracted under magnetic stirring for 2 h following centrifugation at 4,100 rpm for 10 min at 10°C and then the supernatant was separated from the residue. This step was repeated three times. The biomass residue was dried overnight and further extraction was carried out with 3 x 150 ml MeOH. Complete removal of solvent was achieved by rotary evaporation at 40^{o} C (IKA HB 10, Germany). The MeOH extract and hexane extract were weighed and kept in sealed vials in -20°C freezer for further use. To prevent oxidation, all steps were carried out in the dark while the flask was covered with aluminium foil.

2.3 Pathogenic bacteria and fungal strains

Screening for antibacterial and antifungalactivity of methanol and hexane extract was carried out using human pathogenic Gram-positive; *Staphylococcus aureus*(MRSA) ATCC 25923 and*Bacillus anthracis* and Gram-negative; *Pseudomonas aeruginosa* (ATCC 25853), *Escherichia coli* (ATCC 25922) and *Salmonella typhi*and fungi; unicellular *Candida albicans* (ATCC 60192)and *Candida tropicalis* obtained from Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura. Nutrient agar (NA) was used for the cultivation of target bacteria and all bacteria cultures were incubated at 37ºC for 24 hrs and fungal species were grown in potato dextrose agar (Hi-Media) at ambient temperature for 72 h.

2.4 Antimicrobial assay

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method given by Mundt et al. (2001) to assess the presence of antibacterial and antifungal properties of the extract. Bacterial and fungal cultures (which have been adjusted to 0.5 McFarland standard), were used for lawn Muller Hinton and PDA agar plates respectively. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of crude extracts (5µg/disc, 400 µg/disc) were placed on the Mueller- Hinton agar and potato dextrose agar surface. Each test plate comprised four discs. One positive control (Ciprofloxacin (antibacterial), amphotericin B (antifungal) 5µg/disc) which was a standard commercial disc, one negative control (DMSO 100%), and two treated discs.The Petri dish was kept at 4°C about 3 h for pre-diffusion. Afterwards, the plates were incubated for 24 h at 37°C for bacteria and fungi in an inverted position. At the end of the incubation period, the inhibition zones were measured using callipers and expressed as the diameter of the clear zone, with the diameter of the paper disc. The test was repeated three times to ensure reliability.

2.5 Determination of relative percentage inhibition

The relative percentage inhibition with respect to positive control was calculated by using the following formula.

$$\textit{Relative percentage of inhibition of test extract} = \frac{100 \times (a-b)}{C} \quad (1)$$

Where:

a=Inhibition zone of test extract

b=Inhibition zone of the solvent

c=Inhibition zone of the standard drug (Ciprofloxacin, amphotericin B)

2.6 TTC (2,3,5- triphenyl tetrazolium chloride) bioassay

	1	2	3	4	5	6	7	8	9	10	11	12
A	C1											B-control.
B	C1											B-control.
C	C1											B-control.
D	C1											B-control.
E	P											N-control.
F	P											N-control.
G	P											N-control.
H	P											Blank

Figure 1: Preparation of the Microtiter plate for MIC estimation against test bacterial and fungal species.

C1-Highest concentration of crude extract (2.5 mg/ml), P- Highest concentration of Standard antibiotic (cloxacillin 2.5 mg/ml) B- control (Nutrient broth, and bacteria suspension) Negative (N)-Control (PBS buffer, Bacteria suspension).

The minimum inhibitory concentration of crude extract of *Oscillatoria* sp. was determined by broth dilution methods using 96-well Microtiter plates against Gram-positive bacteria (*S. aureus,B. anthracis*) Gram-negative bacteria (*P. aeruginosa, E. coli, S. typhi*) and fungi (unicellular *C. albicans* (ATCC 60192), *C.tropicalis*). For the broth dilution method, inocula of the microbial strains were prepared from the overnight nutrient broth cultures for bacteria and potatoes dextrose broth for fungi. Bacterial and fungal suspensions were adjusted to 0.5 McFarland standard turbidity. The crude extract was dissolved in PBS buffer to get stock solutions C1 (2.5 mg/ml) and the positive control was prepared using standard cloxacillin (2.5 mg/ml) for bacteria andamphotericin B (2.5 mg/ml) for fungi. Different concentrations of extract were prepared using two-fold dilution techniques and transferred to each well of 96 well plate. Bacterial and fungal suspension and PBS buffer were used as negative control (N-control) and PBS, nutrient broth or PDA broth with bacteria and fungi suspension was used as B control. 100 µl of the standardized bacterial and fungi suspension was pipetted into all wells from 1st row to 11th row. The well H12 was kept empty as a photometric blank (Figure 1) and the plate was incubated at 37°C for 24 h.After the incubation, microbial growth was determined by adding 20 µl of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution following 30 min incubation (Sartoratto et al. 2004).

Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the samples inhibiting visible growth (red coloured solution of the wells) after addition of TTC. The OD was measured using multiskan ex, microplate reader at 480 nm.

2.7Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC/MFC is defined as the concentration of the antimicrobial agent that results in the 99.9% reduction in CFU/ml, compared with the organism concentration in the original inocula. The MBC was determined by using the test plates which were previously used for MIC test. 50 µl aliquots from each well were spread separately on the entire surface of the sterile nutrient agar and PDA plates with the help of sterile glass spreader and incubated in inverted position overnight at 37^{o}C for bacteria and three days for fungi at room temperature. The test was run in duplicate and the aliquots of wells that were not given a visible growth defined the MBC/MFC of the compound.

2.8Brine shrimp cytotoxicity assay

Brine shrimp lethality test was carried out according to the method described by Piyathilaka et al., (2015) with minor modification. Brine shrimp eggs (*Artemia salina*) obtained locally were hatched in artificial seawater (38 g/l) for 24 hours. Following 24 hr of hatching, 10 larvae were collected and transferred into 24-microwell plate. A stock solution (500 mg/ml) of the extract was prepared by suspending dried extract in salt water and the suspension was mixed for 5 min. Different concentrations of extract was prepared from stock solution (500, 250, 50, 25, 12, 6 mg/ml) and 5 ml was transferred into each well. Following 24 hr of incubation, the number of dead larvae was counted with the aid of a light microscope (Labomed, USA) and then the lethal concentration (LC_{50}) was determined.

2.9Gas Chromatography/Mass Spectrometry (GC/MS)

The volatile constituents of methanol and hexane extracts of *Oscillatoria* sp. were analysed by GC/MS using a Thermo Scientific Capillary Gas Chromatography (an Agilent Technologies 7890 A GC/5975C mass selective detector (MSD). The dried extract was re-dissolved in extracting solvent and subjected to GC/MS analysis. The GC/MS analysis was performed on A 30-m × 0.25-mm i.d. DB225MS capillary column under the following conditions: oven temperature program from 40 °C (3 min) to 280° C at 5° C/min, then isothermal at 280ºC for 5 min, flow rate of carrier gas (Helium) was 1 ml/min, the injected sample volume was 1 µl, splitless injection technique, and ionization energy of 70 eV in the electron ionization (EI) mode. Identification was carried out by comparing the retention indices and fragmentation pattern in mass spectra.

The antibacterial activity of the methanol and hexane extracts of *Oscillatoria* sp. is presented in Table1.The extracts showed different degrees of antimicrobial activity against test microorganisms. The antibacterial and antifungal activity of the extracts were classified based on the average zones of inhibition into four levels namely resistant (IZ=6-16 mm), moderate activity (IZ=16-20 mm) and strong activity (IZ=22< mm) against 400 µg/disc concentration according to the National Committee for Clinical Laboratory Standards guidelines(NCCLS). The methanol extract showed moderate antibacterial activity against all the test bacteria and low activity against fungi. n-hexane extract exhibited low antibacterial activity only against *S. aureus*.

3. Result and Discussion

Table 1: Antibacterial and antifungal activities (average zone of inhibition in mm±SD) of the methanol and hexane crude extracts of the *Oscillatoria* sp. within 24 hr by disc diffusion assay.

Pathogenic bacteria and fungi	*Oscillatoria* sp. crude extract				Diameter of inhibition zone (Positive control)
	Diameter of inhibition zone				
	Methanol extract		Hexane extract		
Bacteria	400 µg/disc	5 µg/disc	400 µg/disc	5 µg /disc	5 µg /disc
S.aureus	19 mm ± 2	9 mm ± 1	14 mm ± 1	7 mm ± 1	30 mm ± 1
P.aeruginosa	16 mm ± 1	12 mm ± 2	ND	ND	38 mm ± 1
S.typhi	16 mm ± 1	7 mm ± 1	ND	ND	30 mm ± 1
E.coli	16 mm ± 1	10 mm ± 1	ND	ND	40 mm ± 1
B.anthracis	17 mm ± 1	10 mm ± 1	ND	ND	25 mm ± 1
Fungi					
C.albicans	10 mm ± 1	ND	ND	ND	15 mm ± 1
C.tropicalis	9 mm ± 1	ND	ND	ND	14mm ± 1

Positive control: 5 gµ/disc of ciprofloxacin to bacteria and amphotericin B to fungi

ND: Not detected

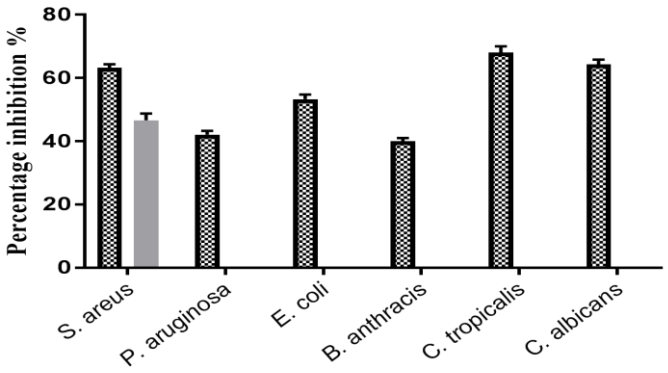


Figure 2: Relative % inhibition of methanol extract and n-hexane extract of *Oscillatori*a sp. against positive control (Black bars- Methanol extract, Hass bars- hexane extract).

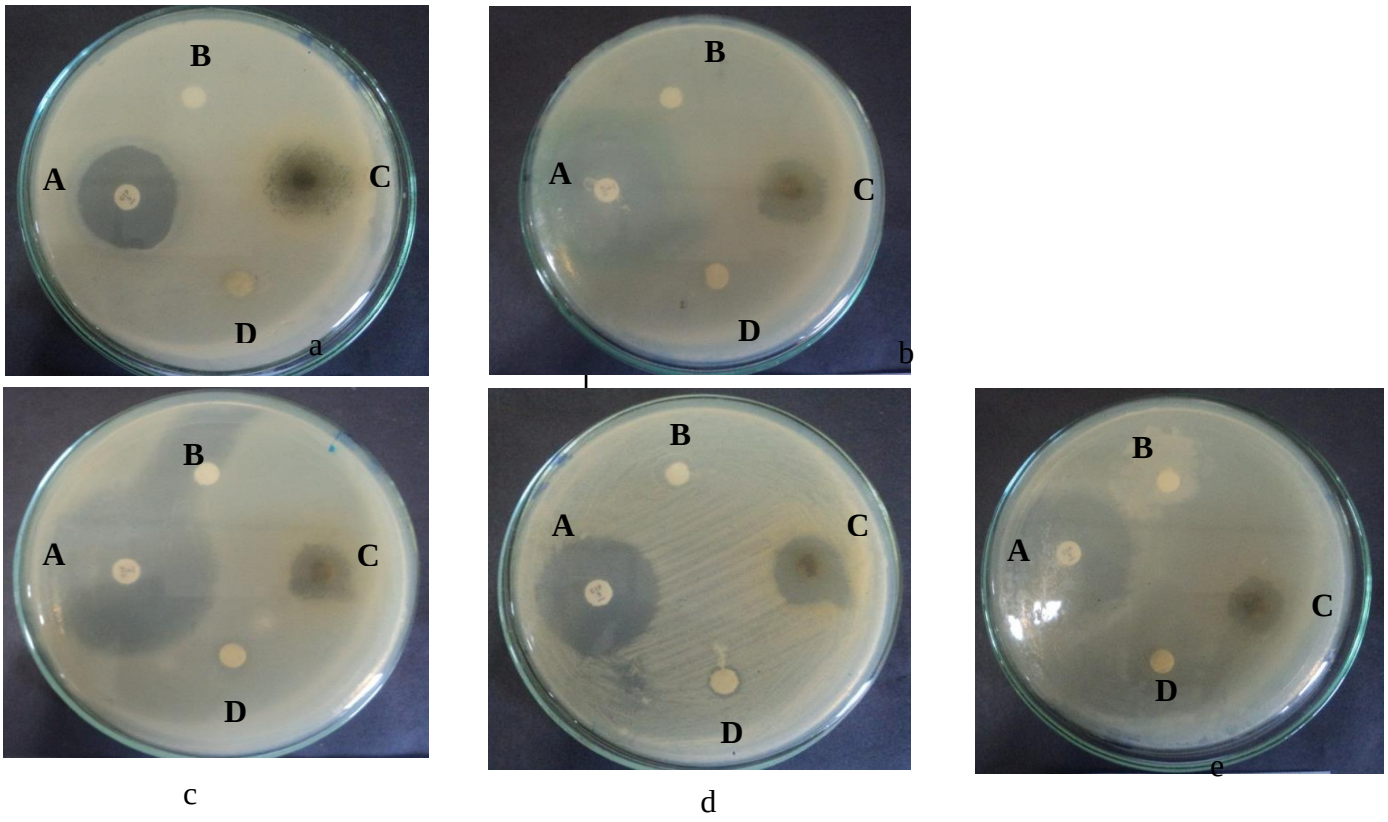
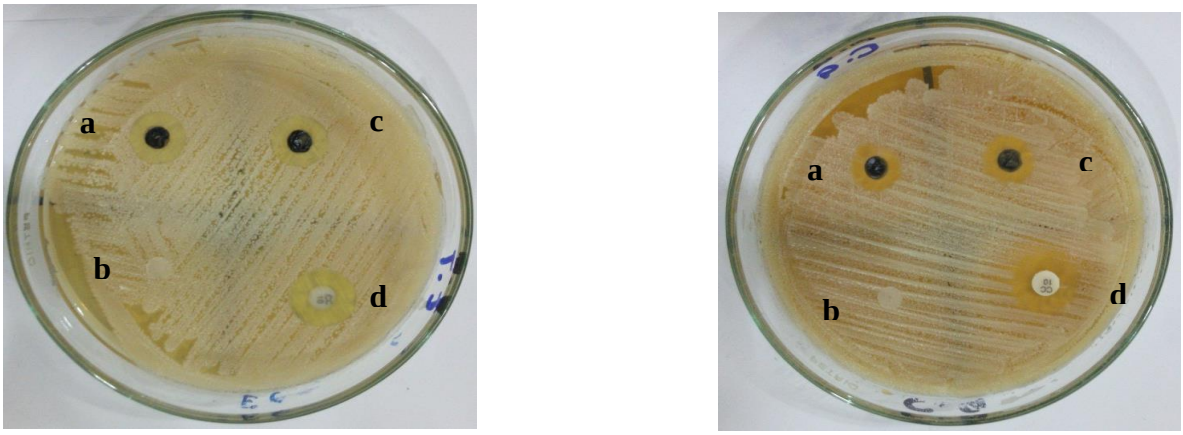


Figure 3: Zone of inhibition of methanol crude extractof *Oscillatoria* sp. against test bacteriaPositive control (5µg/disc ciprofloxacin), B- Negative control, C- Crude extract (400 µg/disc), D- Crude extract (5 µg/disc) a- *B. anthracis* b- *P. aeruginosa* c- *E.coli* d- *S. aureus* e-*S.typphi.*



A **B**

Figure 4: Zone of inhibition of methanol crude extractof *Oscillatoria* sp. against testfungal species.(a-Methanol extract, b- Negative control, c- Methanol extract, d-Positive control (amphotericin B), A-*Oscillatoria* sp. methanol extract against, *C.tropacalis*, B-*Oscillatoria* sp. methanol extract against *C.albicans*).

The figure shows relative % inhibition of methanol extract and n-hexane extract of *Oscillatoria* sp. against test bacteria and fungi. The highest relative % inhibition was recorded (68 %) in the methanol extract against *B. anthracis*.

Table 2a:Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Oscillatoria* sp. crude extractagainst test bacteria species.

Test microorganisms Bacteria species	Antimirobial susceptible test	
	MIC (µg/ml)	MBC (µg/ml)
S. aureus	156.25	625
P. aeruginosa	312.50	1250
S. typhi	312.50	1250
E. coli	312.50	1250
B.anthracis	312.50	1250

Table 2b:Minimum Inhibitory Concentration (MIC) and MFC Minimum Fungicidal Concentration (MFC)of *Oscillatoria* sp. crude extract against test fungal species.

Fungi species	Antimirobial susceptible test	
	MIC (µg/ml)	MFC (µg/ml)
C.albicans	312.50	1250
C.tropicalis	625.00	2500

The methanol crude extract was subjected MIC assay and showed potent MIC and MBC property and the results were correlated with the zone of inhibition recorded by disc diffusion (Table 1). On screening the MIC activity, the lowest MIC was obtained as 156.25 µg/mL against *S. aureus* followed with 312.50 µg/mL against *P. aeruginosa*, *S. typhi*, *E. coli* and *B.anthracis*. On the other hand, methanol extract showed lower MBC against *S. aureus* and theconcentration was 625 µg/ml.

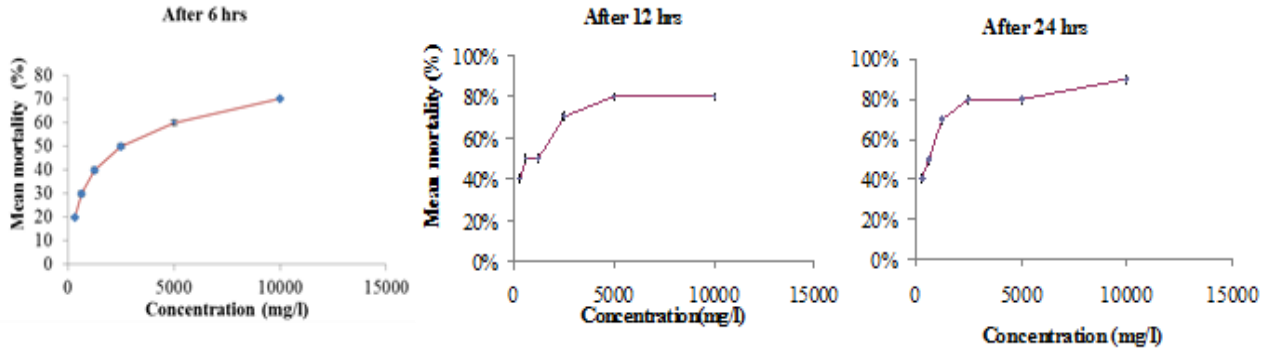


Figure 5: Mean mortality of *Artemia salina* against methanol crude extracts of *Oscillatoria* sp. After 6 hrs, 12 hrs and 24 hrs of incubation.

Mean mortality of Artemia against *Oscillatoria* sp. methanol crude extract was evaluated (figure 5). IC 50 value of *Oscillatoria* sp. methanol crude extract at 6, 12, 24 hrs recorded 2.5 g/l, 1.25 g/l and 0.63 g/l respectively. Similarly, assessment by Maruthanayagam et al., (2013) showed for *Pseudoscytonema* sp.and *Oscillatoria* sp. had weak toxicity, while the *Geitlerinema* sp. and *O. boryana* extracts exhibited stronger toxicity against *Artemia salina* with LC 50 of 32, 61 µg /ml. Further, the compounds has LC 50 values lower than <10 µg/ml are considered as very actively toxic whereas the LC50 between 100-500 µg/ml is was considered moderately toxic and LC50 values greater than 1000 µg/ml was non-toxic for Artemia assay (Babajide et al., 2010; Meyer et al., 1982).

Table 3: GC-MS analysis of the different chemical components in Methanol and hexane extracts of *Oscillatoria* sp.

No	Compounds	Retention time (min)	Area (%)
Methanol extract			
1	Methyl tetradecanoate	31.01	0.261
2	13-Tetradecynoic acid, methyl ester	34.72	0.128
3	1-Butene, 2-ethyl-3-methyl	34.90	0.134
4	Hexadecanoic acid, methyl ester	35.12	0.769
Hexane extract			
5	Bis(2-ethylhexyl hydroxypyridine oxide	22.32	0.995
6	1 2-benzenedicarboxylic acid mono(2-ethylhexyl)ester	45.82	1.581
7	Phthalic acid 6-ethyl-3-octyl heptyl ester	47.14	0.147
8	Phthalic acid dodecyl nonyl ester	48.57	0.169

Chemical components in methanol and hexane extract of *Oscillatoria* sp. were analyzed using gas chromatography-mass spectrometry method. Table 3 shows the chemical components in methanol and hexane extract. According to the mass spectroscopy reading, most of them are fatty acid compounds with more than 97% similarity.

4. Discussion

With regard to increasing human pathogenic bacterial and fungal diseases and rising antimicrobial resistance, the current antimicrobial drug development is not adequate to combat bacterial and fungal diseases. In the present study, the potential of isolated cyanobacteria isolate to produce substances inhibiting the growth of some selected human pathogenic bacteria and fungal species were evaluated.It was documented that different screening methods can lead to dissimilar results due to different sensitivities of the methods and permeability of bioactive substances into the test organisms (Pawar and Puranik, 2008). Thus, in the present study antimicrobial screening of intracellular substances, isolated from freshwater cyanobacterium *Oscillatoria* species was carried out using the agar diffusion method and broth microdilution method.

High metabolite yield was obtained in the methanol extract (60%) compared with hexane (10%) extract, this may be due to the presence of moderately polar compounds in methanol extract. A similar result was recorded by Mundt et al., 2001 for *Chlorococcus* species in methanol extract. It was foundthat high percentage of methanol extracts with positive effects against *S. aureus* indicated a

higher chance of finding antimicrobial chemicals in methanol extracts. The highest effective zone of inhibition by methanol extract of *Anabaena variabilis* against *S. aureus*, *E. coli*, *P. aeruginosa* and *S. typhi* were recorded by Kaushik et al., (2009). In the present study, similar impressed results of antibacterial and antifungal properties were found in methanol extract compared to hexane extract. Cyanobacteria belong to the Gram-negative bacteria; therefore, their metabolites have stronger activity against Gram-positive bacteria and yeasts (Mundt et al., 2001). This fact supports the assumption that these metabolites can be produced to protect cyanobacteria themselves from competing for organisms (Piccardi et al., 2000; Mundt et al., 2001; Bhadury and wright, 2004; Martins et al., 2008). Furthermore, activity against Gram-positive bacteria and rare activity against Gram-negative bacteria were documented (Cannell et al., 1988b; Moore, 1996; Kreitlow et al., 1999; Mundt et al., 2001; Svircev et al., 2008; Medina-Jaritz et al., 2011). In the present study, methanol extract showed strong antibacterial activity whereas hexane extract showed the antibacterial activity only against *S. aureus* (Table 1). Previous studies also showed poor antibacterial activity in hexane extract (Moore, 1996).

However, there are certain drawbacks of the disc diffusion method. Aspaper disc retains the active component and does not allow it to diffuse into the Muller Hinton Agar, diffusion of the active compound depends on the starch content of the agar medium, depth of the solid agar medium and diffusion capacity of the active compound. Hence, in the present study, broth microdilution assay was used to overcome the practical issues of the disc diffusion assay.

Broth microdilution assay was carried out using TTC (Tri phenyl tetrazolium chloride). Use of 2, 3, 5-Triphenyltetrazolium chloride (TTC) for determination of MIC is possible due to its ability to change the colour of the broth from light yellow to pink. When present with bacteria, TTC act as the artificial electron acceptor, which was changed from colourless to red triphenyl formazan (TPF). TTC is reduced to red formazan which is directly proportional to the viable active cells. The inhibitory effects of *Oscillatoria* sp. extract concentrations were remarkably higher against *S. aureus* and *C.albicans* than other test bacteria and fungal species tested which was indicated by the wider diameter of inhibition zones (Table 1). The correlation between relative formazan absorbance and relative colony count was more evidenced in *S. aureus* than in other tested bacteria species, especially in the low values (Table 2a), and that may be attributed to the higher activity against Gram-positive bacteria to reduce TTC into red formazan, compared to Gram-negative species.

Determination of the MIC and MBC of present extracts was apparent for effective antimicrobial property which reasonably correlates with recent records of Kaushik et al., (2009) and Pramanik et al., (2011). Kaushik et al., (2009) reported for effective antimicrobial activity against extracts of cyanobacterial for *S. aureus*, *E.coli*, *P.aeruginosa* and *S. typhi* with the lowest MIC value of 256 μg/ml. In a similar study, Pramanik et al., (2011) demonstrated that the MIC value for crude extracts of *Lyngbya*, *Phormidium*, *Oscillatoria* and *Synechocystis* against *S. aureus*, *E. coli*, *B. subtilis* was 250 μg/ml whereas 500 μg/ml for *P. aeruginosa*. Further investigation on ethyl acetate extracts of *Anabaena* sp. showed strong antibacterial activity against pathogenic *Aeromonas* species at the lowest MIC of 55.30 μg/ ml (Abdel-Raouf and Ibraheem, 2008).

The brine shrimp (*A. sauna*) lethality assay is considered a useful tool for preliminary assessment of cytotoxicity. Literature data suggested a good correlation between the brine shrimp assay and some tumour cell lines (Soils et al., 1993) as well as hepatotoxic activity (Kiviranta et al., 1991). The high IC_{50} value recorded in the present study by *Oscillatoria* extract against *Artemia* after 24 hr incubation (650 μg/ml) indicates the low cytotoxic effect of the methanol crude extract.

According to the GC-MS result (Table 3), eight compounds were identified and they were constituting 96.45% of the total component. Tetradecanoic acid and hexadecanoic acid detected in the present study is to be the common major volatile component in many other cyanobacteria species. The compounds such as tetradecanoic acid and hexadecanoic were found in both algae and plants showed anticancer, antioxidant and antimicrobial activity (Bergsson et al., 1999; Benkendorff, 2010, Galbraith et al., 1971). Unsaturated hydroxylated fatty acids and linolenic acid synthesized by cyanobacteria are involved in defence reactions against cohabitants in the biotope (Mundt et al 2003). Fatty acids are able to change the permeability of the cell membrane, interact with proteins and lipids of the cell membrane, inhibit special enzymes or form a layer around the cells. It was hypothesized by Lampe et al., (1998) that lipids kill microorganisms by leading to disruption of the cellular membrane because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. Then the chemical found in the methanol extract of *Oscillatoria* sp. is a potential organism to produce pharmaceuticals in near future.

5. Conclusion

The methanol extract of *Oscillatoria* sp. showed better antibacterial and antifungal activity against both Gram-positive and Gram-negative human pathogenic bacteria and pathogenic fungi than hexane extract. Methanol extract also showed low cytotoxicity according to the artemia bioassay. Further, GC-MS result revealed that methanol crude extract contains four fatty acid compounds and antibacterial activity may be due to the presence of fatty acid in the crude extract. An improved knowledge of composition, analysis, and properties of *Osillatoria* sp. with respect to antimicrobial compounds would assist in effort in the pharmaceutical application of this cyanobacteria.

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