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Antagonistic Activity of the isolated Cyanobacteria from Lake of Sidi Mohamed Benali against some nosocomial pathogens bacteria

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ABSTRACT

Cyanobacteria are rich source of primary and secondary metabolites which are important in biotechnology and pharmaceutical industries. The Cyanobacteria plays important role for forming the earth's oxygen atmosphere and their contribution to many other attributes important to human life. Due the importance of this groups of bacteria, the main aim of the present study was the screening and the identification of the bacterial strains belonging to the cyanobacteria, isolated from water of lake of Sidi Mohamed Benali located in the region of Sidi Bel Abbes West of (Algeria)and the evaluation of their antagonistic activity against pathogenic bacteria responsible for nosocomial infection. The antagonistic activity of the isolated, selected cyanobacteria against pathogenic bacteria strains such as Escherichia coli ATCC 25922, Salmonella typhimurium ATCC 6538, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 43300, Enterobacter aerogenes ATCC 35029, Proteus vulgaris ATCC 6896, Klebsiella pneumonia ATCC 10031 was evaluated by the using agar well diffusion method and the study of the bacterial growth in the in the absence and in the presence of the supernatant of Ps. aeruginosa ATCC 27853.Furthermore, the obtained antagonistic activity of the selected cyanobacteria, extracted by the using of different organic solvents such as ethanol, acetone, diethyl ether and methanol was compared. The obtained results showed that the diethyl ether extract of Lyngbya. sp, the methanol extract of Oscillatoria sp and the diethyl ether extract of Phormidium.sp, indicated an important antagonistic activity against Aspergilus niger and Candida albicans. Where, the methanol and the acetone extracts of Oscillatoria sp have manifested the highest antagonistic activity against S. aureus, K. pneumonia respectively. Furthermore, the investigated methanol and the acetone extracts of Oscillatoria sp yielded the highest antagonistic activity against S.aureus, K. pneumonia by methanol extract and against E. aerogenese, Acetone extract of Phormidium sp showed a high antagonistic activity against S. aureus, P. vulgaris, K. pneumonia.

Keywords: Antagonistic activity, *Oscillatoria sp, Phormidium sp, E.aerogenese*, pathogenic bacteria.

INTRODUCTION

Cyanobacteria, the blue green algae are morphologically diverse group of Gram-negative eubacteria. They are characterized by their capacity to perform biological nitrogen fixation, oxygenic photosynthesis and used as important food for other organisms [1]. Cyanobacteria are a very old group of organisms and represent relics of the oldest photoautotrophic vegetation in the world that occur in soil, fresh and marine waters and they are excellent source of vitamins and proteins [2-3]. Their survival is affected by many factors including incubation temperature, PH, kind of medium, incubation period, medium composition and light intensity [4].Cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents [5] and have been found as one of the most promising groups of organisms to be able of producing primary and secondary metabolites with diverse biological activities such as antibacterial[6], antifungal [7], antiviral [8], anticancer[9], antiplasmodial [10] and immune suppressive activities[11].

Generally, the isolation of bioactive compounds from cyanobacteria has been achieved for two main objectives. One is to discover new compounds for pharmaceutical, agricultural or bio control application. The second objective was the understanding of the interactions of individual organisms within their natural communities. For each of these purposes, it was necessary to screen new organisms [12].

The screening of cyanobacteria for antibiotics production and other pharmacologically active compounds, has received ever-increasing interest as a potential source for new drugs [13, 14].

The achieved research for the identification of the antimicrobial compounds produced by microalgae against human pathogens as well as other pathogens, has recently received considerable attention as a new source of novel antimicrobial substances [15].

Recently, literature has reported that *Spirulina subsalsa*, *Phormidium tenu* [16], *Chrococcus minor*, *Microcystis aerouginosa*[17], and *Calothrix parietina*, *Oscillatoria angustissima*[18] have been selected for their capability to produce antimicrobial agents. Ethyl acetate extract of *Spirulina platensis* consisted of heptadecane and tetradecane, which can inhibit some Gram positive and negative bacteria and *Candida albicans* [19].Furthermore, the purified Lipopeptidases from *Anabaena spp*[20]and the fatty acids, tetraamine, spermine produced by *Oscillatoria spp*.[21] have manifested an antimicrobial activity.

Therefore, the main objective of this work werethe isolation of bacterial strains belonging to the Cyanobacteria from fresh water of lake of Sidi Mohamed Benali and Sarno located in the region of SidiBel Abbes West of (Algeria)and the investigation of their antagonistic activity

Against such pathogenic bacteria responsible for nosocomial infection, where the study was limited on some filamentous cyanobacteria.

MATERIALS AND METHODS

The fresh water samples were collected in the summer period between Juin-August 2014, from various sites lakeof Sarnoand Sidi Mohamed Benali, located in the region of SidiBel Abbes(Algeria).The samples were cultivated in BG11 culture medium agar and isolated by the using two methods, such as the filtration method, employed by the using of membrane filters of nitrate de cellulose with a diameter of 0.45um and the streak plant method [22].

The isolated cyanobacteria were transferred after that on the same above described culture medium. Furthermore, the uni-algal cultures were prepared by the using of subculturing methods [23]. The isolated *Cyanobacterium* was inoculated in volume of 150 ml of BG-11 culture medium, containing 500 ml flask, incubated at temperature 25°C without shaking, for a period of 30 days, in the presence of illumination at 2000 lux with aphotoperiod of 12/12 Light/dark.

Preparation of cyanobacterial extracts

In order to prepare the cyanobacterial extracts, the cultivated isolated cultures for a period of 30 days were harvested by centrifugation at 5000 rpm for 15 min. The aqueous supernatant was collected and the algal pellet was weighted. 0.2 g dry powder was extracted for tree times with a volume of 15 ml of organic solvent such as acetone, ethanol, methanol or diethyl ether. The obtained solvent extracts were dried under reduced pressure at temperature of 40°C for methanolic extract, at 78°C for ethanolic extract, at 56.5°C for acetone extract and 34.6°C for diethyl ether extract respectively and kept at 4°C until their use for further investigation.

Microbial indicators and growth conditions

The used microorganisms in this work were Gram-negative bacterial strains such as *E. coli* ATCC 25922,*Salmonella typhimurium* ATCC 6538, *Ps. aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 6896, *Klebsiella pneumonia* ATCC 10031 and Gram-positive strains such as *Staphylococcus aureus* ATCC 43300, *Enterobacter aerogenes* ATCC 35029. The used yeast was *Candida albicans* ATCC 20408 and the investigated filamentous fungi's were *Aspergilus niger* ATCC 6275, *Aspergilus flavus* ATCC 10124.Bacterial strains and yeast were kindly provided from institute of Pasteur of Algeria, the filamentous fungi were obtained from INRAA of SidiBel Abbes(Algeria).All bacterial strains were inoculated into nutrient broth, incubated at temperature of 37°C for 24hours.The yeast and fungal strains were inoculated into glucose peptone broth, incubated at 25°C for period of 5 days.

Antibacterial and Antifungal assay

The antagonistic activities of cyanobacterial extracts were investigated against some nosocomial pathogens of Gram-positive and Gram-negative strains, yeast and filamentous fungi. The antimicrobial activity was carried out by

the using of agar-well diffusion method. Where, the introduced Petridishes with a volume of 20 ml of Muller-Hinton were inoculated with a volume of 100 µl of the 24 hours old culture broth of the investigated bacteria, adjusted at 10^6 UFC/ml and spread on the solid BG-11culture mediumand plates respectively with the help of sterile spreader. The inoculated plates were allowed to dry and sterile cork borer of diameter 8.0 mm was used to bore wells in center of inoculated agar plates.A volume of 20ml of the prepared Sabouroud culture medium agar was inoculated with a volume of 100 µl of 5 days old culture on glucose peptone broth culture adjusted to10^8 UFC/ml of tested fungi and yeast. The indicated antagonistic microorganisms were spread on agar plates with sterile diffusion. Wells (8mm) were made and filled with a volume of 50µlof each extract. The Petridishes were placed for 2hours at 4°Ctill the diffusion of the released metabolites into the medium [24], incubated at temperature 37°C for 24hours for bacteria, at 30°C for 3 days for fungi and yeast. The lecture of the obtained results was recorded by the measuring of diameter of the inhibition zone in mm and antimicrobial activity of cyanobacteria was compared with standard antibiotic (penicilline) and fungicide (nistatine).

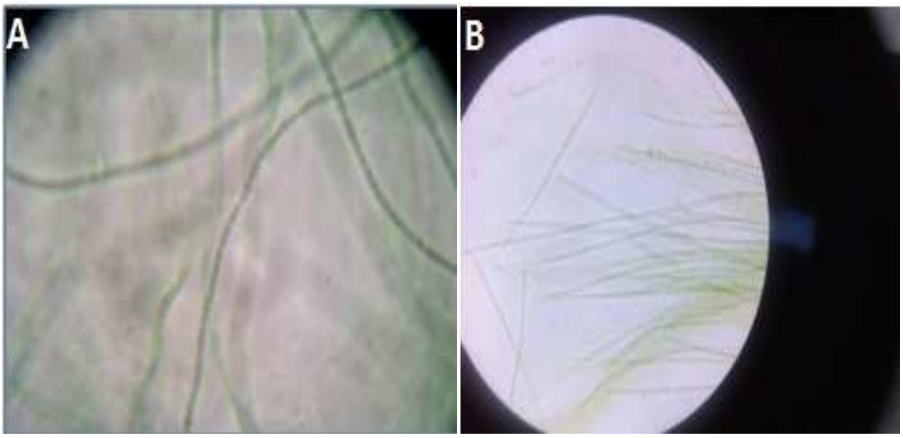
RESULTS

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including cyanobacteria [16]. Due the importance of this groups of bacteria, the main aim of the present study was the screening and the identification of a large bacterial strains belonging to the cyanobacteria, isolated from water of lake of Sidi Mohamed Benali located in the West region of SidiBel Abbes(Algeria)and the evaluation of their antagonistic activity against pathogenic bacteria, which were responsible for nosocomial infection and the study was limited on some filamentous cyanobacteria.For this purpose, the antagonistic activity of four isolated, selected Cyanobacteria against pathogenic bacteria strains such as *E. coli* ATCC 25922, *S.typhimurium* ATCC 6538, *Ps. aeruginosa* ATCC 27853, *Sta.aureus* ATCC 43300, *E. aerogenes* ATCC 35029, *P. vulgaris* ATCC 6896, *K.pneumonia* ATCC 10031 has been investigated by the using agar well diffusion method.

The used external morphology such as the microscopic observation according to the described methods by Anagnostidis Komarek and Sant´Anna [25-26-27] for the identification of the four isolated Cyanobacteria strains, indicated their membership to the family of Cyanobacteriaceae, Oscillatoriaceae, Nostocaceae respectively (Table 1, Figure 1).

Table 1:The identification of the isolated Cyanobacteria strains from fresh water samples of lake of Sarnoand Sidi Mohamed Benali by the use of the external morphology microscopic observation

Strains	Family	Sites of collection
Oscillatoriasp	Oscillatoriaceae	Water of lake of Sidi Mohamed Benali
Anabeanaspherica	Nostocaceae	Sarno dam.
Lyngbyasp	Oscillatoriaceae	Sidi Mohamed Benali lake
Phormidiumsp	Oscillatoriaceae	Sidi Mohamed Benali lake



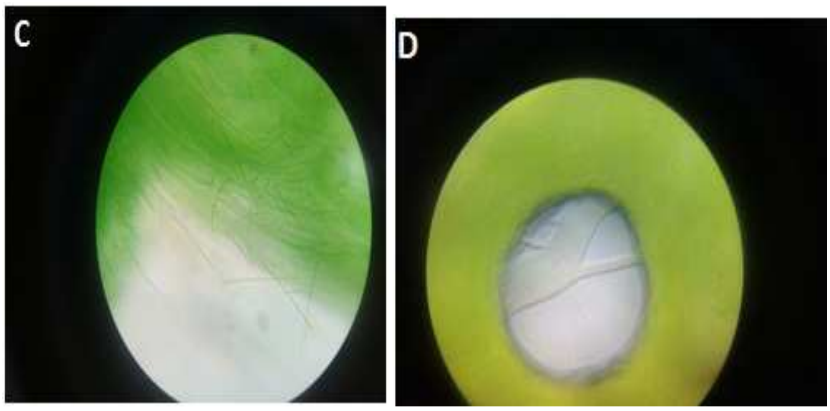


Figure:The identification of the four isolated Cyanobacteria from fresh water of Lake Sidi Mohamed BenaliGx1000, A-*Anabeanaspherica*, B-*Phormidiumsp*, C-*Oscillatoriasp*, D-*Lyngbyasp*by the use of the microscopic observation

Antibacterial activity

In the present study, the antagonistic activity of the extracted crude of cyanobacteria (methanolic, ethanolic, aceton, diethyl ether) against pathogenic bacteria strains such as *E. coli* ATCC 025922, *S.typhimurium* ATCC 6538, *Ps.aeruginosa* ATCC 27853, *Sta.aureus* ATCC 43300, *E. aerogenes* ATCC 35029, *P.vulgaris* ATCC 6896, *K. pneumonia* ATCC 10031 has been investigated by the using of agar-well diffusion method, based on the measure of the formed diameter of the zone inhibition.

From the study, it's clear that the diameter of the inhibition zone depends mainly on the type of the algal species, type of the used solvent and the tested bacterial and the fungal organisms.

The obtained results (table 2) indicated clearly that methanol and acetone extracts of *Oscillatoriasp*yielded the highest antagonistic activity against *S.aureus* (35mm) and *k.pneumonia*(33mm) by methanol extract and against *E.aerogenese* (29mm) by acetone extract. Whereas the ethanol extract showed moderate activity against all bacterial species except *P. vulgaris*, *E.aerogenes* and *E.coli*, with highest value in case of *K.pneumonia* (9 mm) and *S.typhimurium*(7 mm).At the same time, the results of ethanol and methanol extract of *Lyngbyasp* revealed their antibacterial effect against all the investigated bacteria except *E.coli* with the highest value in case of *k. pneumonia* (16 mm) by methanol extract. Methanol extract of *Anabeana spherica* had antibacterial effect toward *E.coli* (10 mm), *S.aureus* (15 mm) and *E.aerogenes* (09 mm),at the same time acetone extract had manifested a feeble effect toward *P.aeroginosa* (5mm) and *S.typhimurium* (4mm). Furthermore, acetone extract of *phormidium sp* showed a has indicated a high antibacterial activity against *S.aureus* (26mm), *P.vulgaris*(25mm) and *K.pneumonia*(20mm).Where, the investigated of the diethyl ether extract of cyanobacteria was recorded any antagonistic activity with except the crude extract of *Phormidiumsp* against *S. typhimurium* (15mm), *K. pneumonia* (9 mm) with a maximal diameter of inhibition zone of 15, 9 mm respectively.

Table 2: Illustration of the antagonistic activity of the extracted crude of cyanobacteria (methanolic, ethanolic, aceton, diethyl ether) against pathogen bacterialby the produced zone inhibition (mm)

Strains	Used solvent	*E.coli*	*S. typhimurium*	*Ps. aeroginosa*	*S. aureus*	*E. aerogenes*	*P. vulgaris*	*K. pneumonia*
Oscillatoriasp	Methanol	*R*	*2*	*2*	*35*	*3*	*3*	*33*
	Ethanol	*R*	*7*	*9*	*8*	*R*	*R*	*9*
	Aceton	*R*	*2*	*2*	*3*	*29*	*2*	*2*
	Diethylether	*R*	*R*	*R*	*R*	*R*	*R*	*R*
Lyngbyasp	Methanol	*2*	*6*	*4*	*4*	*5*	*6*	*16*
	Ethanol	*2*	*5*	*8*	*7*	*7*	*4*	*4*
	Aceton	*R*	*4*	*4*	*3*	*R*	*R*	*R*
	Diethylether	*R*	*2*	*2*	*3*	*R*	*2*	*R*
Phormidiumsp	Methanol	*R*	*4*	*4*	*R*	*R*	*R*	*6*
	Ethanol	*R*	*2*	*6*	*R*	*5*	*4*	*R*
	Acetone	*R*	*2*	*3*	*26*	*R*	*25*	*20*
	Diethylether	*R*	*15*	*2*	*3*	*R*	*R*	*9*
Anabeanaspherica	Methanol	*10*	*6*	*7*	*15*	*9*	*7*	*3*
	Ethanol	*2*	*4*	*4*	*7*	*8*	*8*	*7*
	Acetone	*2*	*4*	*5*	*R*	*4*	*R*	*R*
	Diethylether	*R*	*R*	*R*	*R*	*R*	*R*	*R*

Antifungal activity

In order to explore the effect of the produced molecules by Cyanobacteria, the study of the antifungal activity of the extracted crude (methanolic, ethanolic, aceton, diethyl ether)of Cyanobacteria againstpathogenic fungisuch as *A. niger, A. flavus and C. albicans* has been investigated by the using of agar-well diffusion method. The obtained results (table 3) indicated that the extracts (methanolic, ethanolic, aceton, diethyl ether) of Cyanobacteria has manifested an excellent antifungal activity against pathogenic fungi followed by diethyl ether and methanol with the highest value of the produced inhibition zone by *Candida albicans* (45mm). The tested antifungal activity of the diethyl ether extract of *Lyngbya.sp, Phormidium.sp* against *Aspergilus niger* indicated the diameter of zone inhibition of 40, 40 mm respectively, whereas the used methanol extract of *Oscillatoria.sp* manifested a value of 18mm.Furthermore, *A.flavus* and *C.albicans*has manifested an important resistance against the presence of the acetone extract of the cyanobacteria with the exception that the presence of the acetone extract of *Phormidium.sp* indicated a feeble antifungal activity against *C.albicans*(6mm). Where, the investigated methanol extract of cyanobacteria showed any antagonistic activity against the tested fungal strains.

Table 3: Antifungal activities of different cyanobacteria extracts.

	Used solvent	*A.niger*	*A.flavus*	*C.albicans*
Oscillatoria sp	Methanol	18mm	R	4mm
	Ethanol	R	R	R
	Acetone	R	2mm	R
	Diethylether	5mm	R	3mm
Lyngbya sp	Methanol	3mm	R	R
	Ethanol	R	R	R
	Aceton	R	2mm	4mm
	Diethylether	R	R	45mm
Phormidium sp	Methanol	3mm	R	R
	Ethanol	R	R	R
	Acetone	R	2mm	6mm
	Diethylether	R	R	40mm
A.spherica	Methanol	3mm	R	R
	Ethanol	R	R	R
	Aceton	R	3mm	R
	Diethylether	R	5mm	R

DISCUSSION

A few studies have reported on the ability of members belonging to the genus Cyanobacteria toproduce a various secondary metabolites, with important potential of antagonistic activity against pathogenic bacteria.

In the present study,the antagonistic activity of the extracted crude of cyanobacteria (methanolic, ethanolic, aceton, diethyl ether) against pathogenic bacteria such as *E. coli* ATCC 25922, *S.typhimurium* ATCC 6538, *Ps.aeruginosa* ATCC 27853, *St.aureus* ATCC 43300, *E.aerogenes* ATCC 35029, *P.vulgaris* ATCC 6896, *K. pneumonia* ATCC 10031 has been investigated by the using of agar-well diffusion method. The obtained results (table 2) indicated that the bacterial growth of *S.aureus, k.pneumonia, E.aerogenese, P.vulgaris, S.typhimurium, P. aeruginosa* was considerably inhibited in the presence methanol and acetone extracts of *Oscillatoriasp,* with a high value of the produced diameter of zone inhibition of (35, 33, 29, 25, 15, 9 mm) respectively. Furthermore, thestudy of the antifungal activity of the extracted crude of cyanobacteria (methanolic, ethanolic, aceton, diethyl ether) against such pathogenic fungi such as *C.albicans* and *A. niger* has drastically reduced the fungal growth, with a high value of the produced diameter of zone inhibition of (45, 18 mm) respectively. Whereas, the bacterial and fungal growth of *E.coli* and *A.flavus* was manifested an important resistance against the extracted crude of cyanobacteria (methanolic, ethanolic, aceton, diethyl ether).The presence of extracted crude of *A.spherica* by the culture *E.coli* and *A.flavus* induced the formation of the diameter of zone inhibition of (10, 5 mm) respectively. The highest value of inhibition zone (45mm) was obtained in the presence of the extracted crude of diethyl ether of *Lyngbia.sp* against *C.albicans*, and the minimum inhibition zone (2mm)in the presence of the acetone extract of *A.spherica* against *S.typhimurium*.In conclusion and based on the obtained results, the inhibition of the bacterial and fungal growth was depended on the following parameters such as the investigated genus of the Cyanobacteria and the used organic solvent and the tested pathogenic bacteria and fungi. Furthermore, the four isolated from water of lake of Sidi Mohamed Benali located in the West region of SidiBel Abbes(Algeria) were investigated for their antagonistic activity against pathogenic bacteria, which were responsible for nosocomial infection.

Regarding the effect of the extracted crude of cyanobacteria, the results showed further that methanol and acetone are the best used solvents for extraction of the produced molecules, responsible for the antagonistic activity of *Oscillatoria*, A similar results has been reported by Madhumathi[17], where the acetone extract of *Oscillatoria*

latevirens manifested a high antagonistic activity against *St.aureus* and *Str.mutans*[28]**.** Furthermore, the methanol extract of *Oscillatoriasp* induced highest antagonistic activity against *E.coli, K.pneumonia* and *S.mutans*. The obtained results indicated further that the methanol extract induced the largest inhibition zone by *S.aureus* and *K.pneumonia* and the acetone extract of *Phormidium*.sp has considerably inhibited the bacterial growth of *S.aureus, K.pneumonia, P.vulgaris,* with a larger inhibition zone. Where, the bacterial growth of *E.aerogenese, P. aeruginosa* manifested only a feeble diameter of zone inhibition.

researchers have reported that *Phormidium tenue*; has manifested an excellent antagonistic activity by *St. aureus, S.typhi* and *E. coli* [16], where the acetone was the best used organic solvent for extracting antibacterial molecules produced by *Phormidium corium* [17], and the methanol extract from *Phormidium sp* induced the inhibition of both Gram positive and Gram negative bacteria [24].In further studies, researchers have reported that the methanol and acetone extracts of *Lyngbya sp* indicated a maximum zone inhibition against *E.coli, S.mutans, S.aureus* and *K.pneumonia*[28]. Furthermore, the investigated methanol and acetone extracts from *Lyngbya Aestuarii* showed an important antagonistic activity against *St.aureus, S.typhi*[29], Whereas, the methanol and acetone extracts from *Spirulina platensis* indicated more similarity inhibition zone against *St.aureus* and *S.typhimurium*[30].The used acetone was the best organic solvents for the extraction of the antibacterial molecules from *Lyngbyam artensiana*[17]. The methanol extract of *Lyngbya.sp* showed a maximum inhibition zone against *Proteus vulgaris, Ps. aeruginosa* and *St. aureus*. Furthermore, the *Anabeana sp* has manifested important antibacterial properties against *St.aureus, E. coli, Ps.aeruginosa, Kl. pneumonia* and *S.typhi*[31]. The used acetone and methanol extracts of *Anabeana spherica* indicated an excellent antagonistic activity against *E.coli, Enterococcus facium, St.aureus* and *S.senftenberg*[33]. Furthermore, the methanol extract of *Anabeana spherica* showed antagonistic activity against *E.coli, S.aureus, P.vulgaris* with the highest obtained value by *S.aureus*[32]. For antifungal activity**,** Cyanobacteria are known to produce antifungal compounds, which have been studied by various research groups and indicated promissing results by the inhibition of the pathogenic fungal growth, which were responsible for nosocomial infection. Tiwari and their co-workers have reported about the wide assay for the exploitation of Cyanobacteria as agents to prevent the pathogenic bacterial and fungal growth[34].The acetone extract of *Phormidium corium*, methanol extract of *Lyngbyamartensiana* and diethyl ether extract of *Microcystis aeruginosa* manifested the largest inhibition zone by fungal pathogenic [17]. Where the methanol extract of *Oscillatoria salina* and *Phormidiumtenue* showed an important antifungal activity against *Fusarium solani, Rhizoctonia solani*[16, 35].Furthermore, Sakthivel[16] has reported that cyanobacterial strains *Oscillatoria, Anabaena, Nostoc, Nodularia, and Calothrix* manifested antifungal activity againstseven pathogenic fungi causing diseases. Where, the diethyl ether extract of *Lyngbya.sp,* methanol extract of *Oscillatoria* sp, the diethyl ether extract of *Phormidium.sp*, showed maximum inhibition zone by *Aspergilus niger* and *Candida albicans.*

CONCLUSION

From our results, it was concluded that the extracts of some cyanobacterial showed antagonistic activity against pathogenic bacterial and fungal growth, which were responsible for the nosocomial infection. It would be of interest to find out which functional group is responsible for the antagonistic activity and also whether any of them is a novel compound with antimicrobial activity which would make it a promising candidate for the production of new antimicrobials.

Therefore, it is suggested a further studies involving the characterization of the natural product responsible for the antibacterial activities, where the basic knowledge may useful in various applications such as pharmaceutics and agricultures, and for farther investigation.

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