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RESEARCH ARTICLE

Evaluation of Some Extra-and Intracellular Cyanobacterial Extracts as Antimicrobial Agents

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Abstract

Increasing the microbial resistance to the current drugs is the one of the major problems nowadays. Therefore, there have been tremendous efforts towards finding new metabolites for the development of new antimicrobial drugs. Cyanobacteria were selected to investigate the antimicrobial activity against some pathogenic and toxigenic microorganisms. Water samples were collected from some water plants and different sites of River Nile during the period from December 2011 to August 2012. Many species of cyanobacteria were detected in the River Nile. Among them, three representative species were isolated and identified as *Fischerella*, *Oscillatoria* and *Anabaena*. Cyanobacterial mass, filtrates and organic extracts from each of it were examined for inhibitory activity against seven tested microorganisms; tow Gram-positive bacterium (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 33018) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhimurium* ATCC 14028) and two fungi included one strain as molds; *Aspergillus niger* nrrl 326 and one strain as yeast; *Candida albicans* ATCC 1023.The methanol extract showed more potent activity than hexane extract. The mehanolic extract from mass of *Oscillatoria* scored a high inhibitory effect against *E. coli*, *Ps. aeruginosa*, *B. cereus* and *Sta. aureus*. Generally, the broth microdilution assay gave minimum inhibitory concentrations (MIC) values ranging from 0.016 to 0.5 µl µl $^{-1}$.

The antibacterial activity of methanol extract from both cyanobacterial filtrate and mass was decreased or completely lost after boiling for 10,20,30 min or pasteurization at 80^0 C/15 min. On the other hand, the activity was stable or enhanced after previous heat treatments.

INTRODUCTION

Increasing the microbial resistance to the obtainable drugs and spreading the multi-drug resistant microorganisms (MDROs) as methicillin resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumonia* (PRSP), vancomycin resistant *Enterococcus* (VRE), multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB) and *Pseudomonas aeruginosa* indicate the loss of conventional antibiotics efficiency. These factors lead to incessant search for discovery of new antimicrobial agents from new sources because there is a large number of unexploited microbes, which produces potent bioactive compounds. In this respect, the natural products from cyanobacteria are most likely to offer newer source of antibiotics (Kumar, 2014).

Cyanobacteria (blue-green algae) are a group of extraordinary diverse Gram-negative prokaryotes that originated 3.5 billion years ago (Kaushik *et al*., 2009). They are characterized by their capacity to perform biological nitrogen fixation and oxygenic photosynthesis. Cyanobacteria represent an exceptionally diverse but highly specialized group of microorganisms adapted to various ecological habitats. They can be found in terrestrial,

glaciers, marine, brackish and fresh water environments and they are very resistant to extreme environmental conditions. For example, they were reported to tolerate the high temperature up to 50ºC. (Malathi *et al*., 2014). They are considered as a main component of phytoplankton in many freshwater and marine ecosystems (Dixit and Suseela, 2013).

Cyanobacteria produce one or a range of bioactive compounds, which are potentially rich source of a vast array of products with applications in feed, food, nutritional, cosmetic and pharmaceutical applications (Tan, 2007). Due to their high chemical stability and water solubility, these compounds have important implications. In this respect, Gerwick *et al*. (2008) found that secondary metabolites extracted from cyanobacteria were from the members of oscillatoriales (49 %), followed by nostocales (26 %), chroococcales (16 %), pleurocapsales (6 %) and stigonematales (4 %). Cyanobacteria such as *Anabaena*, *Nostoc*, *Microcystis*, *Lyngbya*, *Oscillatoria*, *Phormidium* and *Spirulina* produce variety of high value compounds such as carotenoids, fatty acids, lipopeptides, polysaccharides and other bioactive compounds. Also, Ghasemi *et al*. (2003) found that *Fischerella* species had ability to produce broad-spectrum of antimicrobial substance.

Most species of cyanobacteria are known to produce bioactive compounds with diverse biological activities in two ways either within the cell biomass *i.e.* intracellular metabolites or towards the environments *i.e.* extracellular metabolites. Generally, these activities may include cytotoxic, anticancer, antimalarial, anticardiotoxic, immunosuppressive, hypocholesterolemic, antihepatoxic, antialgal, antibacterial, antifungal and antiviral activities (Noaman *et al*., 2004, Dahms *et al*., 2006, El-Sheekh *et al*., 2008, Kaushik *et al*., 2009, Malathi *et al*., 2014 and Mandal and Rath, 2015).

The biomass of cyanobacteria shows bioactivity when extracted with organic solvents. Methanolic extracts from *Tychonema bourrellyi*, *Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii* were antibacterial (Østensvik *et al*., 1998). The methanolic extract of *Chroococcus dispersus* has both antifungal and antituberculosis activities (Ghasemi *et al*., 2007). Bioassays of methanolic extracts from the genera of *Anabaena* and *Nostoc* were found to be antifungal and antibacterial (Drobac-Åik *et al*., 2007). Methanolic extracts of *Oscillatoria* sp. showed inhibition against fungal pathogens, followed by extracts in n-propanol, petroleum ether and water (Pawar and Puranik, 2008). Extractions of bioactive compounds from *Phormidium* sp. in different solvents (hexane, ethanol and water) were found to have antifungal and antibacterial activities (Meizoso *et al*., 2008). Methanolic extract of *Fischerella* sp. showed antibacterial activity (Asthana *et al* .,2006).

The aim of this work is to test the antimicrobial activity of extra and intracellular metabolites extracted from some cyanbacterial species isolated from Egypt. Also, the thermal stability of such active metabolites was evaluated to apply them as biopreservatives in food stuff.

Materials and Methods

1. Water samples collection

A total of water samples (51 samples) were collected from different drinking water treatment plants and Nile water in Monib (6 samples), Gizerat Eldahab (1 sample), Abo Elnomrose (2 samples), Giza (31 samples), Elramad (1 sample), Sakya (1 sample), Hawamdia (3 samples), Om Elmasreen (4 samples). One sample was collected from the Giza Zoo and another one from Faculty of Agriculture, Cairo University. The treated and untreated water samples were represented. The samples were collected during the period from December 2011 to August 2012. All samples were kept in ice box to transfer to the lab and kept refrigerated at 4°C for not more than one day until examined**.**

2. Isolation of cyanobacteria

The liquid enrichment cultures were prepared from different water samples. Twenty five ml of water samples were aseptically added to 100 ml of Allen and Arnon broth (Allen and Arnon., 1955) in 250 conical flasks. The inoculated flasks were incubated at 30˚C under continuous illumination, with Philips Fluorescent white lamps, at a relatively low light intensity (400 -500 lux).

3. Purification of isolated cyanobacteria

The axenic cultures of cyanobacteria were obtained applying two techniques:

a. Several successive transfers

The isolated cyanobacteria, which had been previously growing in flask containing Allen and Arnon liquid medium, were successively subcultured several times on the same medium and incubated for 3 - 4 weeks at 30˚ C until the healthy and homogenous culture were obtained.

b. Single filament isolation

This technique depends on gliding movement and phototaxis of cyanobacteria. The nonaxenic cultures of cyanobacteria were grown on Allen and Arnon agar in Petri dishes to examine the ability of cyanobacterial filaments to grow and swim toward a single source of light to pick a single filament of cyanobacteria. Once the single filament

moved a sufficient distance on Allen and Arnon agar, it was removed under sterilized conditions and placed into a separate flask contained fresh liquid Allen and Arnon medium.

All pure isolates were maintained under photoautotrophic growth conditions in Allen and Arnon medium (Guillard, 1973).

4. Identification of isolated cyanobacteria

After wet mount preparation, the cyanobacteria morphotypes such as filamentous nature, size, shape of vegetative cells, presence of heterocyst and akinetes were identified and photographed using light microscope (Rippka *et al*.,1979).

5. Studying the antimicrobial activity of cyanobacteria

a. Indicator microorganisms

The microorganisms used as indicators to assay the antimicrobial activity of cyanobacteria were obtained from Cairo University Research Park. These microorganisms represented G^+ bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 33018), G^- bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeroginosa* ATCC 9027 and *Salmonella typhimurium* ATCC 14028) and fungi. The fungal strains included one strain as molds; *Aspergillus niger* nrrl 326 and one strain as yeast; *Candida albicans* ATCC 1023.

The strains were maintained on agar slants at 4°C. Ten milliliters of nutrient broth were inoculated with a loopful of each bacterial strain. The inoculated media were then incubated at the optimum temperature for each bacterial strain (37°C for *Staph. aureus, Sal. typhimurium, E. coli* and *Ps. aeroginosa*, and 30°C for *B. cereus*) for 24 hours to enhance the growth. The same procedure was used to activate the growth of fungal strains using glucose broth and incubation at 30°C / 24 h for *C. albicans* and 3 days for *A. niger*.

b. Screening of cyanobacteria for the antimicrobial activity

The antimicrobial activity of isolated cyanobacteria was tested by the disc diffusion assay (Bauer *et al*., 1966). The disc diffusion or Kirby-Bauer disc method is a commonly used assay for microbial susceptibility. In this method, the antimicrobial substance-impregnated discs are placed on the agar surface inoculated with the test microorganism. The discs release antimicrobial substance into the surrounding medium and an inhibition zone occurs if the microorganism is susceptible to this substance.

The antagonistic effect of cyanobacteria was studied for 30, 45 and 60 days old broth cultures of cyanobacteria**.** Before testing, the cyanobacterial mass was removed using filter paper, followed by filtration of cyanobacteria-free supernatant (extracellular extract) through a cellulose acetate membrane filter with pore size 0.22 µm. The resulting material is considered as a crude cyanobacterial filtrate.

To carry out the disc diffusion assay, agar medium (1.5% agar) was inoculated with 10% v/v of bacterial or fungal culture broth. The sterile paper disc with a diameter of 5 mm was saturated with crude cyanobacterial filtrate and placed on the inoculated agar medium. The plates were incubated at the optimum temperature for each indicator strain and tested after 24h for tested bacteria and yeast and after 3-5 days for tested mold. Growth inhibition was scored positive in presence of a detectable clear zone around the disc.

6. Successive extraction of cyanobacterial filtrate and mass

The cyanobacterial mass and cyanobacteria – free supernatant obtained from forty five days old broth culture were extracted successively with three different organic solvents which increasing polarity starting with hexane, methanol and water (Cannell *et al*., 1988).

Cyanobacteria – free supernatant and corresponding mass were extracted by shaking with hexane (5 ml g^{-1} wet weight for mass and equal volume for free supernatant) for 2 h, centrifuged and the supernatants were collected. The remaining pellet was further extracted in the same manner with methanol and then water. Each extract was tested for its antagonistic effect as mentioned before.

7. Determination of the minimum inhibitory concentration (MIC)

Based on the results of testing the antagonistic effect of solvent extracts, the antimicrobial activity of methanol extract was quantified through determination of MIC, which was determined as the lowest concentration of the methanol extract inhibiting the visible growth of each sensitive microorganism. The broth dilution method was applied to determine the MIC. The extract was filtered and serially diluted two fold using microtiter plates. The final volume in each well was 200 µl.

8. Thermal stability of antibacterial activity of methanol extract

The thermal stability of methanol extract activity was determined by heating the extract at 100°C for 10, 20 and 30 min, and by pasteurization at 80°C for 15 min. The residual activity was evaluated applying the broth dilution method.

Results and discussion

1. Isolation of cyanobacteria

Microscopic examinations of the original sample showed low number of cyanobacteria belonging to different species. This might be attributed to lack of some trace elements required for growth of cyanobacteria. Allen and Arnon medium (Allen and Arnon., 1955) was used to enhance their growth in water samples. Result in Table (1) indicate that the presence of cyanobacteria was more pronounced in May and June 2012 when the temperature ranged between 28 and 35^0 C in Sakya and Monib B1 , B2 and A3 samples. Table (1) represents the relative occurrence of cyanobacteria in different water samples.

Meanwhile, data in Table (1) revealed the absence and / or weak occurrence of cyanobacteria during winter season i.e. December, January and February samples. This is most lively due to the climate change scenarios in rivers and lakes that experience increased/decreased temperature, longer periods of thermal stratification, modified hydrology and altered nutrients loading. Such environmental drivers could have substantial effects on freshwater phytoplankton species composition and biomass, potentially favoring cyanobacteria over other phytoplankton. (Carey *et al*., 2012).

2. Identification of cyanobacterial isolates

A total of 14 cyanobacterial isolates were purified and identified morphologically according to Rippka *et al.* (1979). Microscopic images of the cyanobacteria observed in this study are shown in Figs (1 and 2). The purified isolates comprised three genera of cyanobacteria; *Anabaena* (one isolate), *Fischerella* (3 isolates) and *Oscillatoria* (one isolate).

***Anabaena* sp**. filamentous heterocystous cyanobacteria. Heterocysts are intercalary or terminal; and akinetes is variable. Vegetative cells are ovoid or cylindrical. Reproduction by random trichome breakage, or by germination of akinetes.

***Oscillatoria* sp**. is a filamentous non heterocystous cyanobacteria , cells composing trichome are disc-shaped and not separated by deep constriction. Reproduction by trichome breakage. trichome motile, either not ensheathed or thinly sheathed . They do not produce heterocysts or akinetes. Cells do not contain gas vacuoles.

***Fischerella* sp**. is a filamentous heterocystous cyanobacteria divide in more than one plane. The hormogonia composed of small cylindrical cell which enlarge and become rounded, heterocysts develop exclusively in an intercalary position. Cells in the mature trichome divid in more than one plane to produce apartly multiseriate trichome with lateral uniseriate branches. Heterocyste in the primary trichome are terminal or lateral. Hormogonia are produced from the ends of trichomes or from lateral branches

3. Antimicrobial activity of isolated cyanobacteria

The inhibitory spectrum of the crude cyanobacterial filtrate (extracellular extract), obtained from 30, 45 and 60 days old broth cultures of 3 cultures of *Fischerella* sp.(Ha, Mb, Gd), one culture of *Anabaena* sp.(Sk) and one culture of *Oscillatoria sp*.(Mb), was tested against 7 indicator microorganisms (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 33018, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028, *Aspergillus niger* nrrl 326 and *Candida albicans* ATCC 1023).

Generally, no antifungal effect was recorded against both *Aspergillus niger* nrrl 326 and *Candida albicans* ATCC 1023.

The extracellular extract of *Anabaena* sp. SK isolates had antibacterial activity against all tested bacteria (Table, 2). Generally, the diameter of the inhibition zone ranged from 6.0 to 8.0 mm. Also, the results show there is no noticed difference between the antibacterial activity of the *Anabaena* sp. isolates with different culture ages.

Regarding to *Fischerella* isolates, the results indicate that *Sta. aureus* was resistant to the extracellular metabolites extracted from both of *Fischerella* sp. 1Mb, *Fischerella* sp.2Gd cultures and sensitive for *Fischerella* sp. 3Ha culture, while *S. typhimurium* was resistant only to the culture filtrate of *Fischerella* sp. 1Mb (Table, 2). The filtrates of *Fischerella* sp.2Gd and *Fischerella* sp.3Ha cultures were considered to be the most effective than the corresponding of *Fischerella* sp.1Mb, since *Fischerella* sp. 2Gd has antibacterial activity against all tested bacteria except *S. aureus* with the largest inhibition zone (Ø, ranged between 7.0 and 8.0 mm). and extracellular extract of *Fischerella* sp. 3Ha. had antibacterial activity against all tested bacteria. On the other hand, no remarkable differences between the antibacterial activity of the *Fischerella* isolates with different culture ages were found. While, *Oscillatoria* sp. Mb filtrate has antibacterial effect only against *E. coli* and *P. aeroginosa* with inhibition zone of 7.0 mm. (Table, 2).

Table 1. Relative occurrence of cyanobacteria in tested water samples

Site & Time	**Monib (B1)** June 2012	**Monib (B2)** June 2012	**Monib (B3)** June 2012	**Monib (A1)** June 2012	**Monib (A2)** June 2012	**Monib (A3)** June 2012	**Gizerat Eldahab** June 2012	**Abo Elnomrose 1** August 2012
	+++	+++	-	+	-	+++	+	+
Site & Time	**Abo Elnomrose 2** August 2012	**Elramad** July 2012	**Sakya** May 2012	**The Zoo** July 2012	**Fac. of agri. (water garden)** Mars 2012	**Hawamdia 1** Feb. 2012	**Hawamdia 2** May 2012	**Hawamdia 3** May 2012
	-	+	+++	+	-	+	++	+
Site & Time	**Om Elmasreen Madbah st. 1** August 2012	**Om Elmasreen Madbah st. 2** August 2012	**Om Elmasreen Madbah st. 3** August 2012	**Om Elmasreen Madbah st. 4** August 2012	**Giza (B1)** Dec. 2011	**Giza (B2)** Dec. 2011	**Giza(B3)** Dec. 2011	**Giza(B4)** Dec. 2011
	++	-	+	+	-	+	+	-
Site & Time	**Giza (B5)** Dec. 2011	**Giza (B6)** Dec. 2011	**Giza (B1)** Jan. 2012	**Giza (B2)** Jan. 2012	**Giza (B3)** Jan. 2012	**Giza (B4)** Jan. 2012	**Giza (B5)** Jan. 2012	**Giza (B1)** Feb. 2012
	+	-	-	-	-	-	-	-
Site & Time	**Giza (B2)** Feb. 2012	**Giza (W1)** Jan. 2012	**Giza (W2)** Jan. 2012	**Giza (A1)** Dec. 2011	**Giza (A2)** Dec. 2011	**Giza (A3)** Dec. 2011	**Giza (A4)** Dec. 2011	**Giza (A5)** Dec. 2011
	-	-	-	+	+	-	-	+
Site & Time	**Giza (A6)** Dec. 2011	**Giza (A1)** Jan. 2012	**Giza (A2)** Jan. 2012	**Giza (A3)** Jan. 2012	**Giza (A4)** Jan. 2012	**Giza (A5)** Jan. 2012	**Giza (A1)** Feb. 2012	**Giza (A2)** Feb. 2012
	+	-	-	-	-	-	+	+
Site & Time	**Giza (A3)** Feb. 2012	**Giza (A4)** Feb. 2012	**Giza (A5)** Feb. 2012					
	-	+	-					

+++, good; ++, moderate; +, weak; -, no growth; B, before water plant; A, after water plant; W, from water plant

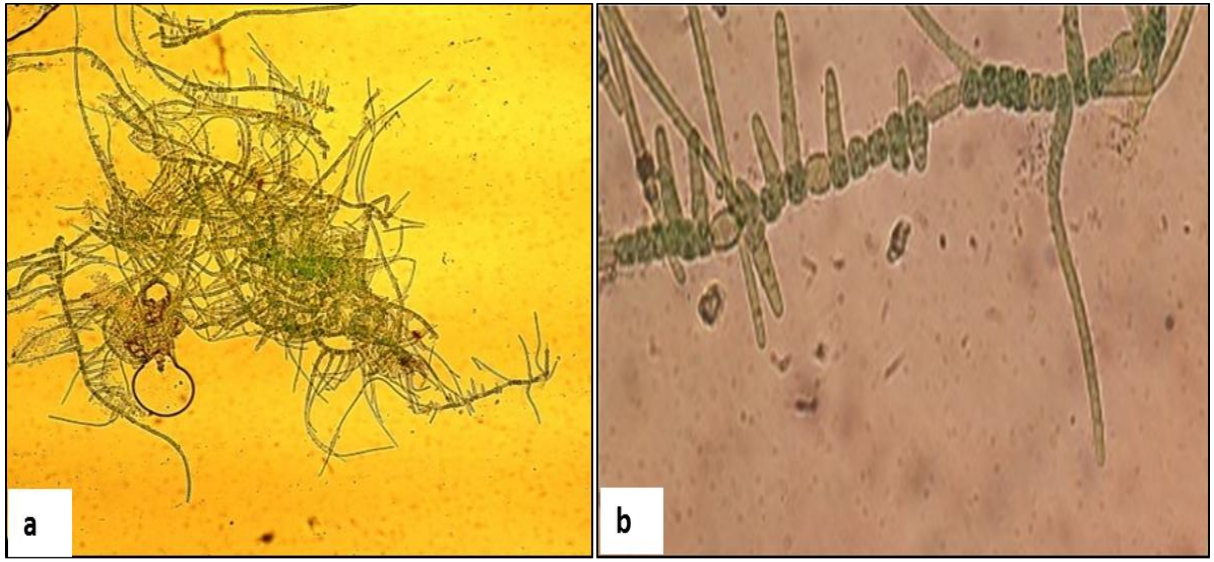

Fig.1. Light micrograph of 15 days old Culture of *Fischerella* sp. culture isolated from Hawamdia (a: 10x, b: 60x).

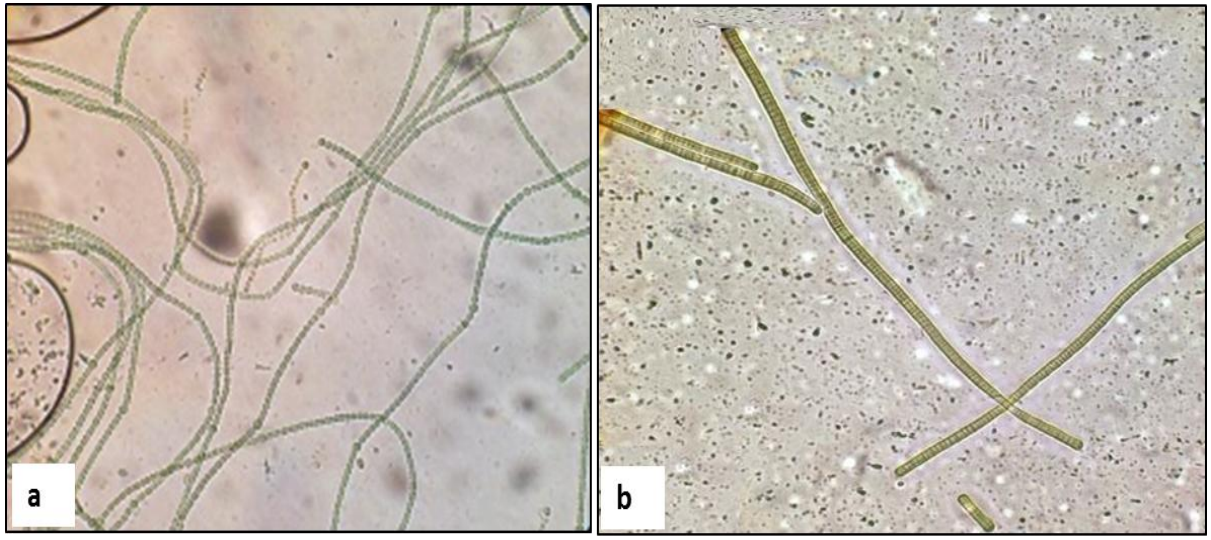

Fig.2. Light micrograph of 20 days old culture of a: *Anabaena* sp. and b:*Oscillatoria* sp.(x60) isolated from Sakya and El-Monib, respectively.

Table 2. Antibacterial activity of 30, 45 and 60 old days cyanobacterial filtrates against G^+ and G^- bacteria as presented by inhibition zone diameter (mm).

Indicator microorganism	*Fischerella* sp. 1Mb			*Fischerella* sp. 2Gd			*Fischerella* sp.*3 Ha*			*Oscillatoria* sp. Mb			*Anabaena* sp. Sk		
	30	**45**	**60**	**30**	**45**	**60**	**30**	**45**	**60**	**30**	**45**	**60**	**30**	**45**	**60**
E. coli	8.0	8.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
P .aeruginosa	8.0	8.0	7.0	8.0	8.0	8.0	7.0	7.0	7.0	7.0	7.0	7.0	8.0	8.0	7.0
S. Typhimurium	-	-	-	8.0	8.0	7.0	6.0	6.0	6.0	-	-	-	6.0	6.0	6.0
B. cereus	6.0	6.0	7.0	7.0	7.0	7.0	6.0	6.0	7.0	-	-	-	7.0	7.0	7.0
S. aureus	-	-	-	-	-	-	6.0	6.0	6.0	-	-	-	6.0	6.0	6.0

-, no inhibition (resistant microbe)

In this respect, Ibraheem *et al*. (2012) tested *Nostoc calcicola*, *Nostoc commune, Nostoc entophytum, Nostoc minutum, Nostoc palndosum, Nostoc passerianum, Nostoc punctiforme, Anabaena ambigua, Anabaena amomala,* and *Anabaena doliolum* for their allelopathic activities including inhibitory and/or promoting effects against two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Data suggested two types of allelopathic effects: the first always appeared in cyanobacterial medium as in case of *N. minutum* medium that inhibits the growth of all tested bacterial species. The second type is induced only when cyanobacteria are in contact with bacteria; this occurred when the growth of both *B. subtilis* and *S. aureus* were inhibited in co-culture with *N. commune.* Concerning the effect of *N. commune* culture on the tested bacteria, slight inhibitory effect on *E. coli* and slight promoting effect on *P. aeruginosa, S. aureus* and *B. subtilis* were observed. In another report, *Fischerella ambigua* collected from paddy fields excreted parsiguine, a broad spectrum antibacterial and antifungal substances into the culture medium (Ghasemi et *al* 2004).

During this study, filtrates of 45 days old broth cultures of *Fischerella*, *Anabaena* and *Oscillatoria* species, were extracted sequentially with different solvents initially with hexane then methanol which concentration with rotary evaporator and cyanobacterial mass were extracted sequentially with three different solvents initially with hexane, methanol and finally with water. Antibacterial activity of each extract was tested.

No water extract from mass of all tested cyanobacterial cultures showed antibacterial effect against any tested bacteria.

The results in Table (3) demonstrate that the methanol extract from either culture filtrate (extracellular extract) or mass (intracellular extract) has the strongest antibacterial effect comparing to hexane extract as the largest inhibition zones, against all tested bacteria(9.0 - 13mm), were recorded with the methanol extract for *Oscillatoria sp.*Mb mass.

Generally, this may be attributed to that methanol can extract alkaloids, tannis, phenols, steroids, flavonoids, carotenoids, phycocyanins and carbohydrates, and some of these compounds have antimicrobial effect. Sabarinathan and Ganesan (2008) found that C-phycocyanin extracted from filamentous fresh water cyanobacterium *Westiellopsis* sp. has antibacterial effect against *Bacillus subtilis*, *Pseudomonas* sp. and *Xanthomonas* sp. with diameter of inhibition zones varied from 2.2 to 13.1 mm. Also, Fan *et al*. (2013) extracted and purified phycocyanin from *Anabaena cylindrica* to test its antimicrobial activity. They indicated that three kinds of protein ingredients: PC-A, PC-B and PC-C were obtained using HPLC and that PC-C had

Table 3. Antibacterial activity of hexane and methanol extract from filtrates and mass of 45 days old cyanobacterial cultures against G^+ and G^- bacteria as presented by inhibition zone diameter (mm)

Indicator microorganism	*Fischerella* sp.1 Mb				*Fischerella* sp.2 *Gd*				*Fischerella* sp.3 *Ha*				*Oscillatoria* sp. Mb				*Anabaena* sp. SK			
	H_f	H_m	M_f	M_m	H_f	H_m	M_f	M_m	H_f	H_m	M_f	M_m	H_f	H_m	M_f	M_m	H_f	H_m	M_f	M_m
E.coli	7.0	-	10.0	9	-	7.0	8.0	9.0	-	-	8.0	-	8.0	-	9.0	9.0	-	-	8.0	9.0
P. aeruginosa	-	8.0	9.0	-	8.0	7.0	9.0	-	-	-	9.0	9.0	8.0	9.0	9.0	10.0	8.0	-	9.0	-
S. typhimurium	-	-	-	-	-	-	9.0	-	-	-	8.0	-	-	-	-	-	-	8.0	9.0	-
B. cereus	-	-	9.0	-	-	-	9.0	-	-	7.0	9.0	10.0	-	-	-	12.0	-	-	8.0	-
Sta. aureus	-	7.0	-	10.0	-	-	-	8.0	8.0	10.0	9.0	10.0	-	9.0	-	13.0	-	-	8.0	10.0

H_f, hexane extract from culture filtrate; H_m, hexane extract from culture mass; M_f, methanol extract from culture filtrate; M_m, methanol extract from culture mass; - ,no inhibition,

certain antibacterial activity against *Vibrio parahemolyticus*, *Bacillus mucilaginosus* and *Sarcina lutea*. In addition, PC-C had certain antibacterial activity against *Vibrio harveyi*. Asthana *et al* (2006) reported the active constituents in the methanolic extract of laboratory grown culture of *Fischerella sp.*, isolated from Neem (*Azadirechta indica*) tree bark. They observed inhibitory effect against *Mycobacterium tuberculosis Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli*.

Similarly, Kim *et al*. (2012) extracted three hapalindole-type alkaloids, namely hapalindole X, deschloro hapalindole I, and 13-hydroxy dechlorofontonamide, along with ten known indole alkaloids (hapalindoles A, C, G, H, I, J, and U, hapalonamide H, anhydrohapaloxindole A, and fischerindole L) and fischerellins A and B from *Westiellopsis* sp. (SAG strain number 20.93) and *Fischerella muscicola* (UTEX strain number LB1829). Hapalindoles X and A, and hapalonamide H showed potent activity against both *Mycobacterium tuberculosis* and *Candida albicans* with MIC values ranging from 0.6 to 2.5 µM

Respecting the antagonistic effect of different cyanobacterial species on tested bacteria, it was found that the methanol extract of *Oscillatoria* sp.Mb mass could be regarded to be the most efficient extract as the largest inhibition zone was recorded with this extract against the tested bacteria except *S. typhimurium* (Ø, 9.0 mm against *E. coli*, 10.0 mm against *P. aeruginosa*, 12.0 mm against *B. cereus* and 13.0 mm against *Sta. aureus*). The methanol extract of *Anabaena* sp.SK and *Fischerella* sp.3Ha filtrate has antagonistic effect against all tested indicator bacteria.

Comparing the diameter of the inhibition zone of crude filtrate against susceptible bacteria (Table, 2) with that corresponding to the methanol extract of the filtrate (Table, 3), it was generally noticed that the methanol extraction enhanced the antagonistic effect of the all cyanobacterial filtrates as larger inhibition zones were recorded with the methanol extract. This indicates that the use of organic solvents for preparation of algal extracts provides more appropriate antimicrobial activity. Meanwhile data obtained during this study, are in conflict with those reported by Mathivanan *et al.* (2010); who found that ethanolic extract of *Oscillatoria* showed the maximum inhibition zone against *S. aureus* 21 mm in(30 µl per well) . Also Reginald Appavoo and Darling (2015) reported that *Oscillatoria* sp highest zone of inhibition were observed in ethanol extract against *Staphylococcus aureus* (11.5mm).

The antibacterial activity of the methanol extract of both cyanobacterial filtrate and mass was quantified through determination of MIC (Table, 4). The MIC was measured as µl µl $^{-1}$.

Table 4. **Minimum inhibitory concentration (µl µl $^{-1}$) of methanol extract from cyanobacterial filtrate and mass.**

Indicator microorganism	*Fischerella sp.1* Mb		*Fischerella sp.2 GD*		*Fischerella sp.3 Ha*		*Oscillatoria* sp. Mb		*Anabaena sp.* Sk	
	M_f	M_m	Mf	M_m	M_f	M_m	M_f	M_m	M_f	M_m
E.coli	0.25	0.25	0.25	0.25	0.5	N.D.	0.25	0.25	0.25	0.25
P. aeruginosa	0.5	N.D.	0.5	N.D.	0.5	0.125	0.25	0.125	0.25	N.D.
S. typhimurium	N.D.	N.D.	0.25	N.D.	0.25	N.D.	N.D.	N.D.	0.25	N.D.
B. cereus	0.5	N.D.	0.5	N.D.	0.25	0.031	N.D.	0.031	0.5	N.D.
S. aureus	N.D.	0.125	N.D.	0.25	0.25	0.031	N.D.	0.016	0.5	0.25

N.D. not detected; Mf, methanol extract from culture filtrate; Mm, methanol extract from culture mass.

The results show that there is consistency between the antimicrobial activity expressed as inhibition zone diameter and MIC. That means the lowest concentration inhibiting the growth of the sensitive bacteria was recorded with the extract exhibited largest inhibition zone diameter. The methanol extract of

Oscillatoria sp Mb mass has the lowest inhibiting concentration (0.016 µl µl $^{-1}$) against *S. aureus*. In a similar study, Chandra and Rajashekhar (2013) investigated the antimicrobial activity of two species of cyanobacteria namely, *Phormidium chalybeum* and *Leptolyngbya tenuis* isolated from hospital discharge waste waters near Mangalore. They reported that The MIC of *P. chalybeum* ranged between 30 and 255 µg/ml in all the solvent extracts against tested bacterial strains. A significant MIC is noticed in diethyl ether extract of *P. chalybeum* against *S. aureus* (30 µg/ml). In the case of *L. tenuis*, the MIC value ranged between 50 and 225 µg/ml, whereas least MIC value was observed in ethanolic extract against *B. subtilis* (50.5 µg/ml). It was also noticed that both species showed lesser MIC values against *S. aureus*.

The methanol extracts from cyanobacterial filtrates and mass were assayed for their sensitivity to temperature *i.e.* loss of activity due to different heat treatments. The thermal stability of the methanol extract, after boiling for 10, 20 and 30 min. and after pasteurization, was tested by measurement of the MIC after each heat treatment. The results in Table (5), for methanol extract of filtrate and Table (6), for methanol extract of mass, revealed the following:

Table 5. Minimum inhibitory concentration (µl µl^{-1}) of methanol extract from culture filtrate of tested cyanobacteria after boiling for 10, 20 and 30 min. and pasteurization.

Indicator microorganism	*Fischerella* sp. 1 Mb				*Fischerella* sp.2 GD				*Fischerella* sp.3 Ha				*Oscillatoria* sp. Mb				*Anabaena* sp. Sk			
	Boiling after			Past.	Boiling after			Past.	Boiling after			Past.	Boiling after			Past.	Boiling after			Past.
	10 min	20 min	30 Min		10 min	20 min	30 min		10 min	20 Min	30 Min		10 min	20 min	30 min		10 min	20 min	30 min	
E. coli	-	-	-	0.25	-	-	-	0.25	-	-	-	0.25	-	-	-	0.25	0.5	-	-	0.25
P. aeruginosa	-	-	-	0.5	-	-	-	0.5	-	-	-	0.25	-	-	-	0.25	-	-	-	0.25
S. typhimurium	ND	ND	ND	ND	-	-	-	0.5	-	-	-	0.25	ND	ND	ND	ND	-	-	-	0.25
B. cereus	0.5	0.5	-	0.5	-	-	-	0.25	-	-	-	0.25	ND	ND	ND	ND	-	-	-	0.5
S. aureus	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-	0.5	ND	ND	ND	ND	-	-	-	0.5

Past., pasteurization; ND, not detected; - , activity was lost after heat treatment

Table 6. Minimum inhibitory concentration (µl µl $^{-1}$) of methanol extract from mass cultures of tested cyanobacteria after boiling for 10, 20 and 30 min. and pasteurization.

Indicator microorganism	*Fischerella* sp. 1 Mb				*Fischerella* sp.2 Gd				*Fischerella* sp.3 *Ha*				*Oscillatoria* sp. Mb				*Anabaena* sp. Sk			
	Boiling after			past.	Boiling after			past.	Boiling after			Past	Boiling after			Past.	Boiling after			Past.
	10 min	20 min	30 min		10 min	20 min	30 min		10 min	20 min	30 Min		10 min	20 min	30 min		10 min	20 min	30 min	
E.coli	0.25	0.25	0.25	0.25	0.25	-	-	0.125	ND	ND	ND	ND	-	-	-	0.125	0.25	0.25	0.25	0.25
Ps. aeruginosa	ND	ND	ND	ND	ND	ND	ND	ND	0.125	0.25	-	0.125	-	-	-	0.125	ND	ND	ND	ND
Sal. Typhimurium	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B. cereus	ND	ND	ND	ND	ND	ND	ND	ND	0.063	0.125	-	0.125	0.5	0.5	0.5	0.002	ND	ND	ND	ND
Sta. aureus	0.25	0.25	0.25	0.004	0.25	-	-	0.031	0.063	0.125	-	0.25	0.5	-	-	0.002	0.5	0.5	0.5	0.25

Past., pasteurization; ND, not detected; - , activity was lost after heat treatment

i. The activity of methanol extract of *Fischerella* sp.1Mb filtrate was completely lost, against both *E. coli* and *Ps. aeroginosa*, after boiling for 10, 20 and 30 min. On the other hand, the activity of mass extract was stable against *E. coli*, with boiling and pasteurization.

ii. The activity of methanol extract of *Fischerella* sp.2 Gd filtrate was completely lost, against all susceptible bacteria, after boiling for 10, 20 and 30 min, while the mass extract was stable against *E. coli* and *Sta. aureus* after boiling for 10 min only. In contrast, the pasteurization enhanced the activity of mass extract against *E. coli* and *Sta. aureus* as the lowest concentration inhibiting *E. coli* and *Sta. aureus* decreased from 0.25 to 0.125 µl µl $^{-1}$ for *E. coli* and to 0.031 µl µl^{-1}for *Sta aureus*.

iii. The activity of *Fischerella* sp.3Ha filtrates were completely lost by boiling, but the pasteurization increased the inhibitory effect of *Fischerella* sp.3 Ha filtrate against *E. coli* and *Ps. aeroginosa*. While the pasteurization increased MIC of *Fischerella* sp.3Ha filtrate against *Sta. aureua* from 0.25 to 0.5 µl µl $^{-1}$.

iv. The activity of methanol extract of both filtrate and mass of *Oscillatoria sp.*Mb was completely lost, against *E. coli* and *Ps. aeroginosa*, after boiling for 10, 20 and 30 min. The boiling treatment has negative effect on the activity of mass extract against *B. cereus* as the MIC increased from 0.031 to 0.5 µl µl $^{-1}$.

v. The activity of *Anabaena* sp.Sk filtrates were completely lost by boiling, but the pasteurization stable the inhibitory effect of *Anabaena* sp.Sk filtrate.

Conclusion

Microbial natural products are the important source of new drugs. Among the producers of commercially important metabolites, cyanobacteria have proven to be the prolific source with a surprisingly small group of taxa accounting for the vast majority of compounds discovered till date. The results of this study suggested that River Nile contains several species of freshwater cyanobacteria that have immense potentials. Results indicated that cultures, cultures filtrates and extracts of isolated cyanobacterial showed considerable antimicrobial activity against different microorganisms. Therefore, it could be concluded that Extracts of cyanobacteria can be used as antibacterial agents against common food-borne pathogens.

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