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Screening of Antimicrobial Activity of Selected Egyptian Cyanobacterial Species

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Abstract: The present study aimed to evaluate the antimicrobial activity (against selected bacterial, fungal and yeast strains) of successive extracts from different blue green algae. Aqueous and organic extracts of seven cyanobacterial species were screened against *in vitro* eight human bacterial pathogens and five fungal strains. Chloroform extract of the seven cyanobacterial species showed largest antibacterial inhibition zone diameter against the pathogenic bacterial strains. The chloroform extracts showed a broad spectrum against Gram- negative bacteria (*Escherichia coli*, *Aeromonas hydrophila* , *Salmonella enterica* S 1180, *Klebsiella pneumonia* K 51 , *Vibrio cholera* V116 and *Salmonella paratyphi*) and Gram-positive bacteria (*Staphylococcus aureus* S 1426, *Listeria monocytogenes* L 49). However, the chloroformic extracts displayed also antifungal activity against *Aspergillus terreus* F98, while, none of the extracts of the seven cyanobacterial species demonstrated any activity against *Tirchoderma viride* F94. Meanwhile, the ethanolic extracts of four of cyanobacterial species showed antifungal activity against both yeast strains of *Candida tropicalis* Y26 and *Saccharomyces cerevisiae* Y39. On the other hand, ethyl acetate extracts of all cyanobacterial species showed antifungal activity against *Saccharomyces cerevisiae* YH.

Keywords: Antibacterial activity, Antifungal activity, Blue green algae, Human pathogens, Successive extracts.

1.Introduction

Cyanobacteria(blue green algae) are Gram negative autotrophic bacteria, exhibiting a variety of metabolic capabilities and adaptive mechanisms, including chromatic adaptation, nitrogen fixation (occurs in specialised cells called heterocytes/under microaerobic conditions/through temporal spatiation), and the ability to form symbiotic associations with several eukaryotic hosts such as plants, fungi, and protists (Bergman and Ran 2008). They mainly have chlorophyll a, moreover accessory pigments such as phycobilins, carotenoids (Hedges et al., 2001). Cyanobacterial phycobiliproteins (phycocyanin, phycoerythrin and allophycocyanin). All these pigments have different pharmaceutical applications, as antioxidants, boost the immune system and possibly decrease the risk of heart disease, prevent onset of cancers and protect against age related diseases as cataracts and multiple sclerosis, etc. (Larsson et al., 2007). Blue green algae also, contain nutrients, including vitamin B, vitamin E, beta-carotene, manganese, zinc, copper, iron, selenium, and essential fatty acid such as γ linolenic acid (Gupta et al., 2013). Moreover, they included the potential sources of new polymers, carbohydrates (as glucosyl glycerol, trehalose and sucrose) which are synthesized by cyanobacteria under different osmotic stresses (Santillan, 1982). They have potential applications in diverse areas, especially in agriculture (as biofertilizer, plant growth promoting rhizobacteria and as biocontrol agents).

Their role as food supplements/nutraceuticals and in bioremediation and wastewater treatment is an emerging area of interest. In addition, they are known to produce wide array of bioactive compounds (secondary metabolites) with different biological activities including antibacterial, antifungal, antiviral, antimalarial, antitumoral and anti-inflammatory properties, having industrial, therapeutic and agricultural significance (Gupta et al., 2013). Screening of cyanobacteria for antibiotics and other pharmacologically

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active compounds, has received ever-increasing interest as a potential source for new drugs (Browitzka, 1995; Schlegel et al.,1999).The important compounds identified as antimicrobial are fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols (Kannan et al., 2010). A couple of biologically active compounds were identified among exometabolites, e.g. certain antibacterial diterpenoids in *Nostoc commune* (Jaki et al., 2000) and antifungal peptides in *Tolypotrix byssoidea* (Jaki et al., 2001). The present study aimed to evaluate the antimicrobial potential of successive extracts from seven cyanobacterial species against human pathogenic bacteria, fungi, and yeast.

2. Materials and methods

2.1 Algal species and culture conditions

Seven Cyanobacteria species (*Oscillatoria* sp., *Nostoc* sp., *Nostoc muscorum*, *Nostoc piscinale*, Phormidium sp., *Anabaena flos-aquae* and Spirulina *platensis*) were obtained from the Microbiology Department, Soils, Water and Environment Res. Inst. (SWERI), Agric. Res., Center (ARC), Giza, Egypt. Cyanobacterial species were maintained on BG11 medium (Rippka, 1988) except *Spirulina platensis* which was cultured on Zarrouk medium (Zarrouk, 1966). Cultures of these species were incubated at temperature 25±1°C under illumination of natural light intensity of 30 µE/m2/s and photoperiod 12/12 h. At the late exponential growth phase, algae were harvested by centrifugation at 3500 rpm for 10 min and the pellets were subjected to extraction by different solvents of increasing polarity.

2.2. Successive extract preparation

A known weight (2g) of each algal species was extracted two times by hexane for 30 min.at room temperature (25±1°C) followed by centrifugation at 4500 rpm for 10 min. and the supernatants were combined. The subsequent successive extraction of each algal pellet was performed by the same pervious procedure with the following solvents, chloroform, ethyl acetate, ethanol (70%) and dist. water. So each algal species have five extracts (Fig 1). Solvents were evaporated using rotary evaporator at 40-45^{o}C, while those of ethanol (70%) and water were freeze-dried. Dried residues of extracts belonging to each algal species were dissolved in DMSO (1%) and used for determination of their antimicrobial activities.

2.3. Determination of antimicrobial activity

2.3.1. Antibacterial activity

The Bacterial strains (Gram- positive) *Staphylococcus aureus* S 1426, *Listeria monocytogenes* L 49, (Gram-negative) *Escherichia coli*, *Aeromonas hydrophila* , *Salmonella enterica* S 1180, *Klebsiella pneumonia* K 51 , *Vibrio cholera* V116 and *Salmonella paratyphi*, were kindly provided by Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. These strains were cultured and maintained on L.B agar medium (Martin et al.,1981). Bacterial strains were inoculated in L.B broth medium overnight (16 hours) in shaking incubator at 37^{o}C. the grown culture (2x10^{8} CFU/ml) were used to seed L.B agar plates (by medical swaps) the seeded plates were left for 1 hr, then filter paper discs (10 mm diameter) were loaded by 50 µl of tested extract from each algal species against each bacterial strain . Three replicates were used for each extract. The plates were incubated up right for 24 hrs at 37^{o}C. Amoxicillin was used as standard commercial antibacterial agent (positive control) and dimethyl sulfoxide (DMSO (1%)) as (negative control). The inhibition zones around discs were determined (mm).

2.3.2. Antifungal activity

The yeast strains *Saccharomyces cerevisiae* Y39; *Candida tropicalis* Y26 and *Saccharomyces cerevisiae* YH. Fungal strains *Tirchoderma viride* F94 and *Aspergillus terreus* F98 were kindly provided by Dr. Abo-State (National Center for Radiaition Research and Technology (NCRRT), Cairo, Egypt).Yeast strains were cultured and maintained on Wickersham's medium (Wickersham´s. 1951).Yeast strains were inoculated in Wickersham's broth medium and incubated for 24 hrs at 30 ^{o}C, (2x10^{5} CFU/ml). These yeast cultures were used to seed the Wickersham's agar plates for testing biological activity of algal extracts. Fungal strains were cultured and maintained on Saubouraud agar medium (Oxoid, 1982). and spore suspension were carried out according to Abo-State 2003 , flasks (250ml) containing 100ml Saubouraud agar medium were inoculated and incubated at 27 ^{o}C for 7 days. The spores of each strain were collected by 0.1 % Tween 80 in sterile saline solution (30ml). The collected spore suspensions (2x10^{7}spores/ml) were used to seed Saubouraud agar plates. The seeded plates were left for 1 hr and then sterile filter paper discs (10 mm diameter), loaded by 50 µl of each algal extract were placed on the surface of seeded plates and incubated at 27 ^{o}C for three days. Three replicates were used for each extract. Ultragriseofulvin was used as standard antifungal activity agent (positive control) and dimethyl sulfoxide solvent (DMSO (1%)) as (negative control).The clear inhibition zones around discs were determined (mm) which were used as a measure of antifungal activity of algal extracts.

2.4. Statistical analysis

The experiments were carried out in triplicates and the results were expressed as the means values and standard deviations. The statistical analyses were performed using SPSS version 16.0 for windows.

3. Result and discussion

The extracts of seven cyanobacterial species were tested for their antibacterial activity against eight human pathogenic bacteria and antifungal activity against three yeast and two fungal strains.

3.1. Antibacterial activity

Table (1) and Fig (1, 2) recorded the antibacterial activity of successive extracts of the tested pathogenic bacterial species. The most remarkable results was that of chloroform extracts of all the seven used bacteria showed inhibition zones of variable diameters (11.0 -30.0 mm), while hexane and water extracts recorded negative results. Ethyl acetate extracts of *Nostoc* sp., *A. flos-aquae* and *S. platensis* showed slight inhibition zones (11.0 , 12.0 and 11.5 mm) against *S. paratyphi* and that of *N. musorum* showed similar slight inhibition zone with *S. enterica* S 1180 (11.0 mm) . While, ethanolic extract of all cyanobacterial species demonstrated inhibition zones of variable diameters (11.0 - 15.5 mm) against *E. coli* accept that *N. piscinale* which showed no activity. Ethanolic extracts of both *N. Piscinale* and *S. platensis* showed slight and moderate inhibition zones (11.0 , 17.0 mm respectively) against *A. hydrophila*. Also slight inhibition zones were recorded against *S. aureus* S 1426 by ethanolic extracts of both *N. piscinale* and *A. flos-aquae* (11.0 mm). In addition, ethanolic extract of *N. musorum* illustrated slight antibacterial activity against *V. cholera* V116 (12.0mm).

Table (1): Antibacterial activity of different successive extracts from seven blue green algae

Algal species	Extracts	Inhibition zone diameters (mm)							
		Gram negative						Gram positive	
		E. coli	*K. pneumonia*	*S. paratyphi*	*A. hydrophila*	*V. cholera*	*S. enterica*	*L. monocytogens*	*S. aureus*
Oscillatoria sp.	Hexane	-	-	-	-	-	-	-	-
	Chloroform	23.50± 0.18	22.00± 0.34	-	11.50± 0.35	15.00± 0.19	12.00± 0.26	13.00± 0.17	12.50±0.12
	Ethyl acetate	-	-	-	-	-	-	-	-
	Ethanol (70%)	12.50± 0.15	-	11.00± 0.12	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-
Nostoc sp.	Hexane	-	-	-	-	-	-	-	-
	Chloroform	15.00± 0.34	21.50± 0.40	13.00± 0.17	15.50± 0.33	22.50± 0.16	-	11.00± 0.13	15.50±0.24
	Ethyl acetate	-	-	11.00± 0.54	-	-	-	-	-
	Ethanol (70%)	11.00± 0.27	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-
Nostoc musorum	Hexane	-	-	-	-	-	-	-	-
	Chloroform	16.00± 0.15	20.50± 0.18	14.50± 0.24	13.50± 0.23	15± 0.18	12.00± 0.23	11.00± 0.56	20.00±0.33
	Ethyl acetate	-	-	-	-	-	11.00± 0.17	-	-
	Ethanol (70%)	11.00± 0.16	-	-	-	12.00± 0.42	12.00± 0.34	-	-
	Water	-	-	-	-	-	-	-	-
Nostoc piscinale	Hexane	-	-	-	-	-	-	-	-
	Chloroform	25.00± 0.22	26.50± 0.66	13.50± 0.14	14.50± 0.15	14.50± 0.18	17.50± 0.12	15.00± 0.19	12.50±0.14
	Ethyl acetate	-	-	-	-	-	-	-	-
	Ethanol (70%)	-	-	14.00± 0.26	11.00± 0.13	-	-	-	11.00±0.15
	Water	-	-	-	-	-	-	-	-
Phormidium sp.	Hexane	-	-	-	-	-	-	-	-
	Chloroform	17.50± 0.18	18.50± 0.19	15.00± 0.15	16.00± 0.20	15.50± 0.31	17.50± 0.15	12.50± 0.45	15.50±0.24
	Ethyl acetate	-	-	-	-	-	-	-	12.50±0.17
	Ethanol (70%)	12.00± 0.30	-	11.50± 0.12	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-
Anabaena flosaquae	Hexane	-	-	-	-	-	-	-	-
	Chloroform	15.50± 0.50	30.00± 0.41	14.50± 0.32	16.50± 0.31	22.00± 0.12	17.50± 0.16	15.50± 0.45	11.50±0.23
	Ethyl acetate	-	-	12.00± 0.42	-	-	-	-	12.00±0.18
	Ethanol (70%)	12.50± 0.21	-	13.00± 0.60	-	-	-	-	11.00±0.51
	Water	-	-	-	-	-	-	-	-
Spirulina plateuris	Hexane	-	-	-	-	-	-	-	-
	Chloroform	21.50± 0.13	17.50± 0.13	16.50± 0.15	17.00± 0.52	15.00± 0.24	16.00± 0.27	15.00± 0.15	13.50±0.40
	Ethyl acetate	-	-	11.50± 0.20	-	-	-	-	-
	Ethanol (70%)	13.00± 0.17	-	15.50± 0.24	17.00± 0.13	-	-	-	-
	Water	-	-	-	-	-	-	-	-
Amoxicillin	Standard antibiotic	-	-	-	-	-	-	30.00± 0.23	13.50±0.32
DMSO (1%)	Control	-	-	-	-	-	-	-	-

Results are the means of diameter values ± standard deviation. (-): No activity

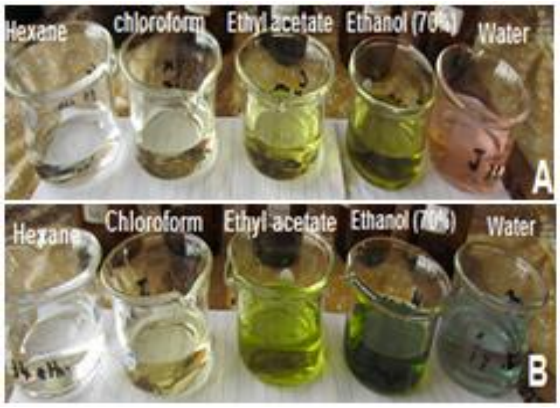

Figure (1): Successive extracts of different cyanobacterial species acquire various colours, *Oscillatoria* sp. (A), and *Phormidium* sp. (B).

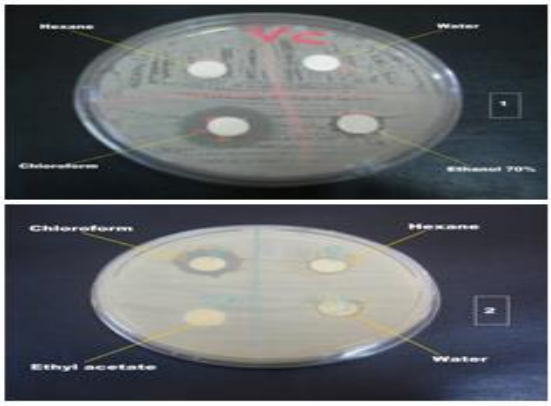

Figure (2): Antibacterial activity: zone of inhibition exhibited by different extracts of cyanobacterial species: *Nostoc* sp. (1: *V. cholera* V116) and *N. piscinale* (2: *Salmonella enterica* S 1180).

3.2. Antifungal activity

None of the tested seven cyanobacterial extracts showed any antifungal activity against *T. viride*. However, the chloroform extract of five cyanobacterial species out of the seven demonstrated antifungal activity against *A. terreus.*While three ethanolic extracts also showed antifungal activity against the same fungus as showed in (Table 2). On contrast *C. tropicalis* Y26 was sensitive against four ethanolic extracts (of *N. musorum* (17.5 mm), *S. platensis* (13.5 mm), *Phormidium* sp (11.5 mm) and *Oscillatoria* sp. (11.0 mm)). However ethyl acetate extracts of the seven cyanobacterial species was effective in inducing weak activities (11.0 -12.5 mm) against S. *cerevicieae* YH. In case of *S. cerevisiae* Y39 the ethanolic extracts of the four cyanobacterial species (*N. musorum* , *N. piscinale*, *Phormidium sp*. and *S. platensis*) showed antifungal activities against this organism (13.0, 13.5, 12.0 and 12.5 mm respectively). Extracts of *Oscillatoria* sp., *Nostoc* sp. and *A. flos-aquae* showed no activity with the most tested fungal and yeast strains. Hexane and water extracts of all the investigated cyanobacterial species showed no activities with the tested fungal and yeast species except hexane extract of *S. platensis* which exhibited very weak activity against *A. terreus* F98.

The cyanobacteria such as *Nostoc commune* (Jaki et al., 2000), *Scytonema hofmanni* (Pignatello et al., 1983), *Anabaena* spp. (Frankmolle et al., 1992), *Nostoc spongiaeforme* (Hirata *et al.*,1996), *Phormidium* sp. (Fish and Codd 1994), have been reported as the main cyanobacterial species producing antimicrobial substances.

Investigations aimed to identify antimicrobial agents in cyanobacteria showed the occurrence of many promising compounds. Some of these substances were identified including Nostocyclyne A (Ploutno and Carmeli, 2000), Nostofungicidine (Kajiyama et al.,1998), Kawaguchipeptin B (Ishida et al.,1997), Nostocin A (Hirata et al.,1996), Ambigol A and B (Falch et al.,1995), Hapalindoles (Moore et al.,1987) and Scytophycins (Ishibashi et al.,1986). Studies have only done as *in vitro* assays and, it is likely that most of these compounds will have little or no clinical application as they are either too toxic or inactive *in vivo* . However, they may be useful compounds for the synthesis of antibiotics or may be used in agriculture applications. For example Tjipanazoles which was isolated from the cyanobacterium, *Tolypothrix tjipanensis*, demonstrated appreciable fungicidal activity against rice blast and leaf rust wheat infections (Browitzka, 1995). The obtained results (antibacterial and antifungal) i n this study were confirmed by the results of other investigators. Ghasemi et al. (2003) found that methanolic extracts and culture supernatants of 21 species of cyanobacteria exhibited significant antibacterial activity and 13 species showed antifungal effects. No antimicrobial activity was detected in the hexane extracts of the cyanobacteria under investigation which may be probably due to the polar nature of the active components. Also, Bhateja et al. (2006) studied the effect of different extracts of nine cyanobacteria against different strains of *Staphylococcus aureus.* Aqueous extract of all the tested blue green microalgal were found to be inactive against in vitro generated vancomycin intermediate resistant *Staphylococcus aureus* (VISA). While, Kreitlow et al. (1999) evaluated the antimicrobial activities of twelve cyanobacterial species against seven microorganisms. However, no inhibitory effects were recorded against the three Gram- negative bacteria, *E. coli*, *Proteus mirabilis* and *Serratia marcescens* and the yeast *Candida maltosa*. Different results have been reported by many authors in this context. For example, Falch et al. (1995) and Hirata et al. (1996) reported active compounds against *E. coli* in the petroleum ether fraction of *Fischerella ambigua* and supernatant of *Nostoc spongiaeforme*. However, Plazaa et al.(2010) found that ethanol was selected as the most appropriate solvent to extract bioactive compounds from two macro and microalgal species with antimicrobial

activities against four microorganisms (*E. coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*). But, Bhagavathy et al. (2011) used the green algal, *chlorococcum humicola* extracts of various organic solvents against seven pathogenic bacteria, one yeast and two fungal strains. From all the investigated organic extracts, only benzene and ethyl acetate extracts showed greatest activity (reached nearly 80% of microbial growth inhibition). Also, Chauhan et al. (1992) reported that ether extract of *Oscillatoria* sp. demonstrated antibiotic activity which may be due to the isolated and identified saturated fatty acids($C_{14:0}$, $C_{16:0}$ and $_{C18:0}$). In the same context, Shanab (2007) studied the antibiotic efficiency of three *Oscillatoria* species (*O. hameli*, *O. rubescens* and *O. platensis*).

Table (2): Antifungal activity of different successive extracts from seven blue green algae

Algal species	Extracts	Inhibition zone diameters (mm)				
		Fungi		Yeast		
		A. terreus F98	*T. viride* F94	*C. tropicalis* Y26	*S. cerevisiae* Y39	*S. cerevisiae* YH
Oscillatoria sp.	Hexane	-	-	-	-	-
	Chloroform	-	-	-	-	-
	Ethyl acetate	-	-	-	-	11.00± 0.18
	Ethanol (70%)	-	-	11.00± 0.18	-	-
	Water	-	-	-	-	-
Nostoc sp.	Hexane	-	-	-	-	-
	Chloroform	11.00± 0.15	-	-	-	-
	Ethyl acetate	-	-	-	-	11.00± 0.30
	Ethanol (70%)	-	-	-	-	-
	Water	-	-	-	-	-
Nostoc muscorum	Hexane	-	-	-	-	-
	Chloroform	11.00± 0.20	-	-	-	-
	Ethyl acetate	-	-	-	-	11.50± 0.35
	Ethanol (70%)	11.00± 0.25	-	17.50± 0.23	13.00± 0.25	-
	Water	-	-	-	-	-
Nostoc piscinale	Hexane	-	-	-	-	-
	Chloroform	11.50± 0.13	-	-	-	-
	Ethyl acetate	-	-	-	-	12.00± 0.12
	Ethanol (70%)	-	-	-	13.50± 0.15	-
	Water	-	-	-	-	-
Phormidium sp.	Hexane	-	-	-	-	-
	Chloroform	11.00± 0.25	-	-	-	-
	Ethyl acetate	-	-	-	-	11.00± 0.14
	Ethanol (70%)	-	-	11.50± 0.15	12.00± 0.23	-
	Water	-	-	-	-	-
Anabaena flos-aquae	Hexane	-	-	-	-	-
	Chloroform	-	-	-	-	-
	Ethyl acetate	-	-	-	-	11.00± 0.21
	Ethanol (70%)	11.00± 0.50	-	-	-	-
	Water	-	-	-	-	-
Spirulina platensis	Hexane	11.00± 0.33	-	-	-	-
	Chloroform	11.00± 0.45	-	-	-	-
	Ethyl acetate	-	-	-	-	11.50± 0.45
	Ethanol (70%)	11.00± 0.25	-	13.50± 0.20	12.50± 0.32	12.50± 0.50
	Water	-	-	-	-	-
Ultragriseofulvin	Standard fungicide	-	-	-	-	-
DMSO (1%)	Control	-	-	-	-	-

Results are the means of diameter values ± standard deviation. (-): No activity

The results revealed that the active compounds isolated and identified by GC/MS and TLC contained fatty acids (saturated and unsaturated), the tetramine spermine and piperazine derivatives which may be responsible for the pronounced antimicrobial activity against the Gram-positive , Gram- negative bacteria and pathogenic fungi. The antimicrobial activity manifested by either *Oscillatoria* species in the previous study or by the seven selected cyanobacteria species in the present investigation was in accordance with reported antibiotic activities manifested by different cyanobacterial bioactive compounds as Hapalindoles alkaloids, cyanobacterin, Nostocyclamide, Fischerellin A&B and Norhamane which were produced by various cyanobacteria species (Volk, 2005). Antimicrobial activities of Spermine, Piperazine and fatty acids were reported in many studies (Cushion et al., 2004; Shanab, 2007). Fatty acids (non-polar) are recognized to have antibacterial and antifungal activities against broad spectrum of bacterial and fungal species (McGaw et al.,2002; Barbour et al.,2004 ; Agoramoorthy et al., 2007). Many authors suggested the mechanism of action of antimicrobial substances on different microorganisms. It was reported that Gram – negative bacteria are more resistant to the inactivation by medium and long chain fatty acids than the Gram – positive bacteria which are more susceptible; this may result from impermeability of the outer membrane of Gram –negative bacteria which is considered as an effective barrier against hydrophobic substances (Sheu and Freese, 1973). The inhibitory effect of antifungal compounds may be due to the inhibition of spore germination or the inhibition of synthesis of B- (1,3)-D-glucan or inhibition of integral component of fungal cell wall and their effect on fungal cell membrane which alter its permeability (Gupta *et al.*, 2001). In the same context, it was also reported that, antifungal compounds may inhibit lipid synthesis in the tested fungal species. This may be due to a decrease in the ratio of unsaturated to saturated fatty acids or inhibition of ergosterol biosynthesis (Georgopapadakou *et al.*,1987).

Further studies are needed concerning the analysis of the promising extracts of the tested cyanobacterial species, comparing between their contents and identifying the active

substance which may be the responsible agent(s) for the recorded antimicrobial activity.

4. Conclusion

In this work, antifungal activity is very weak compared with the antibacterial activity of selected cyanobacterial species. Also, Hexane and water extracts of all the investigated cyanobacterial species showed neither antibacterial nor antifungal activities against the tested microorganisms. Chloroform extracts exhibited pronounced antibacterial activity against Gram- positive and Gram-negative bacterial species. Most of ethyl acetate extracts have no activity except those of *Phormidium* sp. and *Anabaena flosaquae* which showed weak activity against the Gram- positive *S. aureus*. *T. viridi* showed general resistance against all the tested extracts. Analysis of tested cyanobacterial chloroform extracts may reveal the common presence of one or more antimicrobial compounds to which attributed the recorded activities.

References

[1] A. Ploutno, S. Carmeli, Nostocyclyne A, a novel antimicrobial cyclophan from the cyanobacterium *Nostoc* sp. *J. Nat. Prod.* **63**, 1524-26, (2000).

[2] B. Jaki, J. Heilmann, A. Linden, B. Volger, O. Sticher, Novel extra cellular diterpenoids with biological activity from the cyanobacterium *Nostoc commune*, *J. Nat. Prod.* **63**, 339-343, (2000).

[3] B. Jaki, O. Zerbe, J. Heilmann, O. Sticher, Two novel cyclic peptides with antifungal activity from the cyanobacterium Tolypothrix byssoidea (EAWAG 195). *J. Nat. Prod.* **64**, 154–158, (2001).

[4] B. R. Volk, Screeing of microalgal culture media for the presence of algicidal compounds and isolation and identification of two bioactive metabolities excerted by cyanobacteria *Nostoc insulare* and *Nodularia harveyana*, *J. Appl. Phycol.* **17**, 330-47, (2005).

[5] B. S. Falch, G. M. König, A. D. Wright, O. Sticher, C. K. Angerhofer, J. M. Pezzuto, H. Bachmann, Biological activities of cyanobacteria: evaluation of extracts and pure compounds, *Planta. Med*. **61**, 321-328, (1995).

[6] C. P. Gupta, R. S. Dabey, S. C. Kany, D. K. Mahashwari, Antibiosis mediated recrotrophic effect of *Pseaudomonas* GRC2 against two fungal plant pathogens. *Curr. Sci.* **81**, 91-94, (2001).

[7] C. Santillan, Mass production of *Spirulina*, *Experientia* **38**, 40–43, (1982).

[8] C. W. Sheu, E. Freese, Lipopoly saccharide layer protection of Gram-negative bacteria against inhibition by long chain fatty acids. *J. Appl. Bacterial* **115**, 869-875, (1973).

[9] C. Zarrouk, Contribution á l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthése de Spirulina maxima (Setch. Et Gardner) Geitler. PhD Thesis University of Paris France, (1966).

[10] E. K. Barbour, M. A. Shorif, V. K. Sagherian, A. N. Habr, R. S. Talhank, S. N. Talhouk, Screening of selected indigenous plants of Lebanon for antimicrobial activity, *J. Ethnopharmacol* **93**, 1-7, (2004).

[11] G. Agoramoorthy, M. Chandrasekaran, V. Venkatesala, M. J. Hsu, Antibacterial and antifungal activites of fatty acid methyl esters of the B lind-your-Eye mangrove from India. *Brazilian J. Microbiol* **38**, 739:742, (2007).

[12] G. K. Dash, P. Swresh, S. K. Sahu, O. M. Kar, S. Ganapaty, S. B. Panda, Evalution of *Evavulus alsinoides* Lim. For anthelmintic and antimicrobial activities, *J. Nat. Remedies.* **2**,182-5, (2002).

[13] I. Schlegel, N. T. Doan, N. De Chazol, G. D. Smith, Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *J. Appl. Phycol.* **10**, 471-479, (1999).

[14] J. J. Pignatello, J. Porwoll, R. E. Carlson, A. Xavier, F K. Gleason, J. M. Wood, Structure of the antibiotic cyanobacterin, a chlorine-containing γ-lactone from the freshwater cyanobacterium *Scytonema hofmanni*, *J. Org. Chem.* **48**, 4035-38, (1983).

[15] K. Hirata, J. Takashina, H. Nakagami, S. Ueyama, K. Murakami, T. Kanamori, K. Miyamoto, Growth inhibition of various organisms by a violet pigment, Nostocin A, produced by *Nostoc spongiaeforme*. Biosci. Biotech. Biochem. **60**, 1905-06, (1996).

[16] K. Ishida, H. Matsuda, M. Murakami, K. Yamaguchi, B. Kawaguchipeptin, An antibacterial cyclic undecapeptide from the cyanobacterium *Microcystis aeruginosa*. *J. Nat. Prod*. **60**, 724-726, (1997).

[17] L. Bergman, D. G. A. Ran, Cyanobacterial–plant symbioses: signalling and development, in: A. Herrero, E. Flores (Eds.), The Cyanobacteria, Molecular Biology,Genomics and Evolution, *Caister Academic Press*, Norfolk, UK, pp. 447–473 (2008).

[18] L. J. McGaw, A. K. Jager, J. Vanstadem, Evaluation of antibacterial fatty acidas from Schotia brachypetala, *Fitoter* **73**, 431-433, (2002).

[19] L. J. Wickersham´s, Taxonomy of yeasts. US Dept Agriculture Technical bulletin No.**1029**, 1-56, (1951).

[20] M. A. Browitzka, Microalgae as sources of pharmaceuticals and other biologically active

compounds. J Appl Phycol 7, 3-15, (1995).

[21] M. A. M. Abo-State, Production of Carboxymethyl cellulase by *Fusarium oxysporum* and *Fusarium neoceras* from gamma-pretreated lignocellulosic wastes. Egypt. *J Biotechnol*, **15**, 151-168, (2003).

[22] M. Ishibashi, R. E. Moore, G. M. L. Patterson, Scytophycins, cytotoxic and antimycotic agents from the cyanophyte *Scytonema pseudohofmanni. J. Org. Chem.* **51**, 5300-06, (1986).

[23] M. Plazaa, S. Santoyo, L. Jaime, R. G. Garcia-Blairsy, M. Herrero, F. J. Se˜norans, E. Ibanez, Screening for bioactive compounds from algae. Journal of Pharmaceutical and Biomedical Analysis **51**, 450–455, (2010).

[24] M. T. Cushion, P. D. Walzer, M. S. Collins, S. Rebholz, J. J. E. Vanden, A. Mayence, T. L. Huang, Highly activie anti-preamocystis corinii compounds in library of novel piperozlne-linked bisbenzamidines and related compounds. *Antimicrobial Agents Chemotherapy* **48**, 4209-16, (2004).

[25] N. H. Georgopapadakou, B. A. Dix, S. A. Smith, J. Frrendenberger, P. T. Funke, Effect of antifungal agents on lipid biosynthesis and membrane integrity in *Candida albicans.* Antimicrob. *Agents Chemother*. **3**, 46-51, (1987).

[26] Oxoid, Manual of culture media, ingredients and other laboratory services. Published by Oxoid Limited Wade Road Basingstoke Hampshire, RG24OPW,UK, (1982).

[27] P. A. W. Martin, J. R. Lohr, D. H. Dean, Transformation of *Bacillus thuringiensis* protoplasts by plasmid deoxyribonucleic acid. *J. Bacteriol*. **145**, 980-983, (1981).

[28] P. Bhateja, T. Mathur, M. Pandya, T. Fatma, A. Rattan, Activity of blue green microalgae extracts against in vitro generated *Staphylococcus aureus* with reduced susceptibility to vancomycin, *Fitoterapia* **77**, 233–235, (2006).

[29] R. E. Moore, C. Cheuk, X. G. Yang, G. M. L. Patterson, Hapalindoles, antibacterial and antimycotic alkaloids from the cyanophyte *Hapalosiphon fontinalis.* J Org Chem **52**: 1036-43, (1987).

[30] R. R. R. Kannan, R. Arumugam, P. Anantharaman, In vitro antioxidant activities of ethanol extract from *Enhalus acoroides* (L.F.) Royle, *Asian Pac. J. Trop. Me.* **3(11)**, 898-901, (2010).

[31] R. Rippka, Isolation and purification of cyanobacteria, *Methods Enzymol* **167**, 3-27, (1988).

[32] S. A. Fish, G. A. Codd, Bioactive compound production by thermophilic and thermotolerant cyanobacteria (blue-green algae), *World. J. Microb. Biotech.* **10**, 338-347, (1994).

[33] S. B. Hedges, H. Chen, S. Kumar, D. Y. Wang, A. S. Thompson, H. Watanabe, A genomic time scale for the origin of eukaryotes, *B. M. C. Evolutionary Biology* **1**, 4, (2001).

[34] S. Bhagavathy, P. Sumathi, J. S. Bell, Green algae *Chlorococcum humicola*, a new source of bioactive compounds with antimicrobial activity, *Asian Pacific Journal of Tropical Biomedicine* S1-S7, (2011).

[35] S. C. Larsson, L. Bergkvist, I. Naslund, J. Rutegard, A. Wolk, Vitamin A, retinal, and carotenoids and the risk of gastric cancer: a prospective cohart study, *American Journal of Clinical Nutrition* **85** 497–503, (2007).

[36] S. Kajiyama, H. Kanzaki, K. Kawazu, A. Kobayashi, Nostifungicidine, an antifungal lipopeptide from the field-grown terrestrial blue-green alga *Nostoc commune*, Tetrahedron Lett. **39**, 3737-40, (1998).

[37] S. Kreitlow, S. Mundt, Lindquist, Cyanobacteria—a potential source of new biologically active substances. J. Biotechnol. **70**, 61–63, (1999).

[38] S. M. M. Shanab, Bioactive allelochemical compounds from *Oscillatoria* species (Egptian isolates) *Int. J. Agri. Biol.* **9 (4)**, 617-621, (2007).

[39] S. V. Chauchan, J. B. Marwah, S. N. Bagchi, Effect of an antibiotic from *Oscillatoria* sp. on phytoplankters, higher plants and mice. New Phytol **120**, 251-7, (1992).

[40] V. Gupta, S. K. Ratha, A. Sood, V. Chaudhary, R. Prasanna, New insights into the biodiversity and applications of cyanobacteria (blue-green algae)—Prospects and challenges. *Algal. Res.* **2**, 79–97, (2013).

[41] W. P. Frankmolle, L. K. Larsen, F. R.Caplan, G. M. L. Patterson, G. Knubel, Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa*. l. Isolation and biological properties, *J. Antibiot.* **45** 1451-1457, (1992).

[42] Y. Ghasemi, M. Tabatabaei Yazdi, S. Shokrav, N. Soltani, G. Zarrin, Antifungal and antibacterial activity of Paddy fields cyanobacteria from the North of Iran. *J. Sci. I. R. Iran*, **14**, 203-209, (2003).