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Influence of Nitrogen Source in Culture Media on Antimicrobial Activity of *Microcoleus lacustris* and *Oscillatoria rubescens*

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ABSTRACT

The cyanobacterial, *Microcoleus lacustris* and *Oscillatoria rubescens* were cultivated in three different media, BG-11 ($NaNO_3$ as nitrogen source), modified BG-11 (urea as nitrogen source) and SHU (combined between urea and NH_4Cl as nitrogen sources). The cyanobacterial aqueous, methanol, chloroform and hexane extracts were screened for their antimicrobial activities against four pathogenic bacterial strains *(Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*), one yeast (*Candida albicans*) and two fungal strains (*Aspergillus flavus* and *Aspergillus niger*). Both methanol and hexane extracts of *M. lacustris* from the three media exhibited antibacterial activity against all tested bacteria. While, only methanol of *M. lacustris* from BG-11 medium exhibited activity against tested yeast and fungi. Also, *O. rubescens* methanol, chloroform and hexane extracts exhibited highest antimicrobial activity against all tested microorganisms followed by modified BG-11. Generally, highest antibacterial and antifungal activities of both *M. lacustris* and *O. rubescens* were possessed from extracts cultivated in BG-11 medium.

Keywords: *Microcoleus lacustris*, *Oscillatoria rubescens*, antibacterial, antifungal, nitrogen source.

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INTRODUCTION

Cyanobacteria or blue–green algae are photosynthetic microorganisms commonly found in diverse aquatic environments (Catherine et al., 2013). Cyanobacteria, have received growing attention as producers of a diverse array of biologically active compounds with potential applications in biomedicine, as well as implications for environmental health (Berry et al., 2004). Several strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal, antiviral and anticancer activity (Ayyad et al., 2003). Different important factors are influencing antimicrobial agent production from cyanobacteria such as temperature of incubation, pH of the culture medium, phosphate concentration, incubation period, salinity, medium constituents and light intensity (Ame et al., 2003). Also, carbon and nitrogen sources in culture medium may exhibit significant roles in orienting the secondary metabolites pathway (Noaman et al., 2004).

A large number of cyanobacterial extracts and/or extracellular products have been found to have antimicrobial, *i.e.* antibacterial, antifungal, antialgal and antiprotozoal activity. The identified antibacterial and antifungal substances include fatty acids, phenolics, terpenoids, N-glycosides, lipopeptides, cyclic peptides and isonitrile-containing indole alkaloids (Mo et al., 2009). Secondary metabolites with antibacterial activity are widely produced by Cyanobacteria. These compounds are effective against Gram-positive and/or Gram-negative bacteria (Dixon et al., 2004). Antibacterial effects of extracts from *Fischerella* sp., *Spirulina platensis*, *Anabaena variabilis*, *Nostoc* sp., *Oscillatoria*, *Anabaena* and *Nostoc*, *Synechocystis* and *Synechococcus* and other species belonging to the orders of Chroococales, Pleurocapsales, Oscilatoriales, Nostocales, Stigonematales (Abed et al., 2009; Patil *et al*., 2009).

Frankmölle et al. (1992) reported that crude ethanolic extracts from *Anabaena laxa* inhibited the growth of *Aspergillus oryzae, Candida albicans, Penicillium notatum, Saccharomyces cerevisiae* and *Trichophyton mentagrophytes.* Moon et al. (1992) isolated and determined the structure and fungicidal activity of a compound named calophycin, produced by *Calothrix fusca*. This compound was effective against *Aspergillus oryzae, Candida albicans, Penicillium fiotatum, Saccharomyces cerevisiae, and Trichophyton mentagrophytes.* Smitka et al. (1992) isolated from *Fischerella ambigua* and *Westiellopsis prolifica*, six hapalindole-type alkaloids with fungicidal properties.

Most studies have focused on the antimicrobial activity of different species of cyanobacteria growing in the same media. The main objective of the current work is to study the influence of the nitrogen sources in culture media on the antimicrobial activity of the same cyanobacterial species to select the suitable nitrogen source that enable this species to possess highly effective bioactive substances.

MATERIALS AND METHODS

Cyanobacteria and culture media condition

Cyanobacteria *Microcoleus lacustris* and *Oscillatoria rubescens* were isolated from Wadi El-Rayian, El-Fayioum Governorate. Purification and identification were achieved in Marine Toxins Lab., National Research Centre, Egypt according to Prescott's (1979) and Rippka et al. (1979). Three different culture media were prepared for cultivation of the two cyanobacterial species includes, BG-11 medium, pH 7.1 (Rippka et al., 1979), modified BG-11, pH 7.1 (El-Sayed, 2004) and (SHU) human synthetic urine medium, pH 6.8 (Gordon, 1982).

BG-11 medium is composed of 1.5 g $NaNO_3$; 0.004 g K_2HPO_4; 0.075 g $MgSO_4.7H_2O$; 0.036 g $CaCl_2.2H_2O$; 0.006 g citric acid; 0.02 mg Na_2CO_3; 0.001 g Na_2EDTA; 0.63 g ferric ammonium citrate and 1.0 ml trace elements (TE) in 1000 ml distilled water. TE (g/l) is combined of 2.86 g H_3BO_3; 1.81 g $MnCl_2.4H_2O$; 0.222 g $ZnSO_4.7H_2O$; 0.39g $Na_2MoO_4.2H_2O$; 0.079g $CuSO_4.5H_2O$ and 0.0494g $Co(NO_3)_2.6H_2O$. Modified BG-11 medium is similar to BG-11 medium in its composition with exception of using 0.53g urea (46.5%N) instead of 1.5g $NaNO_3$. SHU medium is composed of 0.5 g $CaCl_2{\cdot}2H_2O$; 4.12 g K_2HPO_4; 0.47 g $MgCl_2{\cdot}H_2O$; 0.29 g KCl; 4.83 g NaCl; 1.55 g NH_4Cl; 2.37g Na_2SO_4; 1.34 g urea; 1.0 g creatinine and 0.65 g sodium citrate (pH 6.8) in 1000 ml distilled water.

Cyanobacterial species were cultivated in 0.5 L Erlenmeyer flasks using Environ-shaker incubator (MP-7552, cv-cc power supply, hsiHefer, Sanfrancisco), with photoperiod of 12 hours light provided by fluorescent lamps at a light intensity of light intensity 440 w/m^2 and temperature 30±2°C. Experiments were initiated with 10% (v/v) inoculum of each species.

Cyanobacterial species were cultivated in 0.5 L Erlenmeyer flasks using Environ-shaker incubator (MP-7552, cv-cc power supply, hsiHefer, Sanfrancisco), with photoperiod of 12 hours light provided by fluorescent lamps at a light intensity of light intensity 440 w/m^2 and temperature 30±2°C. Experiments were initiated with 10% (v/v) inoculum of each species.

Preparation of cyanobacterial extracts

At the stationary phase of growth, 25 days, cyanobacterial cultures of each species were harvested and dried in a hot air oven at 50°C over night. The dried biomass (5g) extracted with different solvent of aqueous, methanol, chloroform and hexane (HPLC grade). The extracts were sonicated for 20 min using ultrasonic microtip probe of 400 watt (GEX 750, USA) and centrifuged at 4500 rpm for 10 min. Supernatant was retained and the pellet re-extracted as before three times. Combined supernatant was evaporated to dryness at 40°C using rotary evaporator. Dried extracts were stored in labeled sterile vials in a refrigerator till further use (Chauhan et al., 2010).

Antimicrobial activity bioassays of the cyanobacterial extracts

Test microorganisms: The antimicrobial activity of the cyanobacterial extracts were carried out on 4 species of pathogenic bacteria, two Gram-positive bacteria, *Bacillus cereus* (EMCC 1080) and *Staphylococcus aureus* (ATCC 13565), two Gram-negative bacteria, *Escherichia coli* 0157 H7 (ATCC 51659) and *Pseudomonas aeruginosa* (NRRL B-272). These bacterial species were obtained from the Holding Company for Biological Products and Vaccines (VACSERA), Egypt.

The yeast *Candida albicans* (ATCC-10231) and two fungal species (*Aspergillus flavus* NRRL 3357 and *Aspergillus niger* ATCC 16888) were used for antifungal assay,. The fungal isolates were obtained from Applied Mycology Dept., Cranfield Unvi., UK. The stock cultures were grown on potato dextrose agar slant at 25°C for 5 days and then kept in refrigerator till use.

Disc diffusion susceptibility assay: The antimicrobial activity of the cyanobacterial extracts were assayed by using agar disc diffusion (Bauer et al., 1966). From the 24 h incubated nutrient agar slant of each bacterial species, a loop full of the microorganism was inoculated in a tube containing 5 ml of tryptic soy broth (BD, Sparks, USA). The broth culture was incubated at 35^{o}C for 2 - 6 h. Petri dishes were prepared with 20 ml nutrient agar (Fluka, BioChemika, Spain) and the bacterial cultures were uniformly spread using cotton swabs. Each extract was dissolved in dimethyl sulfoxide (DMSO) to give 10 mg ml^{-1} for extract. Sterilized filter paper discs (6 mm) were loaded by extracts and dried completely under sterile conditions and the discs were placed on the inoculated plates by using a sterile forceps. DMSO represented as negative control and tetracycline (500 µg ml^{-1}) was used as positive control. Then the plates were incubated at 37°C for 24h and the inhibition zones (mm) were measured.

Spore suspension ($2x10^8$ cfu ml^{-1} of 0.01% Tween 80) of each fungal strain was plated onto potato dextrose agar (PDA) and incubated for 5 days at 25°C. Petri dishes of YES medium were inoculated with 50 µl of each fungal culture and uniformly spread using sterile L- glass rod. Sterilized filter paper discs (6 mm) were loaded with the extracts and dried completely under sterile conditions and the discs were placed on the inoculated plates by using a sterile forceps. Negative control was prepared by using DMSO while commercial fungicide Nystatin (1000 Unit ml^{-1}) was used as a positive control.

The inoculated plates were incubated at 25°C for 24 - 48 h and the antifungal activity was evaluated by measuring the zone of inhibition (mm) against the tested fungus (Medeiros et al., 2011). All treatments consisted of three replicates and the averages of the experimental results were determined.

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RESULTS

Antimicrobial activity of cyanobacterial extracts

Both *Microcoleus lacustris* and *Oscillatoria rubescens* were cultivated in three reported media different in Nitrogen source; these media were BG-11 ($NaNO_3$ as nitrogen source), modified BG-11 (urea as nitrogen source) and SHU (combined between urea and NH_4Cl as nitrogen sources).

Antimicrobial activity *of Microcoleus lacustris* extracts

Antimicrobial activity of *M. lacustris* extracts that grew in BG-11 medium: The antibacterial activities of *M. lacustris* extracts against different species of pathogenic bacteria and fungi are illustrated in Table (1). The methanol and hexane extracts inhibited the growth of all bacterial species under investigation. The highest activity was recorded with hexane extract against *P. aeruginosa* and methanol extract against *B. cereus* which had inhibition zones of 19.7and 19.3 mm, respectively. These zones were higher than those of using tetracycline for the same bacteria as a positive control. The lowest activity (7.0 mm) was recorded with methanol extract against *P. aeruginosa* and aqueous extract against *B. cereus*. The negative control (DMSO) showed no inhibition whereas positive control (tetracycline, 500 µg ml^{-1}) inhibited the growth of all bacterial strain under study.

Also, Table (1) shows the antifungal activity of *M. lacustris* extracts against different species of yeast and fungi. Generally, lower effects were observed when compared with those of bacteria. Methanol extract inhibited the growth of all tested fungal species, while aqueous and chloroform extracts only inhibited the growth of *C. albicans*. The highest antifungal activity (8.2 mm) was of hexane extract against *C. albicans*.

Table 1: Antimicrobial activity of *Microcoleus lacustris* extracts grown in BG-11 medium.

Microorganism	Inhibition zone mm (Mean±*S.E)					
	-ve control	+ve control	Aqueous	MeOH	CH_3Cl	Hexane
Bacteria						
B. cereus	--	16.7±1.1	7.0±0.28	19.3±0.5	--	17.0±1.5
S. aureus	--	17.3±0.7	--	10.5±0.9	7.7±0.32	13.0±2.1
E. coli	--	18.8±1.6	--	9.0±0.5	8.0±0.5	16.2±1.4
P. aeruginosa	--	19.5±0.5	7.3±0.28	7.0±0.28	--	19.7±1.61
Yeast						
C. albicans	--	18.8±0.6	7.5±0.32	7.0±0.5	8.0±0.5	8.2±0.32
Fungi						
A. flavus	--	16.1±0.7	--	7.7±0.28	--	--
A. niger	--	14.5±0.5	--	8.0±0.5	--	7.7±0.5

n=3, *S.E: standard error, --: No inhibition, MeOH: methanol, negative control: DMSO, positive control: tetracycline (bacteria) and Nystatin (fungi).

Antimicrobial activity of *M. lacustris* grown in modified BG-11 medium: The antimicrobial activities of *M. lacustris* extracts growing in modified BG-11 exhibited different trend when compared with BG-11 (Table 2). The methanol and hexane extracts showed antibacterial activity against all bacteria. Although, hexane extract against *P. aeruginosa* and methanol extract against *E. coli* recorded the highest inhibition zones (15.0 and 14.0 mm, respectively), tetracycline exhibited higher effect (19.5 and 18.8 mm, respectively). The lowest activity was recorded with aqueous extract against *P. aeruginosa* with inhibition zones 7.0 mm. All tested bacteria showed resistance against chloroform extract. Concerning to the effect of extracts on fungi, no inhibition was observed using all extracts.

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Table 2: Antimicrobial activity of *Microcoleus lacustris* extracts grown in modified BG-11 medium.

Microorganism	Inhibition zone mm (Mean±*S.E)					
	-ve control	+ve control	Aqueous	MeOH	CH_3Cl	Hexane
Bacteria						
B. cereus	--	16.7±1.1	8.0±0.5	11.2±0.3	--	13.0±0.5
S. aureus	--	17.3±0.7	--	12.5±0.9	--	12.0±1.1
E. coli	--	18.8±1.6	8.2±0.28	14.0±0.6	--	12.5±1.0
P. aeruginosa	--	19.5±0.5	7.0±0.3	10.0±0.3	--	15.0±0.5
Yeast						
C. albicans	--	18.8±0.6	--	--	--	--
Fungi						
A. flavus	--	16.1±0.7	--	--	--	--
A. niger	--	14.5±0.5	--	--	--	--

n=3, *S.E: standard error, --: No inhibition, MeOH: methanol, negative control: DMSO, positive control: tetracycline (bacteria) and Nystatin (fungi).

Antimicrobial activity of *M. lacustris* grown in SHU medium: The antibacterial activities of *M. lacustris* extracts against different species of pathogenic bacteria and fungi are illustrated in Table (3). The methanol and hexane extracts showed antibacterial activity against all bacteria. The highest inhibition zones were obtained by using hexane extract against (*P. aeruginosa* and *E. coli*) and methanol extract against *P. aeruginosa* recording 17.0 mm. These results were very close to those of using a positive control (19 mm). The aqueous extract had an effect only on *E. coli* and *C. albicans* recording inhibition zones 7.2 mm. The highest antifungal activity of *M. lacustris* extracts was showed against *C. albicans* by using chloroform extract which had inhibition zones 8.2 mm. All *M. lacustris* extracts showed bioactivity against *C. albicans*. While, *A. flavus* and *A. niger* showed resistance against all *M. lacustris* extracts.

Table 3: Antimicrobial activity of *Microcoleus lacustris* extracts grown in SHU medium.

Microorganism	Inhibition zone mm (Mean±*S.E)					
	-ve control	+ve control	Aqueous	MeOH	CH_3Cl	Hexane
Bacteria						
B. cereus	--	16.7±1.1	--	10.0±0.3	8.0±0.28	10.0±0.6
S. aureus	--	17.3±0.7	--	12.0±0.5	--	16.0±0.9
E. coli	--	18.8±1.6	7.2±0.14	13.5±0.6	--	17.0±1.2
P. aeruginosa	--	19.5±0.5	--	17.0±1.3	--	17.0±0.5
Yeast						
C. albicans	--	18.8±0.6	7.2±0.2	8.0±0.3	8.2±0.3	8.0±0.3
Fungi						
A. flavus	--	16.1±0.7	--	--	--	--
A. niger	--	14.5±0.5	--	--	--	--

n=3, *S.E: standard error, --: No inhibition, MeOH: methanol, negative control: DMSO, positive control: tetracycline (bacteria) and Nystatin (fungi).

Antimicrobial activity of Oscillatoria rubescens extracts

Antimicrobial activity of *O. rubescens* grown in BG-11 medium: The antimicrobial activities of *Oscillatoria rubescens* extracts against different species of pathogenic bacteria and fungi are illustrated in Table (4). In general the antimicrobial activity of *O. rubescens* extracts was more efficient than that of *M. lacustris*. In addition to methanol and hexane, chloroform extract showed antibacterial activity against all bacteria. The highest activity was recorded with methanol extract against *P. aeruginosa* and *S. aureus* with inhibition zones of 15.0 and 12.5 mm, respectively. Whereas, the lowest activity was recorded by using chloroform extract

against *S. aureus* recording 7.3 mm inhibition zone. All tested bacteria showed resistance against aqueous extract.

Like bacteria, methanol, chloroform and hexane extracts showed antibacterial activity against all tested fungi. The highest antifungal activity of *O. rubescens* extracts was showed against *A. flavus* by using chloroform extract which had inhibition zones 12.2 mm. Whereas, the lowest activity was recorded with aqueous extract against *C. albicans* with inhibition zones 7.5 mm.

Table 4: Antimicrobial activity of *Oscillatoria rubescens* extracts grown in BG-11 medium.

Microorganism	Inhibition zone mm (Mean±*S.E)					
	-ve control	+ve control	Aqueous	MeOH	CH_3Cl	Hexane
Bacteria						
B. cereus	--	16.7±1.1	--	10.3±0.5	9.3±0.3	9.2±0.3
S. aureus	--	17.3±0.7	--	12.5±0.5	7.3±0.2	8.5±0.5
E. coli	--	18.8±1.6	--	9.0±0.3	8.7±0.5	8.3±0.3
P. aeruginosa	--	19.5±0.5	--	15.0±0.8	8.7±0.6	10.7±0.6
Yeast						
C. albicans	--	18.8±0.6	9.5±0.3	9.0±0.5	8.0±0.2	8.2±0.3
Fungi						
A. flavus	--	16.1±0.7	7.5±0.3	10.7±0.5	12.2±0.8	9.7±0.5
A. niger	--	14.5±0.5	--	9.0±0.5	11.3±0.3	7.8±0.2

n=3, *S.E: standard error, --: No inhibition, MeOH: methanol, negative control: DMSO, positive control: tetracycline (bacteria) and Nystatin (fungi).

Antimicrobial activity of *O. rubescens* that grew in modified BG-11 medium: The antimicrobial activities of *O. rubescens* extracts against different species of pathogenic bacteria, yeast and fungi are illustrated in Table (5). The methanol, chloroform and hexane extracts showed antibacterial activity against all bacteria. Also, all tested extracts had antibacterial activity against *B. cereus*. Whereas, the highest activity was recorded with hexane extract against *P. aeruginosa* which had inhibition zones 15.5 mm. The lowest activity was recorded with chloroform extract against *B. cereus* with inhibition zones 7.3 mm.

The highest antifungal activity of *O. rubescens* extracts was showed against *C. albicans* using hexane extract which had inhibition zones 9.2 mm. While, the lowest antifungal activity was showed against *C. albicans* with chloroform extract which had inhibition zones 7.5 mm. All *O. rubescens* extracts showed bioactivity against *C. albicans*. *Aspergillus niger* showed resistance against all *O. rubescens* extracts.

Table 5: Antimicrobial activity of *Oscillatoria rubescens* extracts grown in modified BG-11 medium.

Microorganism	Inhibition zone mm (Mean±*S.E)					
	-ve control	+ve control	Aqueous	MeOH	CH_3Cl	Hexane
Bacteria						
B. cereus	--	16.7±1.1	8.0±0.5	10.0±0.3	7.3±0.2	10.5±0.5
S. aureus	--	17.3±0.7	--	12.0±0.5	8.3±0.28	9.0±0.6
E. coli	--	18.8±1.6	--	11.0±0.6	8.2±0.5	8.5±0.5
P. aeruginosa	--	19.5±0.5	--	13.0±0.6	7.7±0.3	15.5±1.5
Yeast						
C. albicans	--	18.8±0.6	8.5±0.2	8.0±0.3	7.5±0.2	9.2±0.6
Fungi						
A. flavus	--	16.1±0.7	--	7.7±0.2	8.5±0.5	8.2±0.3
A. niger	--	14.5±0.5	--	--	--	--

n=3, *S.E: standard error, --: No inhibition, MeOH: methanol, negative control: DMSO, positive control: tetracycline (bacteria) and Nystatin (fungi).

Antimicrobial activity of *O. rubescens* that grew in SHU medium: Table (6) illustrates the antimicrobial activities of *O. rubescens* extracts against different species of pathogenic bacteria, yeast and fungi. The methanol and hexane extracts showed antibacterial activity against all bacteria. The highest activity was recorded by using methanol extract against *P. aeruginosa* which had inhibition zones 16.0 mm, whereas, the lowest activity was recorded by using chloroform extract against *E. coli* with inhibition zones 7.2 mm.

Very close results were obtained by growing *O. rubescens* in SHU medium when compared with that grown in modified BG-11 notably against yeast and fungi. The highest antifungal activity of *O. rubescens* extracts was showed against *C. albicans* with methanol extract which had inhibition zones 9.0 mm. While, the lowest antifungal activity was showed against *A. flavus* with methanol extract which had inhibition zones 7.2 mm. *Aspergillus niger* showed resistance against all *O. rubescens* extracts.

Table 6: Antibacterial activity of *Oscillatoria rubescens* extracts grown in SHU medium.

Microorganism	Inhibition zone mm (Mean±*S.E)					
	-ve control	+ve control	Aqueous	MeOH	CH_3Cl	Hexane
Bacteria						
B. cereus	--	16.7±1.1	--	9.0±0.3	8.0±0.3	10.0±0.6
S. aureus	--	17.3±0.7	--	12.0±0.6	--	13.0±0.9
E. coli	--	18.8±1.6	--	11.5±0.4	7.2±0.2	11.0±0.6
P. aeruginosa	--	19.5±0.5	--	16.0±1.2	--	10.0±0.3
Yeast						
C. albicans	--	18.8±0.6	7.5±0.3	9.0±0.5	8.0±0.3	8.3±0.4
Fungi						
A. flavus	--	16.1±0.7	--	7.2±0.2	7.7±0.2	8.0±0.3
A. niger	--	14.5±0.5	--	--	--	--

n=3, *S.E: standard error, --: No inhibition, MeOH: methanol, negative control: DMSO, positive control: tetracycline (bacteria) and Nystatin (fungi).

DISCUSSION

Nitrogen is an essential element comprising about 10% of cyanobacterial cell dry weight. Cyanobacteria can utilize different inorganic and organic nitrogen *e.g.* nitrate, nitrite, ammonium, urea, some amino acids and atmospheric nitrogen (Flores and Herrero, 2005; Perez-Garcia et al., 2011). Ammonia is the preferred nitrogen source for cyanobacteria because its uptake and assimilation consumes less energy (Perez-Garcia et al., 2011). However, the cyanobacterial biomass production or the growth rate using ammonia is similar as nitrate when used as nitrogen source (Park et al., 2010) or even lower (Kim et al., 2013; Lin et al., 2007). Serious constraints when using ammonia for cyanobacterial growth are the potential toxicity and its loss from the cultivation media due to volatilization, especially at higher pH values (Markou et al., 2014).

Cyanobacteria can utilize nitrogen from organic forms such as urea and some amino acids. Urea and amino acids are transported actively into the cells and are metabolized intracellularly (Flores and Herrero, 2005; Perez-Garcia et al., 2011). The most significant organic nitrogen form that could be used as nitrogen source for cyanobacteria cultivation is urea. Generally, urea is hydrolyzed to ammonia and carbonic acid which both can be utilized by microalgae and cyanobacteria. The current study investigated the effect of different nitrogen sources, $NaNO_3$, urea and combined between NH_4Cl and urea on the antimicrobial activity of *Microcoleus lacustris* and *Oscillatoria rubescens*.

Alonso et al. (2000) studied the influence of nitrogen concentration in continuous cultivation on lipid concentration in microalga *Phaeodactylum tricornutum*. They noted that there was an accumulation of saturated and unsaturated fatty acids when the nitrogen source was reduced. Olaizla (1998) reported that the form of the nitrogen source may influence in accumulation rate of secondary carotenoids in microalgae. *Haematococcus* accumulated astaxanthin rapidly if sodium acetate or glycine (amino acid) was supplied in the medium, whereas astaxanthin accumulation was delayed for some weeks if other organic or inorganic nitrogen

sources were supplied. Liu and Lee (2001) indicated that urea is the best nitrogen source for growing of *Chlorella* sp. and *Scendesmus* spp. to produce cell with high carotenoids contents.

Maximum antimicrobial activity by *Micrococoleus lacustris* and *Oscillatoria rubescens* extracts was recorded with BG-11 medium that content sodium nitrate as a source of nitrogen. Thummajisakul et al. (2012) reported that *Micrococoleus* sp. extracts that cultivated in BG-11 medium showed antibacterial activity against *S. enteritis* and *E. coli*. Other microalgae were tested for their antimicrobial activities in different media.

Prosperi et al. (1992), Liotenberg et al. (1996) and Asthana et al. (2006) revealed that the nutrient medium can affect on the growth, metabolism, phycobiliprotein and anti microbial properties. Also, Soltani et al. (2007) indicated that nitrogen source in culturing medium effect on growth rate, chlorophyll and phycobiliproteins content in *Fisherella* sp.

Shaieb et al. (2014) study the impact of various nitrogen concentrations on the growth rate, generation time and antimicrobial activity of cyanobacteria *Nostoc mucorum* and *Spirulina platensis*. They found that there was no obvious trend between the nitrogen concentration and the antimicrobial activity of *N. mucorum*. While, there was a closer relationship between the nitrogen concentration and the antimicrobial activity of *S. platensis*. They found that aqueous and ethanol extracts of *S. platensis* in high nitrogen supplemented medium had wide range of antibacterial activity against *S. aureus*, *E. coli*, *B. cereus* and *K. pneumonia* in addition to the fungus *A. flavus* in comparison to control as nitrogen deprived.

In the present study the highest antimicrobial activity by *Micrococoleus lacustris* and *Oscillatoria rubescens* extracts was recorded with BG-11 medium that contain sodium nitrate as a source of nitrogen, the obtained result was in agreement with that obtained by Noaman et al. (2004) who found that *Synechococcus leopoliensis* grown in BG-11 medium possessed the highest antimicrobial activity when compared to the others media applied (Chu 10 and G medium). *Micrococoleus* sp. extracts that cultivated in BG-11 medium showed antibacterial activity against *S. enteritis* and *E. coli* (Thummajisakul et al., 2012).

CONCLUSION

Nitrogen source in the culture media of caynobacteria may play an important role in orienting the secondary metabolites pathway and hence influence the antimicrobial activities of the cyanobacterial extracts. In the current study two caynobacterial species, *Microcoleus lacustris* and *Oscillatoria rubescens*, were cultivated in three different reported media; BG-11 ($NaNO_3$ as nitrogen source), modified BG-11 (urea as nitrogen source) and SHU (combined between urea and NH_4Cl as nitrogen sources). The *Microcoleus lacustris* and *Oscillatoria rubescens* cultivated in BG-11 medium were possessed the highest antibacterial and antifungal activities in their extracts. The results obtained suggested that BG-11, containing $NaNO_3$ as nitrogen source, is a suitable medium that enable *Microcoleus lacustris* and *Oscillatoria rubescens* to possess highly effective bioactive substances.

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