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Phytochemical and Antibacterial Study of Five Freshwater Algal Species

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Abstract: A phytochemical study of five freshwater algal species isolated from an Egyptian water station and comparing their inhibition activities against three selected bacterial pathogens in order to correlate the biological activity and the chemical constituents of the algae. Five freshwater algal species, *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica* and *Spirulina platensis* (blue-green algae, Cyanobacteria) and *Cosmarium leave* (green algae) were isolated from an Egyptian water station and purified using BG11 media and cultivated. The alcoholic and the aqueous extracts of the five species were evaluated for their inhibitory effect against three bacterial pathogens: *Escherichia coli, Salmonella* Typhimurium and *Streptococcus faecalis* using poured plate method. A comparative phytochemical study was performed to detect the main active components of the tested extracts. The obtained results revealed that both the MeOH and the aqueous extracts of *Spirulina* showed noticeable inhibitory activity against the three bacterial strains: 91.6% (0.7 mg mL^{-1}), 86.2% (0.5 mg mL^{-1}) and 100% (0.3 mg mL^{-1}) for MeOH. and 74.4% (0.9 mg mL^{-1}), 99.3% (0.9 mg mL^{-1}) and 72.6% (0.1 mg mL^{-1}) for H_2O against *E. coli*, *Salmonella* and *Streptococcus*, respectively in comparison with other algal extracts. The latter exerted different inhibition activities depending on the types of the bacteria and the extract. It could be concluded that the antibacterial activity was strongly correlated with the quercitin and pigment contents for the MeOH extract and the carbohydrates and pigments contents for the aqueous extract.

Key words: Freshwater, algae, antibacterial, bioactive compounds

INTRODUCTION

Microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to be produced by chemical synthesis (Borowitzka and Borowitzka, 1989; Goud *et al*., 2007). Nowadays, there is a marked trend in the food industry towards the development and manufacture of functional products (Uccella, 2000; Rodriguez-Meizoso *et al*., 2008). Also as pharmaceuticals and nutraceuticals (Chu and Radhakrishnan, 2008; Kasinathan *et al*., 2009). Many bioactive compounds in microalgae have unique and interesting structures and functions. Inhibitory activities against growth of microorganisms and development of animal and plant cells are common indicators for screening antibacterial, antifungal, antiviral, cytotoxic and antitumor substances (Borowitzka, 1995; Kulik, 1995; Febles *et al*., 1995). Cyanobacterial pigments are not only used as nutritional ingredients and natural dyes for food and cosmetics but also used as pharmaceuticals and fluorescent markers in biomedical research (Branen *et al*., 2002; Shimizu, 2003; Venugopal *et al*., 2005). The ability to produce antimicrobial substances could be used not only as a defensive agent against pathogens but also as pharmaceutical bioactive natural compounds. The aim of this research is to study the antibacterial activity of both methanol and water extracts of the five fresh water algal species and makes a correlation between the chemical constituents and the biological activity.

MATERIAL AND METHODS

Isolation and purification of microalgae species: Five algal species *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica*, *Cosmarium leave* and *Spirulina platensis* were isolated from phytoplankton community structure of River Nile. Algal identification has been carried out according to the keys of identification (Hustedt, 1976; Komarek and Anagnostidis, 1989). Algal isolation and purification were carried out using BG11 media (Carmichael, 1986).

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Cultivation of the isolated strains: Cultivation was carried out in sterilized 5 L conical shoulder flasks containing 3 L of the corresponding culture medium under continuous aeration and continuous illumination. The cultivation time differed from one strain to another depending on the optimum growth rate and it always ranged between 10-15 days.

Preparation of algal extracts for antibacterial testing: Twenty five grams of each of the five powdered algal species were extracted several times with methanol till exhaustion to yield five methanolic extracts: for *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica*, *Spirulina platensis* and *Cosmarium leave*. The residues left were extracted with distilled H_2O at 50°C to give five aqueous extracts: for *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica*, *Spirulina platensis* and *Cosmarium leave*.

The inhibitory effect was performed on three bacterial strains from the Culture Collection of Bacteriological Lab, Water Pollution Research Department, National Research Center. Two gram-ve *Escherichia coli* and *Salmonella* Typhimurium (ATCC 6538) and one gram+ve *Streptococcus faecalis* (ATCC 43845) bacteria. The antibacterial effects of different concentrations of algal extracts were carried out against bacterial strains 24 h aged inoculated in Tryptone Soya Broth (TSB) and incubated at 37°C for 17-24 h. One milliliter from each culture was transferred into 9 mL (0.9% NaCl solution) and diluted to 10^5 CFU (Colony forming Unit) mL^{-1}. Methanol and water extracts were impeded in different percentile concentrations (0.1, 0.3, 0.5, 0.7 and 0.9 mg mL^{-1}) using pour plate method according to APHA (2005).

Experimental procedure: In two sterile tubes, the following constituents were mixed together: 10 mL of Mueller-Hinton Agar (MHA) kept at 45°C, 1 mL of 10^5 CFU 1 mL^{-1} bacterial cultures, algal extract concentration, the whole constituents mixed together and poured in a sterile Petri dish, after solidification, plates were inverted and incubated at 37°C for 17-24 h. Bacterial colonies were counted and compared to the counts of control plates. Inhibition effects were calculated as a factor related to algal extract concentration. The algal water extracts were sterilized by filtration through 0.45 μ membrane before testing.

Media used: Muller- Hinton Agar and Tryptone Soya Broth (TSB) media were used throughout this investigation. pH should be 7.4±0.2 after autoclaving at 121°C for 15 min.

HPLC Determination of quercetin content: The identity of quercetin content was obtained out by using authentic standard and by comparing the retention times and UV-visible spectra. Concentration of the quercetin content was calculated from integrated areas of the sample and corresponding standard.

Absorbance at 200-600 nm of the stock solutions were scanned on UV-Vis Shimadzu Spectrophotometer (UV-1601 PC) spectrophotometer equipped with 1 cm quartz cuvette. Samples were run through a HPLC system (Agilent 1100 series) coupled with UV-Vis detector (G1315B) and G1322A DEGASSER. Sample injections of 10 μL were made from an Agilent 1100 Series auto-sampler; the chromatographic separations were performed on ZORBAX-EclipseXDB-C18 column (4.6×250 mm, particle size 5 μm).

Optimum efficiency of separation was obtained using 0.35 mL min^{-1} of pH 2.5 sulphuric acid (solvent A) and the flow-rate of methanol (solvent B) was increased from 0 to 0.45 mL min^{-1} from 15-40 min and kept at 0.45 mL min^{-1} for a period of 5 min and then reduced to initial conditions in another 5 min. Ten minutes of equilibration is required before the next injection. Other parameters adopted were as follows: injection volume, 20 μL; column temperature, 400°C; detection wavelength, 280 nm.

Determination of pigments in the isolated strains

Phycocyanin: (Silveira *et al*., 2007).

One gram of dried algal cells was mixed with 10 mL dist. H_2O. Samples were placed at rotary shaker at 30°C. Samples were collected at 24, 48, 72 h:

$$P.C. = A\ 615 - 0.474\ A\ 652/5.34$$

where, P.C.: Phycocyanin content, A: absorbances at 615, 652 μm.

Carotenoids: (Shaish *et al*., 1992).

One mL cell suspension centrifuged at 1000 rpm for 5 min. The pellets were dissolved in 3 mL (Ethanol: Hexane 2:1) and 2 mL distilled H_2O and 4 mL hexane, then shaking and centrifugation at 1000 rpm for 5 min. The absorbance of hexane layer was read at 450.

Chlorophyll a content: For maximum standing biomass production, chlorophyll a determination takes place. The fresh sample (25 mL) of each strain was taken every 48 h and filtered through 0.45 μm membrane filter and extracted with hot methanol (Fitzgerald *et al*., 1971) after the addition of 0.5 mL magnesium carbonate solution (1%) in order to prevent chlorophyll degradation.

After the algal sample filtration, the membrane filter was immediately soaked in little amounts (2-3 mL) of hot methanol 90% for two minutes. Soaking was repeated till complete extraction was assured. The extract was completed by methanol to a known volume, then centrifuged for 10 min at 2000 rpm. The clear extract was transferred to a 1 cm cuvette and absorbance at 664, 647 and 630 nm was determined spectrophotometrically. The following equation was used for calculating the concentration of chlorophyll a (as $\mu g\ L^{-1}$):

$$C\ a = 11.85\ (A664)-1.54\ (A647)-0.08\ (A630)$$

Chlorophyll a $\mu g\ L^{-1}$ = Ca×extract volume, (L) volume of sample, (L)

where, A664, 647 and 630 are the absorbance at 664,647 and 630 nm.

Total protein content: Total protein content was determined by Micro-Kjledahl method and then multiplied with a factor 6.25 to give the total protein content according to Chapman and Pratt (1978), as follows:

Catalyst: The 10 g K_2SO_4+1 g $CuSO_4.5H_2O$+0.5 g selenium.

Tashiro's indicator: Methylene blue 0.24 g+0.375 g methyl red in 30 mL methyl alcohol.

Sample analysis: To 0.2 g of sample, 0.5 g of catalyst and 2 mL H_2SO_4 was added. The mixture was digested until the color becomes clear. Two drops of methyl orange indicator and 15 mL of 40% NaOH were added to clear sample and transferred to distillate apparatus. The liberated ammonia is received in 10 mL of 4% boric acid and 2 drops of Tashiro's indicator until the volume reached 50 mL. Titrate against 0.01 N HCl.

Determination of amino acids using amino acid analyzer: Condition of Amino Acid Analyzer LC300 (in Central Lab, National Research Center (Eppendorf- Germany); Flow rate: 0.2 mL min^{-1}; Pressure of buffer: from 0 to 50 bar; Pressure of reagent: from 0 to 150 bar; Reaction temperature: 123°C.

Acid hydrolysis: One mL of 6 N HCl was added then the sample solution was freeze dried (Bhushan, 1991). The hydrolysis tube was sealed and placed in an oven at 110°C for 24 h; cooled, centrifuged in order to precipitate insoluble components. The supernatant was evaporated at approximately 40°C in a rotary evaporator, dissolved with approximately 1 mL of distilled water and evaporate once again in order to remove traces of acid. Dissolve the sample with 1-2 mL of the sample diluting buffer.

Determination of total carbohydrate content: The 0.1 g of sample, 25 mL of 1 N H_2SO_4 was added and the mixture was hydrolyzed for 2 h on a boiling water bath. At the end of hydrolysis a flocculent precipitate was noticed. This was freed of sulphate by precipitation with barium carbonate. Filter and complete to 100 mL. One milliliter of filtrate mixed with 1 mL 5% phenol and 5 mL conc. H_2SO_4 measured at 485 nm (DuBois *et al*., 1956).

Extraction of polysaccharide (Fischer *et al*., 2004): Five grams of algal powder of each species was separately mixed with 50 mL distilled water slightly acidified with HCl, stirred 12 h and left to stand for another 12 h. The solution was passed through folded muslin. The process was repeated three times.

The polysaccharide was precipitated from the aqueous extract by adding, slowly while stirring, 4 volumes of ethanol 95% and ethanol-acetone mixture (1:1). The precipitate obtained by centrifugation was washed several times with ethanol till free of chloride ions. The polysaccharide was then stirred in acetone, filtered and dried in vacuum dissector.

Test for the identity of the isolated polysaccharide

Reaction with potassium hydroxide: To 5 mL of the 1% aqueous solution of each precipitate, 1 mL of 2% aqueous potassium hydroxide was added and the mixture was allowed to stand at room temperature for 15 min, a gelatinous precipitate appeared, indicating the pectic nature of the polysaccharide (Amin and Paleologou, 1973).

Acid hydrolysis: To 0.1 g of the powder of each polysaccharide for the five species under investigation was, separately, heated in 2 mL 0.5 M H_2SO_4 in a sealed tube for 20 h on a boiling water bath. At the end of hydrolysis a flocculent precipitate was noticed. This was filtered off and the filtrate was freed of SO_4 by precipitation with barium carbonate (Chrums and Stephen, 1973).

HPLC analysis of sugars: Juice samples were filtered through a 0.45 μm membrane. Analysis of the carbohydrate in the filtrate was performed by using HPLC, Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A Shimadzu detector, L-C-16ADVP binary pump and PL Hi-Plex Pb column, heater set at 80°C.

The mobile phase was 0.01% reagent grade calcium chloride prepared with deionized water and the flow rate was 0.6 mL min^{-1}.

Standard solutions of individual sugars: glucose, xylose, sucrose, fructose, glucuronic acid, fucose, galactose and galacturonic acid (each of analytical grades) were prepared by placing 2 g of each in 100 mL volumetric flask and diluting to volume with deionized water. Injection volume of each standard was 20 μL.

RESULTS

The inhibition percentages of the antibacterial spectrum of methanol algal extracts were showed in Table 1. Results revealed that MeOH extracts of *Anabaena*, *Chroococcus* and *Spirulina* revealed the highest percentage of inhibition 95, 95, 91.6%, respectively at 0.7 mL concentration, while MeOH extract of *Chroococcus* showed the highest inhibitory effect 92.6% at concentration 0.5 mL. MeOH of *Spirulina* and *Oscillatoria* had the highest inhibitory effect with a percentage of 86.2 and 98.5%, respectively at 0.5 mL concentration on *Salmonella* Typhimurium. The percentages of inhibition of algal extracts against *Streptococcus faecalis* showed that MeOH of *Spirulina* had the maximum percentage of inhibition 100% followed by MeOH of *Chroococcus* (94.7%), *Oscillatoria* (93.3%), *Anabaena* (91%) and *Cosmarium* (42.9%) at 0.3 mL concentration.

The percentages inhibition of water algal extracts on *Escherichia coli* are shown in Table 2. Algal water extract inflict against gram-ve and gram+ve bacteria revealed inhibitory effect in all concentrations studied (Table 2). Aqueous of *Chroococcus* showed the highest percentage of inhibition which is 74.4% at 0.9 mL concentration. Simultaneously, aqueous of *Anabaena*, *Spirulina* and *Oscillatoria* attained percentage of inhibition 69.8, 58.5 and 29.8%, respectively and no effect was noticed for aqueous of *Cosmarium*.

Regarding the inhibitory effect of water algal extract on *Salmonella* Typhimurium, Table 2 showed that the maximum inhibitory effect for the aqueous extract

Table 1: Percentage of inhibition of antibacterial spectrum of different concentrations of algal methanol extracts using gram-ve and gram+ve bacteria

Bacteria	Algal isolates	Control	Methanol extract	Inhibition (%)
Escherichia coli		Concentrations (0.5 mg mL^{-1})		
	Anabaena sphaerica	$1.2\times10^{8}\pm0.3$	$3.0\times10^{7}\pm0.4$	75.0
	Chroococcus turgidus	$2.3\times10^{8}\pm0.35$	$1.7\times10^{8}\pm0.1$	92.6
	Oscillatoria limnetica	$9.8\times10^{7}\pm0.7$	$4.0\times10^{7}\pm0.28$	59.2
	Spirulina platensis	$8.5\times10^{7}\pm0.14$	$2.7\times10^{7}\pm0.3$	68.2
	Cosmarium leave	$2.0\times10^{8}\pm0.0$	$2.0\times10^{8}\pm0.0$	0.0
Salmonella Typhimurium	*Anabaena sphaerica*	$9.3\times10^{6}\pm0.05$	$8.0\times10^{6}\pm0.1$	13.9
	Chroococcus turgidus	$9.3\times10^{7}\pm0.1$	$8.4\times10^{7}\pm0.1$	9.7
	Oscillatoria limnetica	$4.0\times10^{7}\pm0.2$	$6.0\times10^{5}\pm0.1$	98.5
	Spirulina platensis	$6.3\times10^{7}\pm0.1$	$8.7\times10^{6}\pm0.3$	86.2
	Cosmarium leave	$4.0\times10^{7}\pm0.28$	$2.1\times10^{8}\pm0.1$	27.5
Streptococcus faecalis		Concentrations (0.3 mg mL^{-1})		
	Anabaena sphaerica	$3.8\times10^{7}\pm0.1$	$3.4\times10^{6}\pm0.4$	91.0
	Chroococcus turgidus	$3.8\times10^{7}\pm0.3$	$2.0\times10^{6}\pm0.1$	94.7
	Oscillatoria limnetica	$3.0\times10^{7}\pm0.3$	$2.0\times10^{6}\pm0.0$	93.3
	Spirulina platensis	$3.0\times10^{7}\pm0.3$	0.0	100.0
	Cosmarium leave	$7.0\times10^{7}\pm0.3$	$4.0\times10^{7}\pm0.4$	42.9

Table 2: Percentage of inhibition of antibacterial spectrum of different concentrations of water algal extracts using gram-ve and gram+ve bacteria

Bacteria	Algal isolates	Control	Water extract	Inhibition (%)
Escherichia coli		Concentrations (0.9 mg mL^{-1})		
	Anabaena sphaerica	$4.3\times10^{8}\pm0.1$	$1.3\times10^{8}\pm0.05$	69.8
	Chroococcus turgidus	$4.3\times10^{8}\pm0.1$	$1.1\times10^{8}\pm0.5$	74.4
	Oscillatoria limnetica	$9.5\times10^{7}\pm0.07$	$6.7\times10^{7}\pm0.3$	29.8
	Spirulina platensis	$4.1\times10^{8}\pm0.7$	$1.7\times10^{8}\pm0.5$	58.5
	Cosmarium leave	$4.1\times10^{7}\pm0.4$	$3.0\times10^{8}\pm0.0$	0.0
Salmonella Typhimurium	*Anabaena sphaerica*	$4.5\times10^{7}\pm0.06$	$6.1\times10^{6}\pm0.4$	86.4
	Chroococcus turgidus	$4.5\times10^{7}\pm0.07$	$2.2\times10^{6}\pm0.4$	95.1
	Oscillatoria limnetica	$6.4\times10^{8}\pm0.7$	$2.6\times10^{8}\pm0.4$	59.4
	Spirulina platensis	$6.9\times10^{8}\pm0.07$	$4.6\times10^{6}\pm0.3$	99.3
	Cosmarium leave	$6.4\times10^{8}\pm0.7$	$2.3\times10^{9}\pm0.1$	0.0
Streptococcus faecalis	*Anabaena sphaerica*	$1.3\times10^{8}\pm0.1$	$1.1\times10^{7}\pm0.07$	91.5
	Chroococcus turgidus	$1.3\times10^{8}\pm0.1$	$5.1\times10^{7}\pm0.1$	60.2
	Oscillatoria limnetica	$1.5\times10^{8}\pm0.07$	$3.9\times10^{7}\pm0.07$	74.0
	Spirulina platensis	$1.5\times10^{8}\pm0.07$	$3.1\times10^{7}\pm0.3$	79.3
	Cosmarium leave	$3.8\times10^{7}\pm0.1$	$1.5\times10^{7}\pm0.0$	60.5

of *Spirulina* is followed by the aqueous extract of *Chroococcus*, *Anabaena* and *Oscillatoria* with percentages of 99.3, 95.1, 86.4 and 59.4%, respectively at 0.9 mL concentration and no effect for aqueous of *Cosmarium*.

All algal strains have influence inhibitory activity against *Streptococcus faecalis*. Aqueous of *Anabaena* showed the highest percentage of inhibition reached 91.5% at 0.7 and 0.9 mL concentration while aqueous of *Spirulina*, *Oscillatoria*, *Cosmarium* and *Chroococcus* had inhibitory effect at 0.9 mL concentration with percentage of 79.3, 74, 60.5 and 60.2%, respectively. It was noticed that the aqueous of *Spirulina* showed pronounced antibacterial activity, so, it was subjected to testing its effect on algal community assemblages (groups of diatoms, green algae and blue green algae).

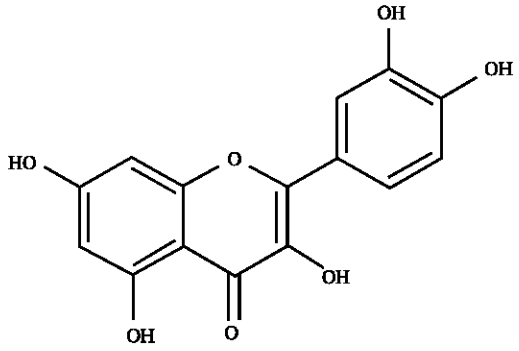
From the above results we revealed the pronounced antibacterial activity of both MeOH and aqueous extracts of *Spirulina* was against all the tested bacterial strains when compared to other algae.

Quercitin content: The results of quercetin (Fig. 1) content demonstrated in Table 3 illustrated that *Spirulina platensis* had the highest quercetin content (40 mg L^{-1}) in comparison with other algal species. Simultaneously, *Chroococcus turgidus* can produce total quercetin content (30 mg L^{-1}) followed by *Oscillatoria limnetica* (20 mg L^{-1}), while *Cosmarium leave* and *Anabaena sphaerica* showed the lowest quercetin content (2 and 0.5 mg L^{-1}, respectively).

Pigment content of the candidate species

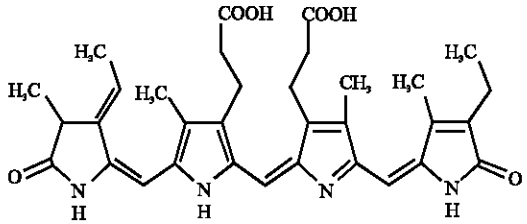
Phycocyanin content: The measurement of phycocyanin pigment (Fig. 2) in blue green algal strains was explained in Table 3. The results showed that the highest phycocyanin content was detected in *Spirulina platensis* (4.38 mg mL^{-1}). In addition the other blue-green algal strains showed no pronounce difference in phycocyanin content and it amounted to 0.5, 0.3 and 0.18 mg mL^{-1} for *Anabaena*, *Chroococcus* and *Oscillatoria* respectively and absent from *Cosmarium leave*.

Total carotenoid content: Determination of carotenoid content emphasizes the same observation as chlorophyll a content where the candidate species differed in its carotenoid content in spite of the algal groups. Table 3 showed the most pronounced carotenoid content in *Spirulina platensis* (1400 mg L^{-1}). Although, *Oscillatoria limnetica* belong to the same algal group as *Spirulina* it produced the lowest carotenoid content (70 mg L^{-1}). The



Quercetin

Fig. 1: Structure of quercetin



C-phycocyanin

Fig. 2: Structure of C-phycocyanin

descending order of carotenoid production of candidate species are *Spirulina*<*Chroococcus*<*Anabaena*< *Cosmarium* <*Oscillatoria.*

Chlorophyll a content: Table 3 revealed that *Cosmarium leave* and *Chroococcus turgidus* continue to grow up to 16th days with maximum chlorophyll a content reached 7718 and 3981 μg L^{-1}, then both species growth began to stationary phase. In addition, *Anabaena sphaerica* the maximum standing biomass of *Anabaena* was attained after 6 days of cultivation. Chlorophyll a content at maximum growth phase amounted to 3162.3 μg L^{-1}, after that the alga growth rate start in decline and stationary phase with biomass content reached 2511.4 μg L^{-1}. *Oscillatoria* growth rate measured as chlorophyll a content showed maximum value at 10th day of culture and reached 401.23 μg L^{-1}. The growth rate start in stationary phase with no decline phase up to 10th day. Furthermore, *Spirulina platensis* chlorophyll a content attain its maximum value 2511.9 μg L^{-1} at 8th day, then the alga growth rate start in decline and stationary phase yielding biomass reached 1995.3 μg L^{-1}.

From the above mentioned data it can be concluded that the maximum biomass (measured as chlorophyll a content) and growth stages differed from one algal strain to another also among the same algal group.

Table 3: Active secondary metabolites contents of all studied species algal species

	Concentrations					
		Pigments				
Algae	Quercetin (mg L^{-1})	Phycocyanin (mg mL^{-1})	Carotenoid (mg L^{-1})	Chlorophyll a (µg L^{-1})	Total carbohydrates (mg L^{-1})	Total protein (mg L^{-1})
Cosmarium leave	2	0.0	88±0.14	7718.9 (16th day)	1.9±0.1	0.36±0.49
Anabaena sphaerica	0.5	0.5±0.04	240±1.4	3162.3 (6th day)	1.1±0.001	0.54±0.35
Chroococcus turgidus	30	0.3±0.001	1100±0.001	3981.1 (16th day)	1.5±0.1	0.31±0.5
Oscillatoria limnetica	20	0.18±0.05	70±0.5	401.23 (10th day)	0.53±0.08	0.15±0.0
Spirulina platensis	40	4.38±0.04	1400±0.07	2511.9 (8th day)	3.2±0.2	0.35±0.2

Carbohydrate content: Total carbohydrate content and different sugars represent the polysaccharide content were measured to the investigated algal species. Table 3 reveals the concentration of total carbohydrate content of each algal strain and it emphasizes that *Spirulina platensis* had the highest carbohydrate content (3.2 mg L^{-1}). Where, *Cosmarium leave* and *Chroococcus turgidus* had total carbohydrates content equal to each other approximately (1.9 and 1.5 mg L^{-1}, respectively). In addition, *Anabaena* and *Oscillatoria* yield the lowest total carbohydrate concentration (1.1 and 0.53 mg L^{-1}, respectively).

Referring to the different sugars content of polysaccharide, results showed that the units of sugars were glucose, galactose, mannose, fructose, xylose, galacturonic acid, sucrose and fucose. Furthermore glucose sugar was the main polysaccharide unit present in all algal species and its concentration differed from one species to another. Although, glucose concentration in *Chroococcus* reached to 45.8 g L^{-1} while in *Cosmarium* it reached to 1.1 g L^{-1}, Fructose sugar is the second type of polysaccharide unit present in all algal species but with varying concentrations different from one species to another. The polysaccharide unit of *Cosmarium leave* represented by three types of units, glucose, galactose and fructose. In general, the polysaccharide units of the other algal strains (blue- green algae) are:

- *Anabaena sphaerica*: glucose, galactose, fructose and sucrose
- *Chroococcus turgidus*: glucose, fructose, xylose, galacturonic acid and fucose
- *Oscillatoria limnetica*: glucose, mannose, fructose, galacturonic acid and fucose
- Finally *Spirulina platensis*: glucose, galactose, fructose and fucose

Total protein content: The results of total protein content registered in Table 3 which explaining that *Anabaena sphaerica* having the highest protein content compared to other algal species. Simultaneously, *C. leave* can produce total protein content nearly equal to that produced by *S. platensis* and *Chr. Turgidus* (0.36, 0.35 and 0.31 mg L^{-1}, respectively). Moreover, *Oscillatoria limnetica* revealed the lowest protein content (0.15 mg L^{-1}) (Table 3).

DISCUSSION

Microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to be produced by chemical synthesis (Borowitzka and Borowitzka, 1989; Goud *et al*., 2007; Kaushik and Chauhan, 2008). Most of those compounds are accumulated in the microalgal biomass; others are excreted during growth into the environment (Jaki *et al*., 2000; Jaki *et al*., 2001). Algal extracts were found to have antibacterial properties against three bacterial strains *Escherichia coli* and *Salmonella Typhimurium* and *Streptococcus faecalis*. It was reported that *Oscillatoria* sp., *Phormidium* sp. and *Lyngbya majuscula* have antibacterial effect against human pathogenic bacteria such as *Streptococcus mutants*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumonia* (Sethubathi and Prabu, 2010).

It was also reported that the phenolic content are active as antibacterial against different types of microorganisms like *Salmonella typhi* (Ouattara *et al*., 2011) and the flavonoids are reported that they are active against several strains like *Streptococcus* (Shu *et al*., 2011); *E. coli* and *Staphylococcus aureus* (Gao and Zhang, 2010). Quercitin compound has antibacterial activity against *E. coli* (Rattanachaikunsopon and Phumkhachorn, 2010). which is in agreement with our finding.

In contrast, methanol extracts of *Oedogonium* sp., *Ulothrix* sp. and *Oscillatoria* sp. showed no inhibitory effects against gram-ve bacteria *Escherichia coli* at the same concentration Goud *et al*. (2007). This indicated that the ability to affect bacteria was considered to be species dependant.

Sabarinathan and Ganesan (2008) evaluated the antibacterial effect of Phycocyanin pigment and proved

its safety. Results of present study showed that the Phycocyanin content of *Spirulina* was the highest (4.38 mg mL^{-1}) in comparison with other algal species.

It was also reported that *E. coli* and *Staphylococcus* are sensitive to polysaccharides (Li-Ya- and Chang-Hong, 2010) and *Spirulina* showed also, the highest content of carbohydrates (3.2 mg L^{-1}).

Furthermore, the results showed that methanol extract of the selected algal species had inhibitory activities against gram+ve bacteria and the results of Tuney *et al.* (2006) showed that the methanol extract of *Gracilaria gracilis* exerted inhibitory effects against gram+ve bacteria *Streptococcus epidermidis* at a concentration of 25 μL. The antimicrobial activity of *Trichodesmium erythraeum*, a genus of filamentous cyanobacteria, showed an inhibitory effect against gram+ve bacteria *Enterococcus faecalis* and *Bacillus subtilis* at a concentration 0.315 μg mL^{-1} (Kasinathan *et al*., 2009).

Many algal strains were examined to discover the effect of their extracts on *Salmonella* species. Umamaheshwari *et al.* (2009) found that methanol extract of *Halophila ovalis* exerted antibacterial effects against *Salmonella typhi* and *Salmonella paratyphi*-B. The results of Goud *et al.* (2007), showed that methanol extracts of several species of freshwater algae including *Nostoc* sp., *Lyngbya* sp., *Anabaena* sp. and *Mougeotia* sp. exerted antibacterial activity against Gram-ve bacteria *Salmonella* Typhimurium. In contrast, methanol extract from other freshwater microalgal species such as *Phormidium* sp., *Cladophora* sp. and *Oscillatoria* sp. showed no inhibitory effects against *Salmonella* Typhimurium at concentration 50 mg mL^{-1}.

In addition, results of the effect of water extracts of the selected algal species showed antibacterial activities against selected bacterial strains. It is clear that water extracts showed inhibitory effects lower than that of methanol. These results are in harmony with the finding of Goud *et al.* (2007) and Sethubathi and Prabu (2010).

From the presented results it could be concluded that, the antibacterial activity of the algae depends on the content of quercitin, phycocyanin pigments for the alcohol extracts and polysaccharides content for the water extracts of the species and the type of bacterial strains. *Spirulina* could be used for management of gram+ve and gram-ve microbial infections. For other algae more work is required for more specifications for their activities.

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