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Original Research Article

Antioxidant capacity and Total phenolic compounds of some algae species (Anabaena and Spirulina platensis).

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Abstract: The contents of phenolic compounds and antioxidant capacity were determined in two algae species (Anabaena and Spirulina platensis). The algae samples were growth in laboratory in different types of saline solutions .Simple , rapid and sensitive methods were applied in this study to determine the selective parameters .The data recorded that the antioxidant activity values were : (4.05 and 6.30 ppm) and the phenolic compounds were (33.52 and 28.76 ppm) for Anabaena and Spirulina platensis , respectively.
Keywords: Anti-oxidant – phenolic, Algae species

INTRODUCTION

The growing interest in natural foods has raised the demand for natural ingredients that in addition to give basic nutritional and energy are capable to contribute with additional physiological profits as antioxidants, antimicrobial, etc, that is, functional foods or nutraceuticals. In this work, the microalgae are investigated as natural source of antioxidants, an important kind of compounds for the food industry because of their usefulness as a preservation method and their known beneficial effects for health. One of the research lines of our group is to obtain and characterize natural compounds with antioxidant properties which can be used as ingredients in the food industry [1-5].

Phenolic compounds:-

Microalgae are microscopic organisms capable to convert solar energy to chemical energy via photosynthesis. They contain numerous bioactive compounds that can be harnessed for commercial use. Components with antioxidant activities can be found in only a few species of algae. Although the occurrence of phenolic compounds in plants is well known and these groups of compounds possess antioxidant activity in biological systems [6], the antioxidant characteristics of algae are poorly known. Some studies reported that cancer was prevented by alga extracts, because of their antioxidant properties [7].

Several bioactive metabolites produced by cyanobacteria and algae have been discovered by screening programs, employing target organisms quite unrelated to those for which the metabolites evolved[8]. Many of these chemicals have diverse range of biological activities and chemical structures, which affect many biochemical processes within the cell. Such chemicals are presumably related to the regulation and succession of algal and bacterial populations [9]. These chemicals are expected to be synthesized under stress conditions and low growth rate and released at concentration large enough to be effective.

The medicinal value of cyanobacteria was appreciated as early as 1500 Bc, when strains of Nostoc were used to treat gout, fistula and several forms of cancer (Cyanobacteria are a rich source of potentially useful natural products)[10]. Over 40 different Nostocales species, the majority of which are Anabaena and Nostoc spp. Produce over 120 natural products (Secondary metabolites) having activities such as anti-HIV, anticancer, antifungal, antimalarial and antimicrobial.

MATHERIALS AND METHODS

The growth of the studied algae was occurred according to the following methods:

Specific Medium of Anabaena:-

Isolation of Anabaena sp.

The cultures were isolated and purified by repeated plating on solid Chu 10 medium and colonies of different morphologies were identified according to morphological properties and pigment composition, [11],(Table , 1).

On the other side the composition of the macronutrient solution was shown in Table 2.

Table 1: The saline solutions salts

Salts	**gl^{-1}**	**Salts**	**gl^{-1}**
$Ca(NO_3)_2$	0.04	Na_2CO_3	0.02
K_2HPO_4	0.01	$Na_2SiO_3.5H_2O$	0.025
$MgSO_4.7H_2O$	0.025	$FeCl_3$	0.0008

Table 2: Composition of the macronutrient solution

Salts	gl^{-1}	Salts	gl^{-1}
$NaNO_3$	1.5	Ferric ammonium citrate	0.006
K_2HPO_4	0.04	EDTA (disodium-salt)	0.001
$MgSO_4.7H_2O$	0.075	Na_2CO_3	0.02
$CaCl_2.2H_2O$	0.036	*Micronutrient solution	1 ml
Citric acid	0.006	Distilled water	1L

The nutrient medium was prepared by using one ml of each of the stock macronutrient solution and one ml of the micronutrient stock solution and making it up to one liter by distilled water. The final pH was then adjusted to 7.2. Potassium phosphate solution was autoclaved separately and then added aseptically to the sterilized medium to avoid phosphate precipitation. Algal culture was grown in Erlenmeyer pyres-glass flasks capacity of 250 ml containing 100 ml culture medium. The flasks were grown under controlled light and temperature culture chamber. Culture experiments were conducted under a regime of 16 h. light/ 8 h. darkness.

Production of purified algal biomass

Pure culture was maintained by sub culturing in 250 ml Erlenmeyer flasks containing 100 ml of sterile Chu 10 medium (liquid) and incubated under florescent light (3000 lux) at a temperature of 25 ± 1°C. The culture was harvested by centrifugation (4000 rpm for 15 minutes) after 15 days of inoculation [12].

2- Medium Spirulina platensis

Algal growth media:

Spirulina platensis was grown in Spirulina medium [13]. It consists mainly of the salts which illustrated in Table (3).

Also the micronutrients salts of the solutions were given in Table (4).

One ml from stock solution b + 1.0 ml from stock solution c were added to each 1000ml of solution a. * This salt was neglected in our work; this is because EDTA is a strong chelator for metal cations and it can sequester metal toxicity very much. Actually EDTA is used for detoxification of metal poisoning [14].

Table 3: The media of the growth algae

Macronutrients (Solution a)	Quantity (g.)
1- NaCl	1.0
2- $MgSO_4$. $7H_2O$	0.2
3- $CaCl_2$. $2\ H_2O$	0.04
4- $FeSO_4.7\ H_2O$	0.01
5- Na-EDTA*	0.08
6- K_2HPO_4	0.5
7- $NaNO_3$	2.5
8- K_2SO_4	1.0
9- $NaHCO_3$	16.8
10- Distilled H_2O	1000ml

Table 4: The stock solution of the micronutrient salts.

Micronutrient stock solution			
Stock solution b		**Stock solution c**	
1- NH_4NO_3	0.023 g	1- H_3BO_3	2.82 0g
2- $K_2Cr_2\ (SO_4)_2$. $27\ H_2O$	0.096 g	2- $MnCl_2$. $4H_2O$	1.810 g
3- $NiSO_4$. $7H_2O$	0.044 g	3- $ZnSO_4$. $7H_2O$	0.222 g
4- Na_2SO_4. $7H_2O$	0.018 g	4- $CuSO_4$. $5H_2O$	0.077 g
5- Ti $(SO_4)_3$	0.040 g	5- MoO_3	0.015 g
6- Co $(NO3)_2$. $6H_2O$	0.044 g	6- Distilled H_2O	1000ml
7- Distilled H_2O	1000ml		

V- Culture conditions:

Spirulina platensis was grown in conical Erlenmeyer pyrex-glass flasks (capacity 250 ml). Each flask contained 50 ml culture medium. The inoculated medium was adjusted to optical density above 0.1 units in order to yield a linear growth curve with a lag phase. The cultures were grown under controlled laboratory conditions (temperature at 25˚C ± 1˚C and light at 80 m mol m^{-2} s^{-1}) in a controlled culture chamber. Temperature inside the growth chamber was kept as possible within 25˚C through periodical ventilation.

Culture experiments were conducted under a regime of 16 hour light/ 8 hour dark. Each experimental culture flask was regularly swirled daily by hand to detach adhered algal cells from the walls of the flask. After each mixing, the flasks were returned back to a different position on the glass shelves inside the controlled chamber to remove any bias due to the illumination or temperature gradient on the shelves. The culture period lasted for 18 days. The number of replicates was three separate conical flasks for each measure.

VI- Harvesting of cultures for analyses:

At different periods of culturing which depend on the type of the tested metabolic compound, the cells of Spirulina platensis were harvested by centrifugation at 5000 r p m for 30 min using angle rotor centrifuge. The supernatants were discarded and the remaining pellets were used for the determination.

Dry weight method

Algal samples from the different salt concentrations (10 ml) were filtered under vacuum through 0.45 µm filter membrane and washed several times with distilled water. Then, algal cells were dried at 100°C for 30 min and weighed [14].

Determination of antioxidant power by Prussian blue method

One gram of powder was defatted with petroleum ether. The defatted powder was then extracted sequentially by stirring with 10 ml methanol twice, then with 10 ml 1% hydrochloric acid: methanol (v/v).The three combined extracts were evaporated under vacuum and the residue was dissolved in 10 ml methanol. Half ml of the solution was diluted with 3 ml distilled water, 3 ml 0.008 M $K_3Fe(CN)_6$ was added, 3 ml 0.1 M HCl, and 1 ml 1% $FeCl_3$. The blue color is allowed to develop for 5 min and the absorbance is measured at 720 nm against the blank. Construct a calibration curve within 1-10 µg/ml tannic acids as shown in Figure 1.

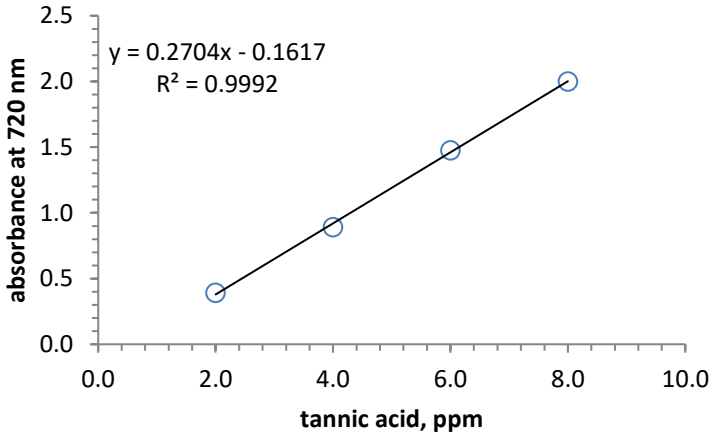

Fig 1: Calibration curve of tannic acid using Prussian blue method

Determination of total phenols by Folin Ciacalteu Method

Aliquots of the extracts were taken in a 10 ml flask and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteu reagent (1:1 with water) and 2 ml Na2CO3 (20%) were added. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of tannic acid from 4 to 20 µg/ml as shown in Figure 2.

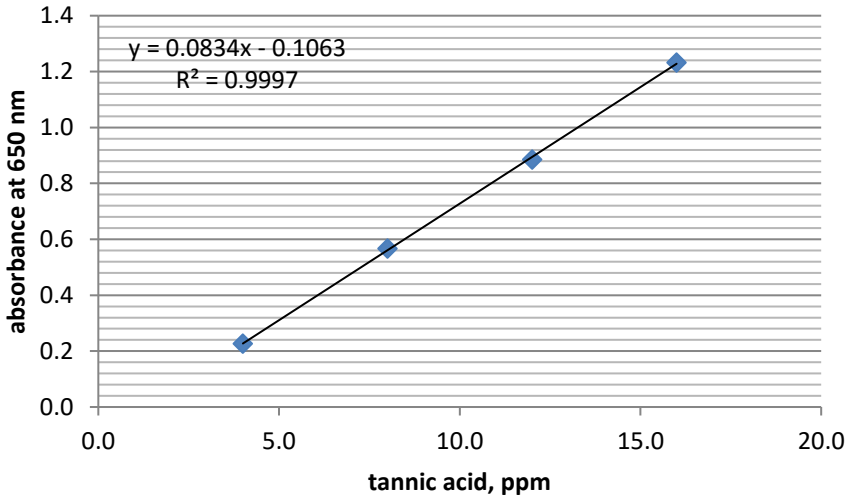

Fig 2: Calibration curve of total phenols using Folin Ciacalteu method.

RESULTS AND DISCUSSION

The contents of antioxidant and phenolic compounds of the growth algae were illustrated in Table (5) &Figure (3). The data showed that the high contents of antioxidant in the two studied algae is not correlated with phenolis contents , where the values were 28.7 ppm and 33.52 ppm of phenolic and 6.30 ppm and 4.04 ppm antioxidant values in the Anabaena and Spirulina platensis species , respectively. It was reported that the one of most compounds which mainly gave antioxidant activity is according into the phenolic compounds, in this study also recorded importance values of phenolic compounds, also some phenolic as tannic, rutien and Gallic acid gave high capacity of antioxidant, so this study not gave the fractions of the phenolic compounds which this study highly recommendation to study the major fractional of the phenolic compounds in the algae's species. The difference between the antioxidant ant total phenolic compounds mainly attributed to the presence of some compounds which have antioxidant activity .where, some studies indicate that many acids and vitamins gave antioxidant activity as ascorbic (Vitamin C), caffeine acids derivates and other.

Table 5: The phenollic compounds antioxidant activity contents.

Species Compounds	Total Phenoilc (ppm)	Antioxidant activity (ppm)
Anabaena	28.76	6.30
Spirulina platensis	33.52	4.055

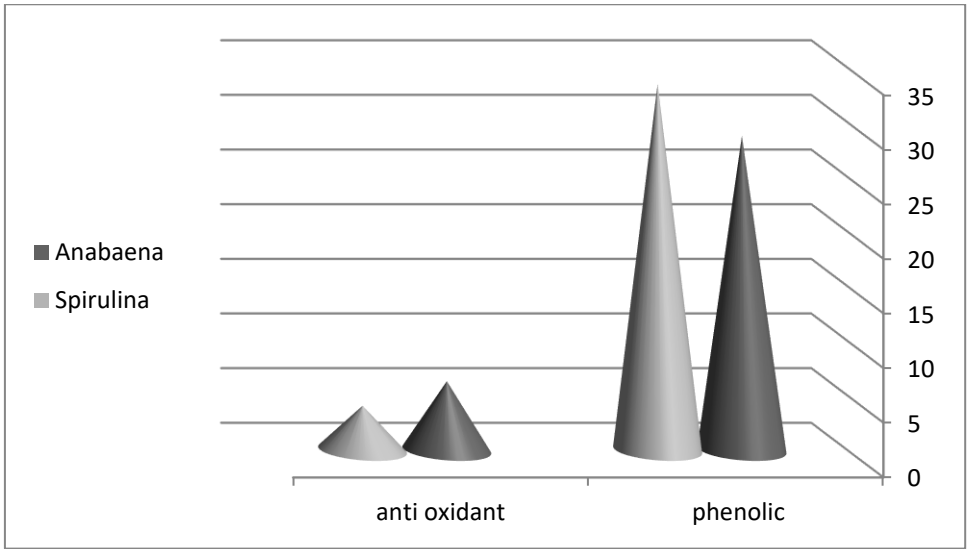

Fig 3: The histogram of phenolic compounds and antioxidant activity of anabaena and spirulina algae species

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