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# Evaluation of Different Biological Activities of *Spirulina platensis* Extracts

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*SPIRULINA* is aquatic and photosynthetic blue green algae and it is a good source of c-phycocyanin pigments. It is widely utilized as food supplement because it contains significant amount of proteins. This investigation is an effort to study some of medicinal applications of *S. platensis* such as antioxidant capacity, antimicrobial, cholesterol- reduction and anticancer. Antimicrobial activity of methanol, acetone, chloroform and petroleum ether extracts of dried *S. platensis* was assayed against two fungi (*Aspergillus flavus* and *Aspergillus niger*) and four bacterial species (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium*). The *S. platensis* extracts exhibited significant activity against all tested bacteria and fungi. The highest inhibition zone (1.35 ±0.04cm) was observed in acetone extract against *S. typhimurium*. While, petroleum ether extract of *S. platensis* was recorded the minimum activities of all tested antimicrobial and the lowest inhibition zone against *Staphylococcus aureus* (0.52±0.01cm). The antioxidant potential of different solvent extracts clearly that acetone extract of *S. platensis* showed highest percentage of DPPH (2, 2 diphenyl-1-picrylhydrazyl) scavenging activity (75%) and highest values of phenolic contents (0.562 mg/g DW). *S. platensis* displayed anticancer activity due to the productions of phycocyanin, allophycocyanin and other valuable products. Calf Serum was used to evaluate the potentiality of various *S. platensis* extracts to control the tumor effects in vitro. The cell proliferation assay showed that petroleum ether extract at a concentration of 125 µg $ml^{-1}$ for 24hrs, significantly inhibited the growth of the Fetal Calf tumor cells (13% viability).

**Keywords:** Anticancer, Antimicrobial activity, Antioxidant, Cholesterol reduction, *Spirulina platensis*.

## Introduction

Microorganisms have special properties and considered as a significant natural source of bioactive molecules, because they are capable of producing many bioactive compounds in culture medium, that are very difficult to be formed by chemical synthesis (Goud et al., 2007). Recently, there are remarkable trend in the industry of food for the development and production of functional products as a pharmaceuticals and nutraceuticals (Kasinathan et al., 2009). Microalgae are a large collection of photosynthetic microorganisms which can be isolated from different habitats, and also can be cultivated in commercial large-scale raceway ponds (Shao et al., 2019). Cyanobacteria or blue green algae are an ancient set of prokaryotic organisms which considered as a source for many secondary metabolites. Various strains of blue green algae can provide with many bioactive compounds that have various biological activities like antimicrobial agents, so it can be considered as good source for secondary metabolites, these secondary metabolites have a significant role in protections against many pathogens (Ghasemi et al., 2007). *Spirulina*, a filamentous cyanobacterium, is widely distributed in nature, due to the occurrence in different habitats, there are many biologists and pharmacists found that *spirulina* is an excellent source for many compounds. Previously, *Spirulina* was used as source of protein, some vitamins, specially

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vitamin B12 and provitamin A and fatty acids like γ -linolenic acid.

Further interest has been given for studying the curative effects of *spirulina*, that have the ability to reduce cholesterol level and heavy metals nephrotoxicity, anticancer characteristics and immune system enhancement (Belay et al., 1994). Khan et al. (2005) reported that *spirulina* possess some another biological functions like antimicrobial and antiparasitic activities. *Spirulina platensis* have wide a broad of health benefits as the decrease of cholesterol level, protection against allergenic diseases, antimicrobial and anticancer activities (Koníˇcková et al., 2014).

Chaitra et al. (2015) reported that in large- scale screening, more than 15,000 chemical compounds were isolated from micro algae that grow in marine environments such as steroids, polyphenolics, enzymes, polysaccharides, flavonoid, tannins, alkaloid compounds and many fatty acids, and newly investigations have shown the presence of many antioxidants.

At present, there are many health problems in the world, such as bacterial resistance to antibiotics. There are many efforts to obtain many biologically active compounds from natural sources all over the world, the marine microalgae and cyanobacteria are considered as the best among these natural resources because they can grow efficiently in large-scale raceway ponds or photobioreactors. In addition, the biological activity of algal extracts has been verified in medical fields as antimicrobial (Tuney et al., 2006).

There are many of researchers reported that *Spirulina* has become a medicinal food because it have good proteins , balanced fatty acids, vitamins, antioxidant compounds and many minerals, that make it able to participate in controlling many health problems like high cholesterol, as well as to decrease body weight in humans (Shao et al., 2019) and (Ramamoorthy & Premakumari, 1996).

Cancer is one of the most serious diseases that causes harmful to human health in the world. Chemotherapy is still utilized as a standard treatment till now, most anti-cancer drugs used in chemotherapy are toxic to healthy normal cells and causes a lack of immunity, which affects not only the development of tumors but also it has a negative impact on the health of the patient. It has become necessary to find other new anti-tumor drugs that have limited side effects on the immune system and the general health of the patient (Shao et al., 2019).

The aim of the present study was to evaluate some biological activities of *S. platensis* extracts.

## Materials and Methods

*Organism*

The blue green microalga *Spirulina platensis* (Nordstedt) Geitler (Oscillatoiales) was obtained from the Culture Collection of the Algal laboratory, Faculty of Science, Alexandria University, Egypt. The powdered sample was stored in refrigerator for further use.

*Preparation of extracts*

Algal extracts were prepared according to the method of Kannan et al. (2013).

Extraction of the bioactive algal powder has been carried out by using four different organic solvents such as petroleum ether, chloroform, acetone and methanol as follows; ten grams of powdered sample were soaked in 50ml of mentioned above organic solvents. The samples were kept in the dark for 72hrs at room temperature (cold percolation) with intermittent shaking. After incubation, the solution was filtered through filter paper, and the filtrate was collected (crude extracts) and stored in the refrigerator until further use.

*Evaluation of Antioxidant activity by DPPH free radicals scavenging assay*

The algal extract was tested as antioxidant activity by 2, 2diphenyl-1- picrylhydrazyl (DPPH) test according to the method of Yen & Chen (1995). One ml of 0.003g DPPH in 50ml methanol were mixed to 1.0ml algal extract and incubated for 30 minutes at room temperature, the absorbance was measured at 517nm by Spectrophotometer Unico UV-2000 spectrophotometer. Gallic acid was used as positive control. The antioxidant capacity was calculated according to the following equation:

$$\text{Scavenging activity \%} = (B\text{-}S/B) \times 100$$

where, (S) Absorbance of sample and (B) absorbance of blank.

*Total phenolic content*

The algal extracts were examined of total phenolic content by Taga method (Taga et al., 1984). One ml of extract was mixed with 750µl Folin Ciocalteau's phenol diluted ten times and allowed to stand for 5min then added 750µl of 6% $Na_2CO_3$ and left in dark for 90min at room temperature. Total phenolic contents were measured by spectrophotometer at 720nm. The standard curve was prepared using Tannic acid.

*Cholesterol binding assay*

Various concentrations (200, 400, 600, 800 and 1000µg/ml) of cell lysates and intact cells for *S. platensis* were prepared from stock of 10mg of lyophilised cells per ml suspended in 1ml of cholesterol-ethanol solution (100µg of cholesterol dissolved in one ml of ethanol 60%), shaking well by vortex and incubated at 37°C for one hour in a shaking water bath. Then the mixtures were centrifuged at 1118g for 10min and unrestricted cholesterol in the supernatant was estimated by enzymatic analysis and the tests were repeated in triplicate. The kits of enzymatic colorimetric used for the estimation of cholesterol was obtained from Biodiagnostic Company Dokki, Giza, Egypt (Richmond, 1973). The absorbance of the sample and standard against blank was measured at 517nm. The percentage of cholesterol lowering effect was determined by this equation:

Cholesterol reduction (%)= A517standard - A517 sample × 100/A517 standard.

*Tested of bacteria and fungi*

The microorganisms used for the antimicrobial activity assay including two-gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two-gram negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) and two fungi (*Aspergillus flavus* and *Aspergillus niger*). The microbial strains were provided from National Institute of Oceanography and Fisheries, Alexandria, Egypt. These strains were maintained on nutrient agar slant. The bacterial stock cultures were maintained on Mueller Hinton agar medium at 4°C and fungal cultures were maintained on Sabouraud Dextrose Agar (SDA) medium at 4°C. A loop full of each of the microorganisms was suspended in about 10ml of saline in a Roux bottle. Each of these were streaked on to the suitable culture slants and incubated at 37°C for 24hrs.

*Antibiotic susceptibility test*

The antimicrobial sensitivity of the bacterial and fungal strains was assessed by using the standard CLSI disk diffusion method due to the Clinical and Laboratory Standards Institute (CLSI, 2012). The antimicrobial agents from various classes of antibiotics with the following concentrations were checked: Ampicillin (AMP, 10µg), Amoxicillin (AX, 15µg), Oxacillin (OX, 1µg), Cefixime (CFM, 10µg), Ceftazidime (CZA, 30µg), Ciprofloxacin (CIP, 5µg), Imipenem (IPM, 10µg), Chloramphenicol (C, 30µg), Gentamicin (GEN, 10µg), Erythromycin (E, 15µg), Streptomycin (S, 10µg) Tetracycline (TE, 30µg), Nalidixic acid (NA, 30µg), Ofloxacin ( OF, 5µg), Vancomycin (VA, 30µg), Miconazole (MIC, 25µg), Fluconazole (FLU, 25µg) and Itraconazole (ITR, 25µg). Antibiotic discs were then used to the agar plates and incubated at 37°C for 24hrs then, the different growth inhibition zones of microbes were measured (mm). Calculate the multiple antibiotic resistances (MAR) to each microbe (total number of antibiotics which the microbe is resistant/ Total number of antibiotics checked) (Jayaraman et al., 2012).

*Antimicrobial assay*

The antimicrobial activity of different extracts of *S. platensis* was assessed by using disk diffusion method (Okigbo et al., 2005). Filter paper disks of (5mm) in a diameter were prepared from Whatman filter paper. The antibacterial test using gram +ve and gram -ve bacteria, were carried out using the agar plate method. The bacterial inoculum was grown in nutrient broth and a fixed volume ($1x10^6$) spores /ml of different bacterium was prepared and 0.2 mL spore suspension was inoculated into 10ml aliquots nutrient agar, mixed and then poured over a nutrient agar base in sterile Petri dishes. After bacterial lawn was solidified, the paper disk of 5mm was saturated with 5µL of each extract and placed in it. The plates were incubated at 30°C for 24hrs for bacterial strains. In another set of experiments for bioassay of fungal, sterile filter paper discs of 5 mm diameter were impregnated with 0.1ml/disc of each extract and placed in duplicates onto SDA plates seeded with 0.1ml of fungal suspension. The plates were then incubated at 37°C for 25°C for 5 days (Saadabi et al., 2012). Standard antibiotic disk (Ampicillin and Miconazole) used as positive control and negative controls were prepared by using different solvents. Zones of inhibition were recorded as antimicrobial activity in cm. All experiments were performed in triplicates.

*Cytotoxicity assay*

*Cell line and culture:*

The cancer cell lines Supplied by VACSERA. (Holding Company for Vaccines and Sera). Cancer cells were seeded in flask with RPMI (Roswell Park Memorial Institute) media with 2–10% Fetal Calf Serum (FCS) and incubated at 37°C in a 5% CO2. After 24hrs incubation period the attached cells were trypsinizated. The cells were counted and distributed in 96 well ELISA plate. The plate was incubated 24hrs at 37°C in a 5% $CO_2$ atmosphere to allow the cells attach to the bottom of the well **(**AshaRani et al., 2009).

*MTT Cytotoxicity assay*

Evaluation of cell viability by the MTT [3-(4, 5-dimetheylthiazol-2)-2, 5 diphenyl tetrazolium bromide] colorimetric method with some modifications. Cells ($1\times10^5$/well) were plated in one ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After incubation 48hrs the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO (dimethyl sulfoxide) for 48hrs at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide cells (MTT) solution was added. After 4hrs incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 595nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 595nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. Each experiment was done in triplicate. The relative cell viability (%) related to control wells containing cell culture medium without sample as a vehicle was calculated (Mosmann, 1983):

% cell viability= A595 of treated cells/ A595 of control cells X 100.

*Statistical analysis*

All experiments were carried out three times independently, and data were recorded as the means ± standard deviation (SD) of the three replicates. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version 5. One-way ANOVA test was used to compare the differences between the mean value of each test. Significance of the obtained results was judged at the 5% level.

## Results

*Antioxidant activity assessed by the DPPH method*

Figure 1 denoted that the DPPH radical scavenging activity of *S. platensis* in different solvent extracts with different concentrations. Various extracts of *S. platensis* possessed the ability to scavenge DPPH substantially in a concentration-dependent fashion and a type of solvent dependent used. *S. platensis* acetone extract had the most efficient free radical scavenging capacity, followed by methanol extract and chloroform. The lowest scavenging activity was recorded in petroleum ether extract. The increases in free radical scavenging activity were dependent on the concentration increased in all extracts. The results indicated that the radical scavenging ability of *S. platensis* acetone extract may result in significant antioxidant effects.

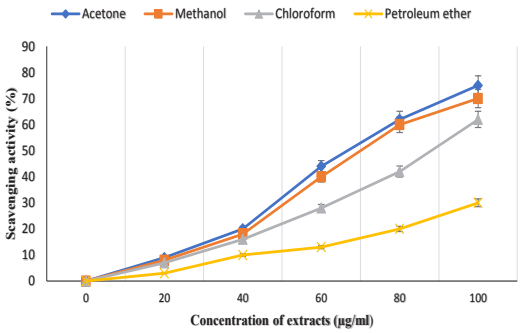


**Fig. 1. DPPH radical scavenging of various extracts of spirulina platensis. The error bars indicate mean± SD (n=3).**

*Phenolic contents*

The results of phenolic contents are presented in Table 1 indicated that the acetone extract of *S. platensis* had more phenolic content, followed by methanol extract, chloroform and petroleum ether (0.562, 0.465, 0.430 and 0.050mg/g DW) respectively, which are significantly different at P> 0.05

*Cholesterol reduction*

The percentage reduction of cholesterol by *S. platensis* was obtained in Table 1. Results indicate that different extracts of *S. platensis* have a high effect on the cholesterol reduction by using various solvents except petroleum ether which recorded a minute effect in the reduction of cholesterol

under all treatments. Maximum percentages of cholesterol reduction were obtained by acetone followed by methanol, chloroform and petroleum ether under all treatments as the following 78.1, 73.0, 65.2 and 5.6%, respectively, which are significantly different at P> 0.05.

*Antibiotic resistant profiles (ARP) to the standard strains*

The resistance of bacterial strains to various tested antibiotics was presented in Table 2. The standard strain of *S. aureus* was sensitive to (AX, CIP, IPM, C, GEN, S, NA and VA) and resistant to all antibiotic checked. The standard strain *B. subtilis* was sensitive to (C, CFM, GEN, S, TE and VA) and resistant to all antibiotic tested. The standard strain of *E. coli* was sensitive to (CIP, IPM, C, E, NA and OF) and resistant to (AMP, AX, OX, CFM, CZA, GEN, S, TE, VA, MIC, FLU and ITR). The standard strain of *S. typhimurium* was sensitive to (CZA, CIP, C, GEN, S, NA and OF) and resistant to all antibiotic tested. The standard strains of *A. flavus* and *A. niger* were resistant to all antibiotic tested except MIC, FLU and ITR. The data of multiple antibiotic resistances (MAR) indicated that all standard tested microbes have a high values of MAR.

**TABLE 1. Phenol contents (mg/g DW) and Cholesterol reduction (%) of *Spirulina platensis* extracted by using different solvents.**

| Test | Methanol | Acetone | Chloroform | Petroleum ether |
|---|---|---|---|---|
| Phenol contents | 0.465+0.22[a] | 0.562+0.3[a] | 0.430+0.1[a] | 0.050+0.23[b] |
| Cholesterol reduction | 73.0+0.1[b] | 78.1+0.2 [a] | 65.2+0.3[c] | 5.6+0.3[d] |

- Mean and standard deviation of three replicates are shown.
- Values in the same row with different superscripts letters are significantly different (P< 0.05).

**TABLE 2. Antibiotic resistant profiles (ARP) to the standard strains.**

| Antimicrobial code | *S. aureus* | *B. subtilis* | *E. coli* | *S. typhimurium* | *A. flavus* | *A. niger* |
|---|---|---|---|---|---|---|
| AMP | R | R | R | R | R | R |
| AX | S | R | R | R | R | R |
| OX | R | R | R | R | R | R |
| CFM | R | S | R | R | R | R |
| CZA | R | R | R | S | R | R |
| CIP | S | R | S | S | R | R |
| IPM | S | R | S | R | R | R |
| C | S | S | S | S | R | R |
| GEN | S | S | R | S | R | R |
| E | R | R | S | R | R | R |
| S | S | S | R | S | R | R |
| TE | R | S | R | R | R | R |
| NA | S | R | S | S | R | R |
| OF | R | R | S | S | R | R |
| VA | S | S | R | R | R | R |
| MIC | R | R | R | R | S | S |
| FLU | R | R | R | R | S | S |
| ITR | R | R | R | R | S | S |
| (MAR) | 0.56 | 0.67 | 0.67 | 0.61 | 0.83 | 0.83 |

R= Resistant, S= Sensitive

*Antimicrobial activity*

The antimicrobial activity of *S. platensis* extracts of different solvents (Methanol, Acetone, Chloroform and Petroleum ether) were studied against *S. aureus, B. subtilus, E. coli* and *S. typhimurium, A. flavus* and *A. niger* are presented in Table 3. The method was used to evaluate the antimicrobial activity by disc diffusion, where the inhibition zone against fungi and bacteria were measured in (cm). Acetone extract of *S. platensis* has exhibited the prominent antimicrobial activity of all the antimicrobial under all treatments but was more susceptible against *S. typhimurium* which recorded the maximum inhibition zone 1.35 ±0.1cm. While, petroleum ether extract of *S. platensis* was recorded the minimum activities of all tested antimicrobial but was less susceptible against *S. aureus* 0.52±0.04cm. The chloroform extract of *S. platensis* also resembles nearly the same activities as acetone extract. Acetone extract was the most potent antimicrobial fractions and also significantly inhibited the growth of microbes used.

*Anticancer activity of Spirulina platensis extracts*

Anticancer activities were evaluated in order to elucidate the effect of different extracts is directly correlated to the induction of cell death or the suppression of cell proliferation. The results of anticancer activity of different extracts of *S. platensis* are summarized in Table 4.

In this investigation, the MTT assay was evaluated to study anticancer activity of *S. platensis* with various concentrations in between 7.8 to 1000µg/ml of different extracts of *S. platensis*. Anticancer effect of different extracts of *S. platensis* was observed in Fetal Calf Serum Cell Line. At low concentration of the different extracts (7.8µg/ml), the viability of the cells was found to be increased, while at a high extract concentrations of *S. platensis,* it was accompanied by decreasing the cell viability. Acetone extract of *S. platensis* exhibited the prominent anticancer activity for all solvents used. The maximum anticancer activities of different extracts were recorded as the following: acetone> Petroleum ether> Chloroform> methanol where the $LC_{50}$ (Lethal concentration) of various extracts were observed at 31, 38, 96 and 98µg/ml, respectively.

**TABLE 3. Antimicrobial activity of *Spirulina platensis* against multidrug resistant standard microbial strains.**

| Pathogenic | Solvent | Mean of inhibition zone (cm) | Antibiotic disc (cm) |
|---|---|---|---|
| *S. aureus* | Methanol | 0.78 ±0.01 | 0.75±0.02 |
| | Acetone | 0.92±0.01 | 1.06±0.3 |
| | Chloroform | 0.89±0.01 | 1.10±0.2 |
| | Petroleum ether | 0.52±0.04 | 1.08±0.1 |
| *B. subtilis* | Methanol | 0.81±0.01 | 0.92±0.5 |
| | Acetone | 0.92±0.01 | 1.02±0.2 |
| | Chloroform | 0.85±0.02 | 1.04±0.05 |
| | Petroleum ether | 0.55±0.01 | 0.72±0.2 |
| *E. coli* | Methanol | 0.69±0.01 | 0.92±0.5 |
| | Acetone | 0.91±0.01 | 0.74±0.2 |
| | Chloroform | 0.88±0.02 | 1.05±0.2 |
| | Petroleum ether | 0.65±0.01 | 0.65±0.1 |
| *S. typhimurium* | Methanol | 0.85±0.02 | 0.83±0.3 |
| | Acetone | 1.35±0.1 ** | 0.78±0.2 |
| | Chloroform | 1.04±0.01* | 0.93±0.1 |
| | Petroleum ether | 0.86±0.03 | 0.72±0.1 |
| *A. flavus* | Methanol | 0.56±0.2 | 1.06±0.22 |
| | Acetone | 0.89±0.04 | 0.73±0.2 |
| | Chloroform | 0.68±0.05 | 1.02±0.5 |
| | Petroleum ether | 0.54±0.01 | 1.05±0.3 |
| *A. niger* | Methanol | 0.84±0.01 | 0.94±0.1 |
| | Acetone | 0.89±0.02 | 0.72±0.2 |
| | Chloroform | 0.65±0.04 | 0.96±0.2 |
| | Petroleum ether | 0.54±0.5 | 1.05±0.1 |

*Significant at P< 0.05.

**TABLE 4. Anticancer effect of *Spirulina platensis* extracted by using different solvents (mean and standard deviation of three replicates are shown.**

| Concentration (µg/ml) | Methanol | | Acetone | | Chloroform | | Petroleum ether | |
|---|---|---|---|---|---|---|---|---|
| | Mean absorbance (O.D) | Cell viability (%) | Mean absorbance (O.D) | Cell viability (%) | Mean absorbance (O.D) | Cell viability (%) | Mean absorbance (O.D) | Cell viability (%) |
| 1000 | 0.07±0.1 | 15±0.1$^{a}$ | 0.07±0.2 | 15±0.1$^{a}$ | 0.13±0.2 | 24±0.2$^{b}$ | 0.06±0.1 | 13 ±0.2$^{a}$ |
| 500 | 0.09±0.4 | 21±0.2$^{b}$ | 0.07±0.1 | 16±0.1$^{a}$ | 0.47±0.3 | 89±0.2$^{c}$ | 0.06±0.1 | 13±0.1$^{a}$ |
| 250 | 0.09±0.2 | 22±0.2$^{b}$ | 0.06±0.2 | 14±0.1$^{a}$ | 0.53±0.2 | 100±0.5$^{c}$ | 0.06±0.4 | 14±0.5$^{a}$ |
| 125 | 0.10±0.2 | 22±0.3$^{b}$ | 0.07±0.1 | 16±0.3$^{a}$ | 0.52±0.2 | 100±0.1$^{c}$ | 0.06±0.2 | 13±0.2$^{a}$ |
| 62.5 | 0.40±0.1 | 90±0.1$^{b}$ | 0.07±0.2 | 15±0.3$^{a}$ | 0.50±0.1 | 95±0.1$^{c}$ | 0.08±0.1 | 17±0.2$^{a}$ |
| 31.2 | 0.43±0.1 | 96±0.1$^{c}$ | 0.22±0.1 | 50±0.1$^{a}$ | 0.52±0.2 | 99±0.2$^{c}$ | 0.28±0.2 | 62±0.1$^{b}$ |
| 15.6 | 0.43±0.2 | 97±0.2$^{c}$ | 0.32±0.2 | 71±0.2$^{a}$ | 0.52±0.3 | 99±0.5$^{c}$ | 0.38±0.3 | 85±0.2$^{b}$ |
| 7.8 | 0.44±0.2 | 99±0.1$^{b}$ | 0.40±.02 | 90±0.4$^{a}$ | 0.54±0.1 | 100±0.2$^{b}$ | 0.42±0.2 | 94±0.2$^{a}$ |
| Cell control | 0.53±0.1 | 100±0.1 | 0.53±0.1 | 100±0.1 | 0.53±.01 | 100±0.1 | 0.53±0.1 | 100±0.1 |

Values in the same row with different superscripts letters are significantly different ($P< 0.05$).

## Discussion

*Spirulina platensis* can be considered as wealthy source of many novel bioactive compounds which have various application in pharmaceutical industries. Bleakley & Hayes (2017) reported that *Spirulina* is the greatest microalgal consumed, which utilized as a source of healthy human food for many centuries, besides it has highly nutritional values, which have many biological activities such as antioxidant, antimicrobial and anticancer. *S.platensis* biomass had high antioxidant activity supporting its use as nutritional supplement **(**Taga et al., 1984). *S. platensis* biomass contains phycobiliproteins, phycocyanin and alophycocyanin, which have antioxidant properties (Estrada et al., 2001). Baicus & Baicus (2007) reported the antioxidant activity was attributed due to the presence of phenolic compounds in the methanol algal extract include of salicylic acid, chlorogenic, trans-cinnamic, caffeic acids, quimic and synaptic; individually or in a synergistic action. Abomohra et al .(2016) reported that gama radiation induce to the (ROS) cellular reactive oxygen species through the signaling purinergic which stimulate the *Arthrospira platensis* cells to increase the production of antioxidants under the oxidative stress. Viswanathan et al. (2014) was recorded there are many literature reports indicated that the manifestation of total antioxidant capacity of sample is based on single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms. My results clarified that *Spirulina platensis* acetone extract had the most efficient scavenging capacity, thus can conclude that the efficacies of antioxidants are often associated with their ability to scavenge stable free radicals. Therefore, the DPPH radical scavenging activity of acetone extract of *S. platensis* demonstrated its oxygen radical absorbance capacity and indicated its potent antioxidant nature.

The results indicated that there is basically a positive relationship between antioxidant activity and total phenolic contents. In particular, the highest total phenolic contents of *S. platensis* acetone extract were related to its highest antiradical-antioxidant capacity. El Salhin et al. (2016) concluded that phenolic compounds have antioxidant characteristics and relevant biological properties. Phenolic compounds such as tannic, rutin and gallic acid gave high antioxidant capacity. Halimoon & Ali (2010) reported that

*S. platensis* contained significant amounts of phenolics compounds. Phenolic compounds found in algae have various health-promoting functions in humans (Islam et al., 2003).

Kamal et al. (2015) studied the treatment with intact cells or cell lysates of *Lactobacillus* and *Spirulina* to stimulate lowering effect of hypercholesteremia. Prakash & Kumari (2011) were utilized *Papaya pulp* and *Spirulina* for preparation of low- fat and high-protein frozen yogurt. My results indicated that *S. platensis* extracts have the potentiality to reduce the level of cholesterol and maximum percentage reduction of cholesterol was obtained by acetone extract followed by methanol extract.

There are many researches have been reported as antimicrobial agents from microalgae, among this *Spirulina*, which can be considered as a rich source of natural antimicrobial agents. Kokou et al. (2012) reported that *Spirulina* have been inhibited growth of six strains of *Vibrio,* so that it can be considered as good antibacterial agents. Also, Özdemir et al. (2004) studied the effect of various extracts of *S. platensis* against bacteria and they were reported that methanol extract is the best for inhibit the growth of bacterial fraction. The present result was in agreement with those obtained by Santoyo et al. (2006) studied the effect of *S. platensis* extracts of ethanol, hexane and petroleum ether as antimicrobial activity against *E. coli, S. aureus, A. niger* and *Candida albicans*. Furthermore, the purified c-phycocyanin pigment of *S. platensis* was inhibited the growth of many strains of bacteria as *S. aureus, E. coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Sarada et al., 2011; Mohamed & Saber, 2019). The results in Table 3 indicated that the acetone extract of *S. platensis* was the best solvent for inhibition the growth of *A. flavus*, *A. niger., S. aureus, B. subtilus, E. coli* and *S. typhimurium,* where the mean of inhibition zone of acetone extract (0.912cm), followed by chloroform extract (0.729cm), methanol extract (0.683cm) and petroleum ether (0.548cm).

There are strong evidences that, *Spirulina* have also with antitumor and anticancer functions. Many investigations were confirming that the potentiality of *Spirulina* extracts for the anti-cancer activities, either sole or in incorporation with other compounds. Schwartz & Shklar (1987) used 20 animals to study the effect of *Spirulina* extracts on the induced squamous cell carcinoma of hamster buccal pouches and the data were shown that phycotene of *spirulina* causes the regression about 30% of the total tumor of the treated animals, whilst β-carotene and canthaxanthin were controlled to the regression of tumor about 20% and 15% of treated animals, respectively. Schwartz et al. (1988) studied how *Spirulina- Dunaliella* extracts prevent oral cancer and they concluded that the extract of *spirulina* enhances and stimulates the immune responses that destroys small foci of developing malignant cells without any side effect to the normal cells. Liu et al. (2000) studied the inhibitory effects of c-phycocyanin from *S. platensis* on the growth of human leukemia $K_{562}$ cells, the study concluded that about 50% of the leukemia cells were controlled after 12 days of treatment use *Spirulina* phycocyanin. Moreover, Subhashini et al. (2004) found that about 49% of the $K_{562}$ cell proliferation was inhibited by using with 50 µM of purified phycocyanin from *S. platensis* through 48hrs. Choi et al. (2013) studied the effect of *Spirulina maxima* grown in deep marine water, which stimulate the immune activity and they are concluded that the higher effective stress of $Bc_{l2}$ gene in $A_{549}$ cells causes the human cancer inhibition in different cells. Kawanishi et al. (2013) reported the complicated polysaccharide obtained from *Spirulina* have the ability to inhibit the growth of glioma cell through lowering regulation angiogenesis by the regulation of production of interleukin-17. All these pervious findings supported our results for anticancer effect of *S. platensis* extracts in Fetal Calf Serum Cell Line. At low concentration of *S. platensis* extracts (7.8µg/ml), the cell viability was found to be increased, whilst at a high concentration of extracts, it was accompanied by decreased of the cell viability. Acetone extract of *S. platensis* has exhibited the prominent anticancer activity of all solvent used.

## Conclusion

There are many scientific reports indicating the possibility of using *S. platensis* in the treatment of many diseases due to the possible synergetic effect of many phytochemicals in the cell. From this study, it can be concluded that *S. platensis* have antimicrobial efficiency, antioxidant capacity, anticancer efficiency and its cholesterol-lowering effects depending on type of solvent used for algal extraction. Acetone was the most effective

solvent for extraction of bioactive compounds from *S. platensis*. So, *S. platensis* can serves as very important potential source of many bioactive products with commercial impact. Further study should be done for isolation and purifying the effective compounds, which will demonstrate their utility in the pharmaceuticals and biological industries.

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*The Conflict of Interest Statement:* The authors declare that they have no conflict of interest.

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## تقييم الأنشطة البيولوجية المختلفة لمستخلصات سبيرولينا البلاتينية

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ان الأسبيرولينا واحدة من الطحالب الخضراء المزرقة وتعتبر مصدر جيد للأصباغ وخاصة الفيكوسيانين ج حيث تستخدم الاسبيرولیناعلى نطاق واسع كمكمل غذائي لائحتوائها على كمية كبيرة من البروتينات وهذا التحقيق هو محاولة لدراسة بعض التطبيقات الطبية لطحلب الأسبيروليبا مثل قدرتها كمضاد الأكسدة ومضاد للميكروبات وكمضاد للسرطان وقدرته على تخفيض الكوليسترول. وقد اجريت التجارب بتحضير أربعة مستخلصات مختلفه من طحلب الأسبيرولينا المجففة باستخدام أربعة مذيبات مختلفة وهي الميثانول والأسيتون والكلوروفورم والإيثر البترولي وقد استخدمت هذه المستخلصات في تقيم النشاط المضاد للميكروبات حيث تم استخدام نوعين من الفطريات وهما *Aspergillus flavus* and *A. niger*.

وكذلك اربعة انواع مختلفة من البكتريا وهي:
*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium*.

ولقد اظهرت هذه المسخلصات المختلفة نشاطا واضحا في السيطرة على نمو جميع انواع الميكروبات من الفطريات والبكتريا المختبرة وقد لوحظ أن مستخلص الأسيتون كان الأكثر سيطرة على نمو الميكروبات حيث حقق أكبر منطقة تثبيط نمو كانت 1,35 سم ضد سيلمونيلا بينما تم تسجيل مستخلص الأثير البترولي بأقل نشاط لجميع مضادات الميكروبات المختبرة وأقل منطقة تثبيط نمو كانت حوالي 0.56 سم مع *Staphylococcus aureus*.

ولقد اوضحت النتائج مدى إمكانيات مستخلصات المذيبات المختلفة كمضادات للأكسدة. فقد تبين أن مستخلص الأسيتون هو أفضل المستخلصات كمضاد للأكسدة ويليه مستخلص الميثانول وقد حقق مستخلص الأثير البترولي أقل القيم وقد سجل مستخلص الأسيتون أعلى قيم في المحتويات الفينولية وهي 0.562 ملجم/ ج وزن جاف. ولقد أظهرت المستخلصات الأربعة نشاطا ملحوظا في السيطرة على الأورام وذلك استخدام خلايا مصل العجل في المختبر. أظهر اختبار تكاثر الخلايا أن مستخلص الأثير البترولي بتركيز125 ميكروغرام /مل لمدة 24 ساعة، حال دون نمو الخلايا السرطانية للعجل الجنيني بشكل كبير.