# Anti-microbial and anti-viral polysaccharides derived from cyanobacteria in different closed lakes

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**Abstract:** The aim of this study is to test the effect of some extraction products derived from algae *Spirulina* against virus (Polio virus) and some bacteria (10 isolates) which cause many diseases for plants, animals and human. *Spirulina Platensis* was isolated from El-Khadra Lake at Wadi El-Natrun, Egypt. The tested bacteria and virus were isolated from different closed lakes at Wadi El-Natrun and NRC respectively. The results showed that antibacterial extracts (80 mg Spirulina extract/ 1000ml) were more effective against Micrococcus sp., pseudomonas sp., and Xanthomonas while less effective against Streptococcus, Staphylococcus, Aeromonas, Citrobacter, and Salmonella sp. The same extracts had moderate effect against Bacillus and Klebsella. On the other hand, the fraction 2 was more active at 50µg/ml, where viral removal was at ratio of 62.5 %. The fractions 2 and 5 caused 50% reduction for the same virus. So, these fractions may be possible for human and animals as a treatment for many diseases.
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**Key words:** Cynaobacteria, Spirulina, antiviral activity, antimicrobial activity.

## Introduction

Cyanobacteria are known as blue green algae, blue green bacteria or cyanophyta that are considered to be the link between bacteria and algae. They are considered to be one of the potential organisms which can be useful to mankind in various ways.

Cyanobacteria can be found in almost every conceivable environment, from oceans to fresh water to bare rock to soil. Cyanobacteria species are receiving increasing attention mainly for their bioactive components such as polyunsaturated fatty acids and many pigments (antioxidants) (Bhat and Madyastha,2000), sulphated polysaccharides (antiviral) and sterols (antimicrobials) which in laboratory tests inhibited bacteria and fungi that incite diseases of humans (Otles and Pire, 2001). *Spirulina platensis* had been chosen from the seven isolated genus in our study for its past history as it is a rich source of nutrients containing up to 70% protein, B-complex vitamins, phycocyanin, chlorophyll, beta-carotene, vitamin E and numerous minerals. Actually it is being widely studied for its possible anticancer, antibacterial and anti-parasitic properties and for several medical conditions such as allergies, ulcers, anemia, heavy metal poisoning and radiation poisoning. *Spirulina platensis* has been also studied for its antiviral properties (Ayehunie et al., 1998) which seem to be related to its sulfated polysaccharide chelating calcium named calcium spirulina (Hayashi et al., 1996). Studies have shown that calcium spirulina (Ca-sp), isolated from *S. platensis* inhibited the replication of several enveloped viruses, including herpes simplex virus type 1(HSV-1), human cytomegalo virus, measles virus, influenza A virus, and human immuno deficiency virus-1(HIV-1). On the other hand, it has been reported that C.phycocyanin from blue- green algae possesses antioxidant and antiflammatory properties (Romay et al., 1999). Extracts from algae also have been demonstrated to have antiviral activity (Serkedjieva et al., 2000).

## Materials and Methods

**Water Sampling:**

*Spirulina* was isolated by collecting subsurface water samples from EL-Khadra Lake at wadi EL- Nutron at 10- 15 cm depth in sterilized bottles and stored in ice- box till analysis at NRC.

**Physical analysis:**

Before collecting water samples the following measurements were done:

- Temperature: using glass thermometer, according to Allen et al, (1974).
- pH value: using a Beckman portable pH meter (A. P.H. A.,1992).
- Electrical conductivity: using of portable conductivity meter (A.P.H.A., 1992).
- Opacity: by using scecik disk (25 cm in diameter) according to Allen *et al* (1974)

**The Morphology and taxonomy:**

**1) cyanobacteria:**

Different types of micro algae found in Wadi EL- Natrun lakes were identified microscopically according to Holmes and Whitton (1981). The scheme of identification was based on the colour of the living cells and their dimensions under microscope. Diameters and length of 100 vegetative cells, 100

heterocysts and a kinetes were measured. In the case of filamentous algae, the diameters and length of basel, median, and appical parts of the vegetative filamentous were also measured.

The taxonomy of Cyanobacteria is extremely difficult due to the paucity of morphological characteristics. Thus names of genera have changed frequently and a number of subspecies are known.

Many species are imbedded in a thick layer of extracelluar polysaccharides. Cyanobacteria are gram negative with a typical peptidoglycan cell wall (Tsuzuki and Miyachi, 1991). The diameter of the cells ranges from 1 to 3 µm in the smaller species and from 3 to 1: µm in the larger one. The *S. p1atensis* cells diameter have 6 to 8 µm.

**2) Bacteria:**

Identification methodology done by using crystal typing system (Becton, Dickinson and compamy Sparks, MD 21152).

**Organism and growth conditions:**

*Spirulina platensis* originally isolated from EL-Khadra Lake at Wadi EL-Natrun live under extreme conditions of pH and salt concentration.

The isolated *S. platensis* was cultivated in batch culture under sterile conditions in Zarouk's medium, at pH 9.5 (Vonshak 1986). The sodium content of the medium was 250m M, most of it is sodium bicarbonate. The flasks kept on a shaker at 30º C and continuously illuminated with cool white fluorescent lambs providing 80 M mole photons / m2

**Purification of *Spirulina platensis:***

Cultures of S. platensis were filtered through sterilized Whatman 11 filter paper and washed three times with modified Zarrouk medium (Zarrouk C. 1966). The washed filaments were suspended in Zarrouk medium covered with alumninum foil and kept in the dark at 35ºC for 2.5 h. To these suspensions, the following ingredients (final concentration) were added : glucose ( 1 % ), peptone ( 0.5 % ), yeast extract ( 0.3 % ), NaCl (0.5 %), ampicillin (100mg / ml ), Cefoxitin ( I00mg / ml ) and imipjnen ( 100mg / ml ) . This culture was incubated at 35º C for 48 h. in the dark, filtered and washed six times and suspended in Zarrouk medium.

**Preparation of *Spirulina* extracts:**

Crud extracts were prepared by using 80% ethanol to remove components of low molecular weight. The residual material was extracted with hot water (100˚c). These times each for 3 hours and the aqueous extracts were collected and concentrated under reduced pressure using rot vapor to give the aqueous which showed the maximum antiviral activity (Zhuang et al., 1995)

**Antimicrobial activities**

Tested microorganisms and culture media:

Antimicrobial activity of crude extracts was tested against the Gram-positive bacteria: *Streptococcus, Micrococcus, Staphylococcus* and *Bacillus*. The Gram-negative bacteria*: Pseudomonas*, *Aeromonas, Citrobacter, Klebsella, Xanthomonas and Salmonella* sp.

The stock cultures of bacteria were maintained on nutrient agar (NA; Difco, Detroit, Ill.) slants at 4°C.

Antimicrobial screening (Agar diffusion method):

The agar diffusion method was used to evaluate the antibacterial effect of the isolated extracts. Briefly, an inoculum of each of the bacterial strains was suspended in 2 ml of nutrient broth and incubated overnight at 37°C. The culture was then diluted with nutrient broth (1:9). To screen for antibacterial activity, sterile nutrient agar plates were used according to the disc diffusion assay (Bauer et al.1966, Barry, 1976). A bent glass rod was used for spreading diluted cultures on the plate after which sterile paper discs of 20 ìg of extract in 0.1mL of water was placed on the inoculated surface. Plates were incubated at 37°C in the dark. Zones of inhibition were examined after 24hr. For positive controls, ampicillin as an antimicrobial agent was used. In addition, for negative controls, dried discs that had been soaked in sterile water served as carrier control. The inhibition zone was measured around the disk.

**Antiviral activity:**

Antiviral activity was measured by plaque infectivity reduction assay for rapid screening.

**Preparation of *Spirulina* extracts for bioassay:**

Extracts were dissolved as 100mg each in 1ml of 10% DMSO (Dimethyl sulfoxide) in water. The final concentration was 100mg/ml (Stock solution). The dissolved stock solutions were sterilized by addition of 50 J.Lg/ml antibiotic-antimycotic mixtures (10,000D penicillin G sodium, 10,000 J.Lg streptomycin sulfate and 250 J.Lg amphotericin B.)

Plaque reduction assay: (Tebas et aI., 1995)

A 6-well plate was cultivated with Vero cell culture (105cell/ml) and incubated for 2 days at 37°C. HSV-l and HAV were diluted to give 104 PFD/ml final concentrations for each virus and mixed with the test compound at the previous concentration and incubated overnight at 4°C. Growth medium was removed from the multiwell plate and virus-compound mixture was inoculated 100ml. After 1h contact time, the inoculum was aspirated and 3ml of MEM with 1 % agarose was overlaid the cell sheets. The plates were left to solidify and incubated at 370˚C until the development of virus plaques. Cell sheets were fixed in 10% formaline solution for 2hrs and stained with crystal violet stain. Control virus and cells were treated identically without chemical

compound. Virus plaques were counted and the percentage of reduction was calculated.

**Result and discussion**

The seven cyanobacteria cultures recorded in Wadi El- Natrun Lake included five genera. Two Spirulina, Oscillatoria, one Nostoc, one Chlorella and one variculs *Spirulina platensis and Spirulina caldaria* were dominant in El- Khadra lake. *Chlorella vulgaris and Noviculs canalis* were dominant in El Gaar lake. *Oscillatoria okei and O. willei were dominant* in El Hamra Lake.

The different types of micro algae community in Wadi El-Natrun lakes are survived differently in water quality of each lake as shown in table (1) that helped to modify the standard medium for the isolation process. The tabulated results of water temperature and opacity of the four closed lakes under study (table 1) showed that the water temperature of the lakes varied during sampling of isolated micro-organisms. It reached maximum 24ºC, while the lowest was 20 ºC. The pH of the water body of the four lakes is indication for light penetration in water. The highest opacity level was recorded in El-Beida Lake, as it reached 11.35, while the lowest value of pH (8.3) was recorded at El-Khadra Lake. The total soluble salts (TSS) of the four lakes were determined. The highest level was recorded in El Baida lake, reached 14.00 m. mohs/cm at 25 ºC, while the lowest level was found in El- Khadra lake, reached 9.5 m moh/cm. No toxicity was detected in all the samples using the mouse bioassay technique.

**Table 1: Physico-chemical analysis of Wadi El Natrun closed lakes**

| Analysis locations | pH values | E.C.m.moh/cm | Temp (ºC) | Opacity (cm) |
|---|---|---|---|---|
| El- Khadra | 8.3 | 9.50 | 24 | 85 |
| El-Gaar | 9.0 | 9.95 | 23 | 350 |
| El-Hamra | 8.5 | 11.25 | 20 | 300 |
| El-Beida | 11.35 | 13.1 | 22 | 35 |

Preliminary experiments had been performed on the fractions isolated from the blue green algae as *spirulina platensis*, to test their antiviral, antimicrobial and anticancer activities.

**Antibacterial activities**

In this study the antimicrobial activities of the native extract was evaluated in vitro against 10 microorganisms. In general the most active with the biggest inhibition zone diameter 20 mm as shown in table 3.

**Table 2: Genus of bacteria isolated from water of different closed lakes:**

| Genus No. | Genus Name | Isolation location |
|---|---|---|
| 1 | Streptococcus | El-Beida lake |
| 2 | Micrococcus | El-Hamra lake |
| 3 | Staphylococcus | El-Beida lake |
| 4 | Pseudomonas | El-Gaar lake |
| 5 | Aeromonas | El-Gaar lake |
| 6 | Bacillus | El-Beida lake |
| 7 | Citrobacter | El-Gaar lake |
| 8 | Klebsella | El-Gaar lake |
| 9 | Xanthomonas | El-Gaar lake |
| 10 | Salmonella | El-Khadra lake |

**Antiviral activity**

The antiviral activity of the fractions isolated from *Spirulina Platensis* was studied using Polio virus as an example of RNA viruses. The results show that only fraction 2 is the most active because of the high percentage of virus reduction (62.5% at 50 µg concentration). Fraction 5 (sup) causes 50% reduction at the same concentration. On the other hand, fraction 5ppt at concentration 20 µg causes also 50% reduction. Furthermore, some of these fractions (2 and 5) were found active against Herpes simplex virus (running work). This research was supported by several studies like those of Hayashi et al. (1992) who found that the aqueous extracts of *Spirulina platensis* inhibited HSV-1 (Herbes simplex virus, DNA virus). Also, Ayehuie et al., (1998) determined that an aqueous extracts of *S. platensis* at concentration nontoxic to human cell inhibited HIV-1 replication in human T-cell lines (HIV) human immunodeficiency virus that causes the acquired immune deficiency disease syndrome AIDS).

**Table 3: The antimicrobial activities of the native cyanobacterial extract against 18 microorganisms for five days**

First day result; Zone of stimulation and or inhibition per (m.m)

| Genus No. | Treatments | | | | | Growth of bacteria |
|---|---|---|---|---|---|---|
| | Cont. | Ex. | 1 | 2 | 3 | |
| 1 | 0 | I 0.5 | I 0.5 | 0 | 0 | +++ |
| 2 | 0 | 0 | 0 | 0 | 0 | Bacteria did not grow yet |
| 3 | 0 | I 2.0 | I 1.0 | S 0.5 | S 1.0 | +++ |
| 4 | 0 | I 0.5 | 0 | S 2.0 | S 0.5 | Bacteria did not grow yet |
| 5 | 0 | I 0.5 | S 0.5 | S 3.0 | S 4.0 | +++ |
| 6 | 0 | I 1.0 | I 2.5 | S 2.0 | I 0.5 | ++ |
| 7 | 0 | I 1.0 | I 1.5 | S 0.5 | I 0.5 | +++ |
| 8 | 0 | I 1.0 | I 1.0 | S 2.0 | S 1.0 | ++ |
| 9 | 0 | I 1.5 | I 0.5 | S 2.5 | S 1.5 | + |
| 10 | 0 | I 0.5 | I 1.0 | S 1.0 | S 1.5 | +++ |

**Notes:**

1: 80 mg Spirulina biomass/1000 ml
2: 60 mg Spirulina biomass/1000 ml
3: 40 mg Spirulina biomass/1000 ml

I: Inhibition
S: Stimulation

Table 3 indicated that agar diffusion method is used to evaluate the effect of extract different concentrations on bacterial growth inhibition zones which measured around the discs when examined after 24h. Most discs examined with the extract impregnated (40, 60, 80 mg/l) showed positive results were compared with control. Control plates contained either Penicillin (ug/ml) showed complete inhibition at the same dilution of the crude extracts after being concentrated. So, the bioactive compounds derived from algae towards the ten tested bacteria on incubating the nutrient agar plates overnight at room temp. The discs impregnated with the control discs, sterilized water or extract were shown are similar to that found stated by; Tuney et al 2006 who stated that *Spirulina* may have pharmaceutical effect.

Results shown in table 4 indicate that antagonistic activity to the Sabine vaccine due to the bioactive compound derived from *Spirulina platensis*, so its extract can be used as antiviral agent; this comes in agreement with Ayehunie *et al* 1998 who stated that isolates of spirulina tends to excrete metabolites with antibacterial activity or the inhibitory effect of *S.platensis* aqueous extract on HIV-1;Human immune deficiency virus replication ; The 2nd fraction at concentration of 50mg/l resulted in removal % of 62.5, 50 when the 4th fraction dilution was 20 and 50 Sabine were inhibited in the 3rd fraction with a ratio of 12.5.

**Table 4: Cyanobacterial antiviral activity**

| Fraction | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| mg/l conc. | Count $x10^9$ | Removal% | Count $x10^9$ | Removal % | Count $x10^9$ | Removal % | Count $x10^9$ | Removal % |
| 20 | 3.2 | 0 | 2.4 | 25 | 3.2 | 0 | 1.6 | 50 |
| 50 | 3.2 | 0 | 1.2 | 62.5 | 2.8 | 12.5 | 1.6 | 50 |

**Refereneces**


1. **Allen, S.; Grimshay, H.M, Parkinson, J.A. and Puarmbt, C. (1974).** "Chemical analysis of ecological materials" (Blackwell, Oxford) pp.565.
2. **American Public Health Association (A.P.H.A.) (1992).** "Standard Methods for Examination of water and waste water". 16th Ed. A WW A WPCF, Washington.
3. **Ayehunie, S., Belay, A., Baba, T. and Ruprecht, R. (1998)**. "Inhibition of HIV-1 Replication by an Aqueous Extract of *Spirulina platensis* (*Arthrospira platensis*)." JAIDS: Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology. 18 (1): 7-12.

4. **Barry, A.L. (1976). "**Principle and Practice of Microbiology". Lea and Febiger, Philadelphia, USA.
5. **Bauer, A.W., W.M.M. Kirby, J.C. Sheries and M. Turek, (1966).** "Antibiotic susceptibility testing by a standardized single disc method". Annl. J. Clin. Pathol., 45: 493–496.
6. **Bhat, V.B. and Madyastha, K.M.** (2000). "C-phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro". Biochemical and Biophysical Research Communications. 275(1): 20-25.
7. **Hayashi, K., Hayashi, T., Morita, N. (1992). "**Mechanism of action of the antiherpesvirus biflavone ginketin". Antimicrob. Agents Chemother. 36, 1890–1893.
8. **Hayashi K, Hayashi T, Kojima** (1996). "A natural sulfated polysaccharide, calcium spiruilna, isolated from *Spirulina platensis*: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities". AIDS Res. Hum Retroviruses; 12:1463-1471.
9. **OTLES, Semih and PIRE, Ruhsen** (2001). Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. Journal of AOAC International. 84(6): 1708-1714.
10. **Romay C, Ledon N, Gonzalez R. (1999).** Further studies on anti- inflammatory activity of phycocyanin in some animal models of in-flammation. Inflam Res 47:334–338.
11. **Serkedjieva J, Konaklieva M, Dimitrova-Konaklieva S, Ivanova V, Stefanov K, Popov S. (2000).** Antiinfluenza virus effect of extracts from marine algae and invertebrates. Z Naturforsch 55: 87–93.
12. **Tebas, P.; Stabell, E. C.; Olivo, P. D. (1995**):" Antiviral susceptibility testing with a cell line which expresses beta-galactosidase after infection with herpes simplex virus". *Antimicrob Agents Chemother*. 39(6):1287-91.
13. **Tsuzuki, M., Miyachi, S. (1991).** "$Co_2$ Syndrome in Chlorella", *Can., J. Bact*. 69, 1003-1007.
14. **Vonshak, A. (1986)**. "Laboratory technologies for cultivation of microalgae". In A. Richmond (ed). Handbook of microalgaal mass culture CRC. Press Baco. Raton. FL. PP: 117-145.
15. **Whitton, B.A., and Holmes N.T.H. (1981)** phytoplankton of four reivers, the Tyne, Wear, Tees and Swale, Dept. of Botany, University of Durham, Durham DID 2LE, Great Britain.
16. **Zarrouk C. (1966),** *Contribution a` l'e´tude d'une* cyano phyce´e. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthe`se de. *Spirulina maxima.* Ph. D. Thesis, Universite.
17. **Zhang J, Zhan B, Yao X, Gao Y, Shong J (1995).** Antiviral activity of tannin from the pericarp of Punica granatum L. against genital Herpes virus in vitro. Zhongguo Zhong Yao Za Zhi, 20(9): 556-558, 576.


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