Antimicrobial activity of aqueous and methanolic extracts from 
*Arthrospira maxima*

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*Arthrospira maxima* is a filamentous, undifferentiated, non-toxigenic cyanobacteria, that has been used as food since ancient times. There have been numerous studies on its antioxidant and anti-inflammatory activities, although antimicrobial action is mentioned, it has not been ascertained. To evaluate the antimicrobial activity of *Arthrospira maxima* different concentrations of aqueous and methanolic extracts were tested by the agar diffusion technique against *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*. The aqueous extracts showed antibacterial activity against all organisms tested, except to *Bacillus subtilis*, while methanol extract showed antimicrobial activity against all microorganisms, even to *Staphylococcus aureus*.

**Keywords** antimicrobial assays; antibacterial; antifungal; cyanobacteria; *Spirulina*; methanolic extracts.

1. Introduction

Cyanobacteria or blue–green algae are photoautotrophic microorganisms largely distributed in nature. Some of them have been used as human food for many years because of their high protein content (35–65%) and nutritional value. *Arthrospira (Spirulina)* is the best known genus and it was consumed by the Aztecs in Mexico Valley and by the Chaad lake population in Africa. At present, some countries are culturing it on a large scale¹. *Spirulina platensis* is a planktonic photosynthetic filamentous cyanobacterium that forms massive populations in tropical and subtropical bodies of water which have high levels of carbonate and bicarbonate, and alkaline pH values of up to 11. This cyanobacterium is recognizable by the main morphological feature of the genus, i.e. the arrangement of multicellular cylindrical trichomes in an open left-hand helix along the entire length of the filaments¹.

For centuries, native peoples have harvested *Spirulina* from Chad Lake in Africa and Texcoco Lake in Mexico for use as a source of food¹, a fact which means that *Spirulina* deserves special attention both as a source of single cell protein (SCP)² and because of its nutraceutical properties. The chemical composition of *Spirulina* indicates that it has high nutritional value due to its content of a wide range of essential nutrients, such as provitamins, minerals, proteins and polyunsaturated fatty acids such as gamma-linolenic acid³. More recently, *Spirulina* has been studied because of its therapeutic properties⁴ and the presence of antioxidant compounds³, ⁵ such as phenolics. The occurrence of phenolic compounds in plants is well documented and these compounds are known to possess antioxidant activity in biological systems but the antioxidant characteristics of algae and cyanobacteria are less well documented, although decreased cholesterol levels have been reported in hypercholesterolemic patients fed *Spirulina*⁶ and the antioxidant activity of phycobiliproteins extracted from *S. platensis* has also been demonstrated⁵.

It has been claimed that consumption of *Spirulina* is beneficial to health due to its chemical composition including compounds like essential amino acids, vitamins, natural pigments, and essential fatty acids, particularly γ-linolenic acid, a precursor of the body’s prostaglandins⁷, ⁸, ⁹, ¹⁰. It has also been reported that some cyanobacteria produce substances that can either promote or inhibit microbial growth¹¹, ¹², ¹³.

Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects¹⁴, ¹⁵. The antimicrobial substances involved may target various kinds of micro-organisms, prokaryotes as well as eukaryotes. The properties of secondary metabolites in nature are not completely understood¹⁶, ¹⁷. Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetic importance. Recently, there has been an increasing interest in cyanobacteria as a potential source for new drugs¹⁸, ¹⁹. Antimicrobial effects from cyanobacterial aqueous and organic solvent extracts are visualized in bioassays using selected micro-organisms as test organisms²⁰, ²¹, ²². Methods commonly applied are based on the agar diffusion principle using pour-plate or spread plate (seeded plates) techniques. Antimicrobial effects are shown as visible zones of growth inhibition (inhibition halos). Bacterial bioassays comprise different target bacteria: *Micrococcus luteus*, *Bacillus subtilis*, *B. cereus* and *Escherichia coli* are commonly used to detect antibiotic residues in food²³, ²⁴.

A large number of microalgal and cyanobacterial extracts and/or extracellular products have been found to have antibacterial or antifungal activity. Pedersen and Dasilva²⁵ have pointed out that the cyanobacterium *Calothrix brevissima* produce bromophenols, which possesses antibacterial activity. Mundt et al.²⁶ have proved that the cyanobacterium *Oscillatoria redekei* produce fatty acids which show antibacterial activity. Materen et al.²⁷ isolated two depsipeptide metabolites, scytoxin A and B from the axenically grown terrestrial cyanobacterium *Scytonema hofmannii*.
PCC 7110 and designated as hofmannolin. Bloor and England\textsuperscript{28} reported that extracellular metabolites produced by \textit{Nostoc muscorum} inhibited the growth of \textit{Bacillus circulans}. Austin \textit{et al.}\textsuperscript{29} reported that the supernatants and extracts derived from a commercial heterotrophically grown spray-dried preparation of \textit{Tetraselmis suecica} (Chlorophyceae) were observed to inhibit \textit{Aeromonas hydrophila}, \textit{A. salmonicida}, \textit{Lactobacillus} sp., \textit{Serratia liquefaciens}, \textit{Staphylococcus epidermidis}, \textit{Vibrio anguillilarium}, \textit{V. salmonicida} and \textit{Yersinia ruckeri} type I. Kim \textit{et al.}\textsuperscript{30} extracted \textit{Hizikia fusiforme} by solvents (hexane ethyl ether and ethanol), these extracts were found to be active against \textit{E. coli} and \textit{Bacillus subtilis}. Ohta \textit{et al.}\textsuperscript{31} have proved that the methanol extracts from \textit{Chlorococcum} strain HS-101 and \textit{Dunaliella primolecta} strongly inhibited the growth of a strain of methicillin resistant \textit{Staphylococcus aureus}, which cause serious problems in Japanese hospitals.

Ostensvik \textit{et al.}\textsuperscript{32} have examined five strains of cyanobacteria for antibacterial activity and they found that the methanol extracts made from \textit{Tychonema bourrellyi}, \textit{Aphanizomenon flos-aquae} and \textit{Cylindrospermopsis raciborskii} showed the most pronounced inhibitory effects against \textit{Bacillus cereus} and \textit{B. subtilis}. Blue green algae (cyanobacteria) have been recognized in the last decades as a source of novel cytotoxic and antifungal metabolites. Some of these metabolites have potential for development of new pharmaceutical compounds. De Cano \textit{et al.}\textsuperscript{12} showed the antifungal activity of phenolic compounds from the terrestrial cyanobacterium \textit{N. muscorum} against \textit{Candida albicans}.

2. Methods

2.1 Propagation of \textit{Arthrospira maxima}

This strain was selected from the strain collection of the Plant Physiology Laboratory, Department of Botany, located in the National School of Biological Sciences, National Polytechnic Institute, and was cultivated in 1 L Erlenmeyer flasks bubbled with air in Zarrouk medium\textsuperscript{33}, under photoperiodic illumination (12/12 photosynthetically active radiation provided by “daylight” fluorescent lamps, at 25 to 28°C.

2.2 Aqueous extracts of \textit{Arthrospira maxima}

The aqueous extract was obtained from 1.5 g or 2.0 g biomass of \textit{A. maxima} of 30 days of growth (harvested by filtration on Whatman No. 1), and resuspended in 10 mL of phosphate buffer pH 7, under aseptic conditions. The cell rupture was reached by freeze-thaw cycles until a change of color was observed. The suspension was centrifuged at 3500 rpm for 45 minutes, the supernatant fluid was withdrawn with a pipette to a sterile container.

2.3 Methanol extracts of \textit{Arthrospira maxima}

Biomass from a 40 mL culture of 15 days of growth was harvested by filtration (Whatman No. 1), and then was weighed, adding, for each gram, 60 mL of methanol and 2mL of acetic acid, and rested for 24 h. After this time the methanol and acetic acid was evaporated to dryness by heating in a water bath. The resulting concentrate was resuspended in 3 mL of methanol.

2.4 Antimicrobial activity tests.

Different concentrations of aqueous and methanol extracts were tested by the agar diffusion technique against \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, \textit{Escherichia coli}, \textit{Proteus vulgaris} and \textit{Candida albicans}. They were cultivated in Müller Hinton solid media which was supplemented with yeast extract. Sterilized Petri dishes containing about 20 mL medium had been inoculated with 0.1 mL of microbial suspension adjusted to tube 1 MacFarland’s nephelometer with saline solution.

Sterilized paper discs (0.6 cm) impregnated with 0.1 mL of the extracted material and air dried, were placed over the agar surface; two discs of each concentration of the extracts were placed on the plates with the tested microorganisms, also a filter paper disk impregnated with Zarrouk media and another disc impregnated with water or methanol (depending on the test extract) were placed.

Two plates were tested per target microorganism. The plates were left 2 h at 4 °C then incubated at 37 °C for 24 h and examined for zones of inhibition around the disc (fig. 1).
3. Results and Discussion

The antimicrobial activity was evaluated as the diameters of the inhibition halos formed as a result of disc assay method. Data present in Table 1 show that the aqueous extract of *A. maxima* was antagonistic to all test microorganisms except *B. subtilis*. The highest antagonism of the aqueous extract was recorded against *S. aureus*. However, inside the inhibition halos there were some resistant colonies, indicating that these colonies represent a variant (mutant) strain.

The methanol extracts showed better antimicrobial activity against all strains used, including *Bacillus subtilis* that was between the most susceptible strains to the methanol extract.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Aqueous</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target microorganism</td>
<td>Inhibition halos (⌀ mm) with 0.15 g/mL</td>
<td>Inhibition halos (⌀ cm) with 0.2 g/mL</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>11.5* 0.6</td>
<td>11.8* 0.5</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>14.0* 2.4</td>
<td>13.0* 2.0</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>11.8* 0.5</td>
<td>11.8* 0.5</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>&gt;50.0 0.5</td>
<td>&gt;50.0 0.5</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
</tr>
</tbody>
</table>

*Some colonies growth in the inhibition areas

Data also show that the three concentrations of methanolic extract of *A. maxima* were antagonistic to all target microorganisms but *S. aureus* to 0.33 g/mL. The highest antagonism of the methanolic extract was recorded against *B. subtilis*, *P. vulgaris* and *S. aureus* (with 0.5 g/mL and 0.66 g/mL).

The larger inhibition halos were with methanolic extract at 0.5 g/mL, in some cases the inhibitory activity decreased while concentration was increased; the inhibition of growth of *P. vulgaris* and *E. coli* was larger with methanolic extracts compared with aqueous extracts. Meanwhile, the inhibition halos against *C. albicans* were similar with both extracts (aqueous and methanolic). The plates seeded with *S. aureus* showed little growth across the board in both tests, indicating susceptibility to water extracts and methanolic extract at 0.33 g/mL.

Clearly, there was a similar inhibitory effect between the last two concentrations and even in most cases the average concentration of 0.5 g/mL resulted in greater inhibition zones, when it was expected that higher concentrations would produce greater inhibition due to the cause-effect relationship between the level of exposure to a substance and the magnitude of the response to this, the toxicity being directly proportional to the dose in most cases. This result can not be fully elucidated, since no one knows the structure of the metabolite responsible for the antimicrobial activity, although it may be due to linoleic acid and other fatty acids whose antimicrobial activity has been tested\(^{34}\) or synergistic effect between these fatty acids\(^{35}\). However, as *Arthospira* as well has harmful or toxic compounds to microorganisms, has compounds that can serve as substrates (vitamins, proteins, carbohydrates, trace elements, fibers, lipids, organic acids, etc.) that stimulate the growth of microorganisms, it is possible that at 0.5 g/mL, the metabolites...
responsible for the antimicrobial activity were qualitatively and quantitatively in the right proportion to deliver antimicrobial activity while at 0.66 the concentration of other metabolites stimulate the growth.

The results above may be the basis for further studies that seek to purify and characterize the metabolite responsible for the activity shown, to propose alternatives therapies (less harmful) for the treatment of opportunistic infections and even for infections caused by pathogenic microorganisms.

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References


