NUTRACEUTICALS FROM FRESHWATER MICROALGAE

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ABSTRACT

Microalgae are excellent reservoirs of adverse range of nutraceuticals. The presence of secondary metabolites, pigments, vitamins makes microalgae unique reservoir of nutraceuticals. Many strains of microalgae are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial and antifungal; in addition, to it, they are also rich in adverse range of antioxidants, vitamins and pigments. There is a drastic increase in cases of antibiotic resistance and exploring the antimicrobial efficacy of microalgae can aid in resolving the problem. In this study, we have investigated the activity of Microalgae Anabaena variabilis, Synechococcus elongates and Spirulina platensis against the human pathogenic bacteria namely Enterococcus sp, Escherichia coli, Pseudomonas sp, Klebsiella sp. Experimental results show that microalgal extracts are capable of inhibiting the growth of pathogenic bacterial strains. Methanol (60%) extract of Anabaena variabilis gave 30mm zone of inhibition against E. coli and methanol extract of Synechococcus elongates gave the highest antimicrobial activity against Enterococcus (32mm inhibition zone) and Pseudomonas sp. (26mm inhibition zone). S. platensis shows highest antimicrobial activity against E. coli with Methanol (100%). As per the formation of zones, ethanol (100%) extract, butanol, water, acetone and methanol (100%) extract gave a high visibility of zone of inhibition. Exploring the nutraceutical efficacy of microalgae can efficiently address the plethora of drug related issues and, in addition, serve as an asource of nutrients.

Keywords: Nutraceutical, Microalgae, Antimicrobial activity, antibiotic resistance.

INTRODUCTION

Microalgae are unique reservoirs of nutraceuticals and produce a potential source of biologically active secondary metabolites, which are compounds that are essential for cell metabolism. The nutraceutical efficacy of the microalgal strains is by virtue of the diverse metabolites, antioxidants enzymes, pigments and vitamins, present in these extraordinary photosynthetic microbes. Many of these compounds possess antibiotic and pharmacological effects such as toxicity for eukaryotic organisms, antitumor, antifungal, antiviral, etc. The high portion of the antimicrobial producing strains may be associated with an ecological role, playing a defensive action to maintain their niche, or enabling the invasion of strain into an established microbial community. Allelopathy defined as the release of chemicals or toxins by plants or microorganisms, that affect their potential competitors for resources. It is a ubiquitous phenomenon in the aquatic ecosystem, and there are diverse organisms involved like angiosperms, macrophytes, and macroalgae. According to Molisch, allelopathy covers biochemical interactions, both stimulatory and inhibitory, among different primary producers or between primary producers and microorganisms. Freshwater microalgae are rich in several bioactive secondary metabolites with diverse chemical structure, which may achieve high concentrations in the aquatic medium when microalgal blooms. Some of the compounds released by microalgae have phytoplankton or aquatic plants.

Extracellular components from cultures of “dominant” microalgae have been reported to be isolated indicating the clear effect of extracellular components in the succession of microalgal populations. Subsequent studies have continued to support the role that these allelochemicals in controlling annual variability in phytoplankton communities. Microalgae are one of the richest sources of known and novel bioactive compounds including toxins with wide pharmaceutical applications. Many compounds from microalgae are useful for the welfare of humanity. Microalgae have been identified as a rich source of biologically active compounds with antiviral, antiplasmodial, antibacterial, antifungal and many more activities. Marine microalgae can serve as an excellent source of large scale production of vitamins like Vitamin E and Vitamin B complex. The pigments like carotenoid and phycobiliprotein extracted from microalgae find wide commercial applications as feed additives, as colouring agents, as drugs and in the cosmetic industry.
The aim of the study reported here is to investigate the nutraceutical potential of three freshwater microalgae as an antibacterial agent against the bacteria of clinical significance.

**MATERIALS & METHODS**

**Microalgal Cultures**

Microalgal strains were isolated from an nearby pond and identified as *A. variabilis*, *S. elongates*, and *S. platensis* pertaxonomic classification. The purified cultures were grown in BG11 medium and kept at optimum conditions.

**Bacterial Cultures**

Four pathogenic strains namely *E. coli*, *Enterococcus sp*, *Pseudomonas sp*, and *Klebsiella sp*, were isolated and cultivated. These isolated strains were cultivated on nutrient agar media and were incubated at 37°C for 24 hours.

**Growth Estimation of Microalgal Cultures**

Cultures were harvested on 16th day, and optical density was observed in every 5th day at O.D. (660 nm) of the incubation period.

**Estimation of Phenolic content**

Estimation of phenolic content was done by Folin-Ciocalteu reagent. Dilute extract of microalgae was mixed with Folin-Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous Na₂CO₃(4ml,1M). The mixtures were allowed to stand for 15 minutes, and the total phenols were determined by colorimetry at 765nm.

**Estimation of Polysaccharides**

The polysaccharide content was determined using Phenol-sulfuric acid method. The concentration of polysaccharides was estimated by harvesting cells and extracting with water (0.5 ml). The supernatant was discarded, and 4M NaOH was added. Initially, it was washed with 100% ethanol and later 90% ethanol was added to the supernatant followed by centrifugation. Later on, 4M NaOH was added to the precipitate and kept for 1 hour. 10% TCA was added to the supernatant and kept overnight.
Estimation of Antibacterial Activity

The antibacterial activity of microalgal extracts was evaluated by agar plate diffusion test and disc method.

**AGAR PLATE METHOD (cup plate method)**

In this technique wells are made in the agar plates by using a screw cork of approximately 10mm diameter. The wells were then filled with the microalgal extracts using a micropipette. The Petri plates were then covered, sealed and incubated at a temperature of 26°C for a period of 48-72 hours. The plates were then analyzed for their antimicrobial activity on the basis of formation of a zone of inhibition.

**DISC METHOD**

In this technique, small discs were cut out from WATTMAN FILTER PAPER having a diameter (8mm). These discs were sterilized and were soaked in microalgal extracts for around twenty minutes and were allowed to dry. These discs were then placed in the Petri plates and were incubated at a room temperature of 26°C for 48-72 hours in an incubator.

**RESULTS & DISCUSSION**

Many microalgae produce compounds with potent biological activities. A number of highly potent microalgal natural products have been uncovered as potential lead compounds for further drug development. In this research, microalgae were collected and cultured in BG11 medium. Spectrophotometrical analysis of the microalgal strains at 660nm by culturing them in BG-11 media depicted the growth. It was observed that *Spirulina platensis* showed maximum growth after the 25th day. Whereas *A. variabilis* attained its maximum growth after the 20th day and in

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### Table 2: Antibacterial activity of *Synechococcus elongates*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>E.coli</th>
<th>Enterococcus sp.</th>
<th>Pseudomonas sp.</th>
<th>Klebsiella sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>15±0.21mm</td>
<td>25±1.5mm</td>
<td>11±0.7mm</td>
<td>10±0.6mm</td>
</tr>
<tr>
<td>Methanol (60%)</td>
<td>23±0.9mm</td>
<td>20±1.8mm</td>
<td>8±0.7mm</td>
<td>-</td>
</tr>
<tr>
<td>Methanol (80%)</td>
<td>26±1.2mm</td>
<td>21±1.9mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol (100%)</td>
<td>30±2.1mm</td>
<td>29±2.1mm</td>
<td>-</td>
<td>20±1.1mm</td>
</tr>
<tr>
<td>Water</td>
<td>18±1.3mm</td>
<td>17±0.8mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetone</td>
<td>22±1.3mm</td>
<td>-</td>
<td>23±1.9mm</td>
<td>27±1.2mm</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>28±1.9mm</td>
<td>16±0.9mm</td>
<td>-</td>
<td>22±1.9mm</td>
</tr>
<tr>
<td>Butanol</td>
<td>30±2.5mm</td>
<td>24±1.8mm</td>
<td>23±1.9mm</td>
<td>-</td>
</tr>
<tr>
<td>Met:Acetone (1:1)</td>
<td>9±0.8mm</td>
<td>-</td>
<td>8±0.4mm</td>
<td>6±0.4mm</td>
</tr>
<tr>
<td>But:methanol (1:1)</td>
<td>12±0.9mm</td>
<td>10±0.8mm</td>
<td>7±0.4mm</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol:Acetic acid (1:1)</td>
<td>14±0.6mm</td>
<td>9±0.5mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>32±2.5mm</td>
<td>30±1.8mm</td>
<td>28±1.5mm</td>
<td>29±1.9mm</td>
</tr>
</tbody>
</table>

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### Table 3: Antibacterial activity of *Spirulina platensis*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>E.coli</th>
<th>Pseudomonas sp</th>
<th>Enterococcus sp</th>
<th>Klebsiella sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (60%)</td>
<td>25±1.8mm</td>
<td>-</td>
<td>32±2.8mm</td>
<td>19±0.8mm</td>
</tr>
<tr>
<td>Methanol (80%)</td>
<td>28±2.1mm</td>
<td>26±1.8mm</td>
<td>27±1.2mm</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>20±1.2mm</td>
<td>-</td>
<td>25±1.8mm</td>
</tr>
<tr>
<td>Butanol</td>
<td>29±1.8mm</td>
<td>30±2.1mm</td>
<td>26±2.1mm</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>33±2.4mm</td>
<td>34±1.9mm</td>
<td>35±2.8mm</td>
<td>30±2.5mm</td>
</tr>
</tbody>
</table>

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### Table 4: Phenol and Polysaccharide content in Cyanobacterial extracts

<table>
<thead>
<tr>
<th>Microalgaes</th>
<th>Phenol</th>
<th>Polysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anabaena variabilis</em></td>
<td>25.12± 0.28 mg/g</td>
<td>80± 1.9 µg</td>
</tr>
<tr>
<td><em>Spirulina platensis</em></td>
<td>33.4± 0.83 mg/g</td>
<td>150± 2.5 µg</td>
</tr>
<tr>
<td><em>Synechococcus elongates</em></td>
<td>25.35± 1.2 mg/g</td>
<td>120± 3.8 µg</td>
</tr>
</tbody>
</table>

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S.elongates it was after the 15th day, indicating the fast growth of S. elongates (Fig. 1).

Based on their growth characteristics, three species were selected for the production of antimicrobial agents against pathogenic organisms like E.coli, Pseudomonas sp., Enterococcus sp., Klebsiella sp. Experimental results show that microalgal extracts are capable of inhibiting the growth of pathogenic bacterial strains. Methanol (60%) extract of Anabaena variabilis gave 30mm zone of inhibition against E.coli (Table 1), and methanol extract of Synechococcus elongates gave the highest antimicrobial activity against Enterococcus (32mm inhibition zone) and Pseudomonas sp. (26mm inhibition zone) as shown in Table 2. S.platensis shows highest antimicrobial

Figure 1: Antibacterial activity of Microalgal Extracts.
activity (Table 3) against E.coli with Methanol (100%). As per the formation of zones, ethanol (100%) extract, butanol, water, acetone and methanol (100%) extract gave a high visibility of zone of inhibition as compared to remaining ones (Figure 1).

As microalgae are very resistant to extreme environmental conditions, they are assuming increasing importance in frontier areas of biotechnology. Microalgae produce potent toxins, but they also produce useful bioactive compounds, including substances with antitumor, antiviral, anticancer, antibiotic and antifungal activity, UV protectants and specific inhibitors of enzymes. There are reports on antimicrobial effects from microagal aqueous and organic solvent extract and have been visualized in bioassays using selected microorganisms. In the investigations, microalgae have demonstrated the antimicrobial effects of many strains like Nostoc, Anabaena, Synechococcus, Oscillatoria against a variety of microbes. Kim showed that 50% of the microalgae genera existed in heterocystous filament form. A total of 100 such samples were taken against 10 different strains of fungi and proved to have fungicidal activity. The unusual metabolites produced by the microalgae have been visualized as either or as products of pharmaceutical importance. Vardi reported the allelopathic relationship between microcystin-producing Microcystis sp. and the dinoflagellate, Peridinium gatunense in the mesotrophic Sea of Galilee. The compounds responsible for the allelopathic response can be attributed to microcystins. The antimicrobial substances involved may target various kinds of microorganisms, prokaryotes as well as eukaryotes.

Phenol and Polysaccharide concentration was observed maximum in S. platensis as compared to A. variabilis and S. elongates. The phenol and polysaccharide content in S. platensis was 33.4 mg/g and 150µg respectively whereas in S. elongates it was 25.35 mg/g and 120µg (Table 4). It was very low in A. variabilis that is 25.12 mg/g and 80µg. Microalgal production of extracellular polymers, mainly EPS is well documented (De Phillips et al., 1998). Exopolysaccharides (EPS) have been reported to play a significant role in the occurrence of phenolic compounds in microalgae is less documented than that in higher plants. Algal phenolic compounds were reported to be potential antioxidants to combat free radicals, which are harmful to our body and food systems. Several epidemiological studies revealed that phenolic compounds present in the diet are helpful in treating coronary heart disease and osteoporosis stroke and other degenerative diseases. Furthermore, phenols have been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory or anti-tumor.

De Phillips has reported the production of extracellular polymers, mainly EPS in microalgae. Polysaccharides play a diverse role in nature and get modified under stress condition. Caiaola have reported the important role of Exopolysaccharides (EPS) by acting as protecting layer in the cell boundary. They also play a role in soil aggregation because they possess gluing properties. There have been reports on the presence of functional groups on EPS, contributing to the binding of heavy metals.

Microalgae are also used in aquaculture, wastewater treatment, food, fertilizers, and production of secondary metabolites including exopolysaccharides, vitamins, toxins, enzymes and pharmaceuticals. Future research should focus on isolating new microalgal strains capable of yielding high-value products and methods to enhance the productivity of these products through genetic engineering. Optimization of incubation conditions and fermenter designs in order to increase productivity are essential for the large-scale industrial production of the microalgal products.

REFERENCES


