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Appendix

Antibacterial Activities of Green Algae (Chlorophyceae) From Narmada River

Shailendra Sharma, Taniya Sengupta

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ABSTRACT

This paper deals with the objective to isolate green algae “Chlorophyceae” from Narmada River and to study its antimicrobial activity. The isolated algae were of two different types one was the fresh algae and the other was the dried form collected from the shore line area of Narmada River. Ethanol, chloroform, water, glycerol, acetone, formaldehyde, phenol and acetic acid crude extract from algae were obtained and tested against one Gram’s Positive bacteria “Bacillus subtilis” and another against Gram’s Negative bacteria “Escherichia coli”. The maximum antibacterial activity was recorded from formaldehyde extracts of both types of algae against both bacteria. No antibacterial activity was recorded from water extract. The minimum activity was recorded from dried algae extract against Bacillus subtilis as compared to fresh one whereas the dried extract was pointed out to give maximum inhibition against Escherichia coli as compared with the fresh one. The present finding indicates the need for detailed work on the aspect, especially with reference to antimicrobial activities against different pathogenic bacteria.

KEY WORDS: Chlorophyceae, Escherichia coli, Bacillus subtilis, Antibacterial activities, Narmada River.

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INTRODUCTION

The Narmada is a river in central India and the fifth largest river in the Indian subcontinent. It forms the traditional boundary between North India and South India and flows westwards over a length of 1,312 km (815.2 mi) before draining through the Gulf of Cambey (Khabat) into the Arabian Sea, 30 km (18.6 mi) west of Bharuch city of Gujarat.[1] It is one of only three major rivers in peninsular India that runs from east to west (largest west flowing river) along with the Tapti River and the Mahi River. It flows through the states of Madhya
Pradesh (1,077 km (669.2 mi)), Maharashtra, (74 km (46.0 mi)) – (35 km (21.7 mi)) border between Madhya Pradesh and Maharashtra and (39 km (24.2 mi)) border between Madhya Pradesh and Gujarat and in Gujarat (161 km (100.0 mi)). The Narmada basin, hemmed between Vindya and Satpuda ranges, extends over an area of 98,796 km² (38,145.3 sq mi) and lies between east longitudes 72 degrees 32' to 81 degrees 45' and north latitudes 21 degrees 20' to 23 degrees 45' lying on the northern extremity of the Deccan Plateau. The basin covers large areas in the states of Madhya Pradesh (86%), Gujarat (14%) and a comparatively smaller area (2%) in Maharashtra. In the river course of 1,312 km (815.2 mi) explained above, there are 41 tributaries, out of which 22 are from the Satpuda range and the rest on the right bank are from the Vindhya range.

Algae are large and diverse group of simple typically autotrophic organisms, ranging from unicellular to multi cellular forms. They have their pigments localised in membrane bounded intracellular bodies (Plastids) have no vascular system and do not develop from an embryo. Algae are an extremely important species they can produce more oxygen then all the plant in the world. They form an important food source for many animals. Thus they are at the bottom of the food chain with many living thing depending upon them. *Chlorophyceae* have primary chloroplasts, i.e. the chloroplasts are surrounded by *two membranes* and probably developed through a single endosymbiotic event. Green Algae have chloroplasts with chlorophyll *a* and *b*.Higher plants are pigmented similarly to Green Algae and probably developed from them.

To date, there is moderately an assortment of reports commerce with the antibacterial activity of solvent extract attained from freshwater algae. Antimicrobial activities in freshwater algae have been reported wide reaching for decades by various scientist (Debro et al;1979, Barchi et al; 1984, De Mule et al; 1991, Ishida et al; 1997, Ozdemir et al;2001, Noaman et al;2004). An effort has been derelict in the present study to explore the antibacterial activities of freshwater algae of Narmada River on both Grams positive and Grams negative bacteria.

**MATERIALS AND METHODS**

The green algae was collected from the shore line areas of Narmada River from Mandleshwar in two forms one is the fresh and the other war the dried form. The plants were washed in tap water and cleaned of all epiphytes and other attached material and salt as soon as possible after collection. Then the plants were air dried for few days in the shade.

2 grams of both fresh and dry algae were soaked into 10ml of each solvent (Acetic acid, acetone, chloroform, ethanol, formaldehyde, glycerol, phenol and water) for five days. The resultant crude extracts were filtered. The crude extracts were kept in 4°C until testing their antibacterial activities against Gram’s negative Bacteria *Escherichia coli* and Gram’s negative Bacteria *Bacillus subtilis* were done. For testing the antibacterial activities Plates of Muller Hinton (Himedia) have been used.
RESULTS AND DISCUSSIONS

Table 01: Antibacterial activity of fresh green algae Chlorophyceae obtained from Narmada Shore line areas.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Extract prepared in</th>
<th>Zone of Inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>1</td>
<td>Acetone</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>Acetic Acid</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>Formaldehyde</td>
<td>6.5</td>
</tr>
<tr>
<td>5</td>
<td>Glycerol</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>Phenol</td>
<td>4.2</td>
</tr>
<tr>
<td>7</td>
<td>Water</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 02: Antibacterial activity of Dried green algae Chlorophyceae obtained from Narmada Shore line areas.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Extract prepared in</th>
<th>Zone of Inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>1</td>
<td>Acetone</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>Acetic Acid</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>Formaldehyde</td>
<td>7.2</td>
</tr>
<tr>
<td>5</td>
<td>Glycerol</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>Phenol</td>
<td>3.8</td>
</tr>
<tr>
<td>7</td>
<td>Water</td>
<td>0.0</td>
</tr>
</tbody>
</table>

On completion of 24 hrs incubation the zone of inhibition were measured in each plate for both the kind of green algae.

Table 01 shows the inhibition zone for each extract prepared from fresh green algae. Algae itself in water does not release any extracellular components for inhibiting the growth of bacteria and in organic solvents except acetone and glycerol the fresh green algae shows the activities against *Escherichia coli*. Against *Bacillus subtilis* the fresh algae also shows remarkable inhibition except chloroform and Glycerol as a solvent. Maximum inhibition had seen in both bacteria from formaldehyde extract that is for *Escherichia coli* and *Bacillus subtilis* 6.5 and 6.0 respectively (Fig 01).

Table 02 shows the results for the dried form of green algae against both selected bacterial species. Alike fresh form the dried form of the green algae also did not show any anti bacterial activities. The other organic extracts show efficient inhibition against *Escherichia coli*, whereas in case of *Bacillus subtilis* the results were positive except acetone and phenol extracts. Here also the most effective results were given by formaldehyde extracts against *Escherichia coli* and *Bacillus subtilis* as 7.2 and 7.8 respectively (Fig 02).
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Formaldehyde Fresh Green Algae

Escherichia coli          Bacillus subtilis

Formaldehyde Dried Green Algae

Escherichia coli          Bacillus subtilis

On comparing it had been concluded that Formaldehyde extracts has been proved to be most acceptable antibacterial agent against pathogenic bacteria. Another thing which have been concluded was that the fresh algae extract was much effective on Gram’s negative bacteria (Escherichia coli) and the dried form shows more response against Gram’s positive bacteria (Bacillus subtilis).

Further work have to be done to determine the effect of different solvents used for extract preparation on the bacterial growth and also other different types of bacteria have to be chosen to find how and to what
extent these extracts can be used as antibacterial agent which could control the growth of lethal pathogenic bacteria.

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